Am J Cardiovasc Dis 2011;1(3):244-254
www.AJCD.us /ISSN: 2160-200X/AJCD1107008

Review Article

Inflammasomes in cardiovascular diseases

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Received July 31, 2011; accepted August 12, 2011; Epub September 10, 2011; published September 30, 2011

Abstract: NOD-like receptors (NLRs) constitute a recently identified family of macromolecules that participate in regulation of innate immune responses. To date, 23 members of the NLR family are identified in humans. Diverse NLRs are stimulated by a broad range of pathogen- or danger-associated molecular patterns, and collectively function as intracellular pattern recognition receptors (PRRs). The most studied inflammasomes are NLRP1 and NLRP3 that process inactive pro-caspase-1 to its active form, allowing the cleavage and subsequent activation of pro-IL-1β and pro-IL-18, and initiation of inflammatory responses. Three models, based upon extracellular ATP/K^+ flux, lysosomal release of cathepsin, and reactive oxygen species, have been proposed to be involved in signaling activation of NLRs and downstream events. In this review, I discuss the current state of knowledge related to the roles of NLRs and inflammasomes in the development of cardiovascular diseases.

Keywords: Cardiovascular diseases, inflammasomes, reactive oxygen species, Interleukin 1 beta, Nod-like receptors

Introduction

Recent advances in cell and molecular biology research have highlighted the physiological role of many multimeric protein macromolecules. Examples include ribosomes that function at the level of protein synthesis, proteasomes and exosomes that constitute machinery for protein and RNA degradation, respectively, and apoptosomes and autophagosomes that determine cell fate (death or survival) [1-3]. A newly discovered complex macromolecule is the inflammasome, which is responsible for activation of the inflammatory cytokines IL-1 and IL-18, and constitutes the first line of host defense [4]. Inflammasomes belong to a family of pattern recognition receptors (PRRs), named Nod-like receptors (NLRs). To date, 23 different NLR genes have been identified in the human genome and at least 34 NLR genes are present in the mouse genome [5]. NLRs have a tripartite domain structure and are characterized by the presence of a central nucleotide-binding oligomerization domain (NOD), also called NACHT domain present in neuronal apoptosis inhibitor proteins (NAIP), and a C-terminal leucine-rich repeats (LRRs) domain of variable length (20-29 amino acids). The N-terminal effector binding region consists of a protein-protein interaction domain, i.e., Pyrin domain (PYD), a caspase recruitment domain (CARD), or baculovirus inhibitor of an apoptosis protein repeat (BIR) domain. Based upon the presence of PYD, CARD and BIR effector domains, NLRs are classified as NLRPs, NLRC, and NAIP, respectively [6]. Currently known members of the NLR family in humans include seven NLRCs (NLRC1-NLRC5, NLRX, and CIITA or NLRA), fourteen NLRPs (NLRP1-NLRP14), and seven NAIPs (NAIP1-NAIP7). Readers interested in the detailed composition of NLRs are referred to two recent, excellent reviews [7-8].

The NOD domain is highly conserved, and has a sequence homology with a nucleotide binding motif of apoptotic protease activating factor-1 (APAF-1) that is responsible for ATP-dependent oligomerization and serves as a platform for recruitment and activation of caspases [9-11]. LRRs are proposed to fold onto the NOD domain, thereby inhibiting its spontaneous oligomerization. LRRs undergo conformational change when sensing PAMPs (pathogen-associated molecular patterns) or DAMPs (danger-associated molecular patterns), triggering oligomerization via the NOD domain and

exposure of the effector CARD or PYD domains that, in turn, induce the recruitment and activation of CARD- or PYD-containing effector molecules, respectively. NLRCs (NLRC1 and NLRC2) interact with RIPK2/RICK (receptor-interacting serine/threonine-protein kinase 2) to induce NF-κB and MAPK (mitogen activated protein kinase) signaling cascades. The CIITA (NLRA) is required as class II MHC transactivator [12]. NLRP1 and NLRP3 form the most studied inflammasomes, activated during early innate responses to pathogens or intracellular danger signals. These NLRPs are thought to recruit the ASC (apoptosis-associated speck-like protein containing CARD) protein to activate caspases. Caspases, along with the cytokines, are produced as a catalytically inactive zymogen (i.e., procaspase) and must be cleaved proteolytically to become active. Four inflammatory caspases that have been described as members of the inflammasome group are caspase-1 (originally named interleukin-1 beta converting enzyme), caspase-4, caspase-5 and caspase-12. Activated caspases are reportedly responsible for converting to active forms pro-IL-1β (31 kDa to 17 kDa) and pro-IL-18 (24 kDa to 18 kDa) [13].

The NLRs are expressed in most cell types of the immune system, but are also reported to be expressed in other tissues. Based upon the expression profile of inflammasome components and cytokines, developed by analyzing the cDNA databases, vascular tissues and the heart were found to express fewer types of NLRs (and toll like receptors (TLRs)) than immune and defense tissues, including blood, lymph nodes, and thymus. The basal expression level of caspase-4, caspase-5 and caspase-12 in the heart and IL-1β in vascular tissue were significantly higher than that detected in other tissues [14]. Yet, based upon the expression levels of other components of the three inflammasomes (NLRP1, NLRP3, and NLRC4), the putative function of which in activating caspase-1 has been shown in human tissues, it was suggested that blood, placenta and thymus, functionally involved in host defense, express inflammasome(s) constitutively. Other tissues (e.g., heart, bone marrow) were suggested to require up regulation of one or two components in order to assemble functional inflammasome [14]. Thus, the heart may only inducibly express inflammasomes and be privileged to prevent uncontrolled inflammatory destruction by inflammasome-mediated innate immune responses. Others have described NLRP3 expression in the chondrocytes, lungs, heart, liver, kidneys, colon and ovaries [15-16].

Stimuli for activation of inflammasomes

Although all their ligands are not yet known, NLR proteins appear to function like their membrane counterpart, i.e., TLRs, in modulating innate immune responses. *In vitro* studies have shown that diverse NLR proteins are activated by viruses (e.g. influenza, Sendai) [17], non-pathogenic or pathogenic bacteria (e.g. *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli*) [18-19], and protozoans (e.g. *Plasmodium*) [20]. NLRC1 (NOD1) recognizes the bacterial toxins, e.g. dipeptide g-D-glutamyl-meso-diaminopimelic acid (iE-DAP) produced by most gram-negative bacteria [21]. Studies in *Nod1*-deficient mice demonstrated a lower expression of proinflammatory genes in response to *Chlamydia trachomatis* infection [22]. Mice with a targeted deletion of the *Nod1* gene displayed an increased susceptibility to *Helicobacter pylori* [23] and *T. cruzi* [24]. NOD1 has also been implicated in priming antigen-specific T cell responses, thereby contributing to the onset of adaptive immunity [25], although the mechanism is poorly understood. NLRC2 (NOD2) is shown to be activated by muramyl dipeptide (MDP), nigericin (*Streptomyces hygroscopicus*), listeriolysin O, and aerolysin (*Aeromonas hydrophila*) or maitotoxin by dinoflagellates [18, 26]. NLRC4 recognizes a common motif in proteins in type III secretion systems of gram-negative bacteria [27] as well as in flagellin [27]. NLRP3 is sensitive to a wide range of agonists including bacterial or viral nucleic acids (DNA or RNA), bacterial double-stranded RNA [28], or endogenous molecules generated due to cellular damage [29]. Likewise, NLRP3 can also be activated by exogenous molecules such as sodium dodecyl sulfate [30], UVB [31], aluminum [32-34] and silica [35-36]. More recently, also shown to trigger inflammation by stimulating inflammasomes were metabolic stimuli, such as uric acid, monosodium urate crystals, pyrophosphate calcium [37], beta-amyloid peptide formed during Alzheimer's disease [38], and hyperglycemia developing during type II diabetes mellitus [39].

Mechanisms of inflammasome activation

Signaling pathways are better known in the case of NLRC1 and NLRC2 inflammasomes.

NOD1 recognizes the dipeptide g-D-glutamyl-meso-diaminopimelic acid (iE-DAP) produced by most Gram-negative and specific Gram-positive bacteria [40-41]. A characterized ligand of NOD2 is muramyl dipeptide (MDP) [42], a component of the wall of virtually all Gram-positive and Gram-negative bacteria [43]. In response to the recognition of ligands by LRRs of NLRC1 and NLRC2, homotypic CARD interactions stimulated RIP2 kinase (RICK) recruitment and polyubiquitylation [44]. Polyubiquitylation of RICK was essential for the recruitment of TAK1 (transforming growth factor β-activated *kinase* 1) for activation of the IkB kinase (IKK) complex and phosphorylation of IκB, allowing the latter to release from the NF-κB complex, translocation of RelA to the nucleus and transcription of NF-κB target genes [45]. The CARD-containing adaptor protein CARD9 was shown to promote activation of MAPKs (p38 and JNK) downstream of NOD2, although it was dispensable for NF-κB activation [46]. Whether NF-κB and MAPK pathways cooperate to regulate the expression of pro-inflammatory molecules, including pro-IL-1β and pro-IL-18, remains to be determined in future studies. Irrespectively, pro-IL-1β and pro-IL-18 require activation by proteolytic cleavage via inflammatory caspases [47-48], which may indicate an interaction with other caspase-containing inflammasomes such as NLRP1, NLRP3 or NLRC4. Indeed, recent findings demonstrate that a direct interaction between NLRC2 and NLRP3 is required for activation of the caspase-dependent cleavage of pro-IL-1β and pro-IL-18 in response to MDP stimulus [49]. In *NOD2*$^{-/-}$ mice, lipopolysaccaride (LPS) - mediated TLR-4 activation was sufficient to induce pro-IL-1β and pro-IL-18 synthesis; however, MDP-mediated NLRP3 activation was not sufficient to promote the production of mature IL-1β, which may mean that interaction between NLRP3 and NLRC2 was required for the activation of caspase-1 and cleavage of IL-1β to a mature form. Similarly, direct interaction via the CARD domain was demonstrated between NLRC2 and NLRP1 by co-immunoprecipitation experiments using cells transfected with constructs containing or lacking the CARD domain [50]. Hetero-oligomerization of NAIP5 with NLRC4 was involved in flagellin-induced caspase 1 activation by *Legionella* [51]. Together, these studies challenge the sequential model of NLR activation and are based on the possibility that the host innate responses might be an outcome of an interactive network of NLR-signaling pathways.

NLRP3 is the most studied inflammasome, yet the precise mechanism of its activation is not clear. One model proposes that extracellular ATP, through activation of the P2X7 (purogenic ionotrophic ATP-gated cation channel), triggers rapid K^+ efflux that is required for activation of inflammasomes in macrophages. ATP alone is not sufficient, and priming of cells with LPS is necessary to induce inflammasome, caspase, and IL-1β activation [52-53]. More recently, it was found that the efflux of K^+ triggered pore formation by pannexin, thereby allowing the delivery of bacterial products into cytosol and NLRP3 activation [54]. Moreover, intracellular K^+ concentration at 150 mM is inhibitory of NLRP3. ATP, a potent activator of NLRP3, decreases intracellular K^+ concentration by 50% to ~70 mM, a level conducible to NLRP3 activation [53]. Inhibition of K^+ efflux by high extracellular K^+ blocked NLRP3 inflammasome activation by multiple agonists (reviewed in [55]). However, thus far there is no report to suggest a direct interaction of inflammasome agonists (pathogen molecules, compounds) and NLRP3, which may mean that other signaling events are also be triggered in inflammasome activation.

Others have suggested that insufficient phagocytosis and clearance of DAMPs (especially large particulate activators such as silica and alum) by phagocytosis results in phagosomal destabilization, lysosome rupture, and cathepsin D release, which triggers inflammasome activation by an as-yet-uncharacterized pathway [33, 38]. This model is supported by findings in cathepsin B inhibitor-treated human cells that exhibited impaired inflammasome activation in response to particulate activators [33]; however, the functional significance of cathepsin B release in inflammasome activation is unclear, as macrophages derived from cathepsin B-deficient mice yielded conflicting results [38, 56]. Finally, NLRP3 inflammasome-activating ligands also stimulate ROS (reactive oxygen species) production, which is known to activate NLRP3, and, subsequently, caspase-1 activation, discussed in detail below.

ROS signaling of inflammasomes

ROS (e.g., H_2O_2, $O_2^{\bullet -}$, and ·OH), due to the presence of unpaired valence shell electrons, are highly reactive. ROS mainly originate as a by-

product of oxygen metabolism in the electron transport chain within the mitochondria, and are also generated through the action of specific oxidases and oxygenases (e.g. xanthine oxidase, NADPH oxidase (NOX)), peroxidases (e.g. myeloperoxidase), and the Fenton reaction in which iron (Fe^{+2})- dependent decomposition of H_2O_2 generate highly reactive hydroxyl radical (·OH) [57]. At the basal level, cellular production of ROS is important for regulation of cell signaling and a variety of physiological responses. ROS production is enhanced in response to invading pathogens or other toxic stimuli, and if not scavenged, can cause cellular injury [58-59]. Glutathione, superoxide dismutases (SOD) and glutathione peroxidases (GPx) have been shown to be most critical in cardiac antioxidant defenses, particularly in protecting the cardiomyocytes from oxidative injury [60]. An imbalance between ROS production and the ability to scavenge these by the antioxidant system can result in oxidative stress-induced pathological processes that have been implicated in hypertension, atherosclerosis, ischemia, and idiopathic, as well as infectious, cardiomyopathies [58-59].

Production of ROS is also crucial to the regulation of innate immune responses. Beyond its function in killing invading pathogens, and maintaining a sterile atmosphere, ROS directs leukocyte recruitment at the injury site and orchestrates inflammatory responses in tissues [61]. Recent findings point to ROS as essential secondary messenger signaling NLRP3/NALP3 inflammasome activation [55, 62]. The use of ROS scavengers can control the response caused by a wide variety of inflammatory agonists, normally through NLRP3 inhibition [49]. It is suggested that inflammasomes, rather than being directly activated by PAMPs, are activated by ROS generated following PAMP's binding to other receptors [39]. Initial studies implicated the activation of NOX as the key source of ROS for NLRP3 activation. For example, the use of a NOX inhibitor (diphenyliodinium, DPI) curtailed inflammasome activation by virtually all NRLP3 agonists [63]. In line with this are the observations that extracellular ATP has been shown to trigger the translocation of cytosolic NOX components to the membrane for the assembly of an active macromolecule complex [64], and NOX2-deficient macrophages are impaired in ATP-mediated ROS production [65]. However, further studies reported that NADPH-dependent ROS is dispensable for inflammasome activation, as NOX2-deficient macrophages exhibited no defects in inflammasome activation with NLRP3 agonists [65]. Likewise, *peripheral blood mononuclear cell*s isolated from patients with chronic granulomatous *disease* (CGD) are deficient in NOX, yet secrete IL-1β in a caspase-1- and NLRP3-dependent manner [66]. Others have shown human NLRP3 activation is independent of NOX1-NOX4 [67]. Since DPI can exert inhibitory effects on mitochondrial ROS production via inhibition of respiratory complex I, recent studies proposed ROS release from mitochondria as the key event in NLRP3 inflammasome activation [68]. One attractive hypothesis is that ATP-induced K^+ efflux associated with NLRP3 activators is involved in ROS generation. Indeed K^+ efflux is shown to generate ROS in human granulocytes [69]. Many of the NLRP3-activating particulate elements, e.g. uric acid crystals, alum, silica, and asbestos, are shown to frustrate phagocytosis and associated ROS production in macrophages [70], though the mechanism by which this occurs is not clear. Ultra-structural studies have indicated that silica and alum trigger damage and rupture of lysosomes [34], and cathepsin B release can possibly mediate inflammasomes. Cathepsin B is shown to trigger ROS production in hepatocytes and neurons [71]. It is, thus, possible that cathepsin B and ionic imbalance work together in activating both ROS and downstream NLRP3, to be determined in future studies.

The next question is how ROS, once produced, are sensed by inflammasomes? One can envision that ROS may either directly be sensed by inflammasomes or indirectly through cytoplasmic proteins that modulate inflammasome activity. In this regard, ATP-mediated ROS production has been shown to induce the PI3K pathway, and pharmacological inhibitors of PI3K inhibited ATP-mediated caspase activation, a finding suggestive of the role of ROS in PI3K signaling of inflammasomes, to be validated in future studies [72]. Others have suggested that ROS modification of antioxidants (e.g. thioredoxin (TRX)) leads to changes in protein-protein interaction. TXN are small proteins found in mammals in three isoforms with natural antioxidant properties. The TRX-1 (MW: 12 kDa) contains many cysteine residues and can, therefore, be oxidized or reduced. At a resting stage,

TRX is bound by TXNIP (thioredoxin-interacting protein). TRX oxidation by ROS releases TXNIP that, in turn, is shown to serve as a ligand to the LRR domain, leading to NLRP3 inflammasome activation. [39]. Consistent with this finding is the observation of decreased ATP-mediated activation of caspase-1 and IL-1β in TXNIP-deficient macrophages [73].

Overall, many studies demonstrate that ROS production by NLRP3 agonists drives inflammasome assembly; however, the mechanism of production and the chemical nature of ROS as well as the mechanism of how ROS triggers NLRP3 activation remain to be further elucidated.

Inflammasome-independent activation of IL-1β and IL-18

Apart from a caspase-1/inflammasome-dependent pathway, IL-1β/IL-18 activation by other pathways, e.g. cathepsin G, elastase, and several matrix metalloproteinases, has also been shown [74-75]. Proteinase 3 (PR3), predominantly present in neutrophils, is one of the most potent enzymes processing IL-1β cleavage [74]. A general concept is that neutrophils are the major source of protease for processing IL-1β during the early phases of bacterial and fungal infections, while caspase-dependent maturation of IL-1β play important roles at later stages of infection. Like IL-1β, non-caspase proteases, e.g. PR3 [76-77] and granzyme (present in cytotoxic T cells and NK cells, and neutrophils) [78] can also cleave IL-18, although it is not clear whether the cleaved product is bioactive.

Role of inflammasomes in cardiovascular disease

The blood vessels, the lungs, heart and blood tissues together participate in a highly organized cardiovascular system. The cardiovascular system is exposed to invading pathogens and pathogen-derived molecules at both systemic and local levels (involving blood and blood vessels). It is recognized that endothelial cells are the first tissue to sense and respond to pathogens (or PAMPs) via the activation of cytokines, chemokines, and dilator hormones [79], facilitating transfer and migration of leukocytes at the site of injury and increased blood flow to promote resolution [80]. In addition to the endothelium, underlying vascular smooth muscle cells (VSMCs) can also sense PAMPs via TLRs and NLRs which result in the activation of vasoactive hormones and increased flow [81]. Cardiomyocytes, the main type of cells in the heart, and heart resident fibroblasts also express TLRs and/or NLRs [82-83]. The role of VSMCs, cardiomyocytes and heart resident fibroblasts in innate immune signaling for control of pathogens is not very clear, although the effect of pathogens or PAMPs clearly contribute to pathology and cardiovascular dysfunction.

The most studied areas of cardiovascular disease/dysfunction and inflammasome activation are atherosclerosis and bacterial septic shock [84]. Several studies have shown that among cytokines produced within the plaque, IL-1β plays an important role. In an *ApoE*$^{-/-}$ murine model of atherosclerosis, deletion of IL-1β led to up to a 30% decrease in the size of atherosclerotic lesions [85]. IL-1 receptor antagonist (IL-1Ra) is an endogenous inhibitor of IL-1. The *ApoE*$^{-/-}$ mice with an *IL-1Ra* deletion ($^{-/-}$) or those heterozygous for *IL-1Ra* ($^{+/-}$) developed significantly larger lesions and an up to 86% increase in macrophage infiltration in the lesion area as compared to *ApoE*$^{-/-}$ x *IL-1Ra*$^{+/+}$ mice [86-87]. In contrast, *ApoE*$^{-/-}$ mice crossed with *IL-1Ra*^{tg} mice either over-expressing a secreted form of IL-1Ra or an intracellular form of IL-1Ra, exhibited a sharp decline in atherosclerotic lesion area within aortic roots and thoraco-abdominal aorta [87]. Administration of recombinant IL-1Ra [88] or IL-1Ra-encoding plasmid [89] in *ApoE*$^{-/-}$ mice also resulted in the decreased development of atherosclerotic lesions. Similar to the effect of IL-1β, the pro-athrogenic role of IL-18 has also been shown. *ApoE*$^{-/-}$ mice with deletion of *IL-18* ($^{-/-}$) or the IL-18 receptor (*IL-18R*$^{-/-}$) exhibited greatly reduced atherogenic plaques [88-89], while administration of recombinant IL-18 in *ApoE*$^{-/-}$ mice increased the atherosclerotic lesion size and lesion-associated T lymphocytes [90]. These data show the atherogenic role of IL-1β and IL-18. The significance of inflammasome activation in the IL-1β/IL-18-mediated pathogenesis of atherosclerosis is, however, debated. A recent study with *ApoE*$^{-/-}$ mice crossed with *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$ or *caspase-1*$^{-/-}$ mice showed no differences in the atherosclerotic plaque surface across the aorta, plaque stability, and recruitment of macrophages to the

plaque site in double knock outs and controls, a finding which may indicate that NLRP3 inflammasome is not the only source of IL-1β and IL-18 in the pathogenesis of atherosclerosis [91]. In another study, mice deficient in the low-density lipoprotein receptor and transplanted with *Nlrp3*-, *Asc*- or *IL-1α/β*-deficient bone marrow cells showed an up to 60% decrease in total lesion size at the aortic sinus when compared to controls, and thus the authors proposed an implication of the NLRP3 inflammasome in disease progression [92].

Many bacterial pathogens are capable of activating inflammasomes either by fragments of their walls (LPS, MDP etc.) or by the toxins they secrete. This underscores the fact that the presence of periodontitis increases significantly the risk of atherosclerosis [93-94], and, therefore, coronary heart disease and stroke [95]. The spirochete *Treponema denticola,* associated with periodontal disease as well as cardiovascular injury, is shown to induce the endothelial expression of heat shock protein (Hsp70) and hemoxygenase 1 (HO 1) [96], and one can speculate also the activation of inflammasome, like that noted in *Treponema pallidum* infection model [97].

Cytokine IL-1β has been implicated in the causation of ischemia reperfusion (I/R) injury during myocardial infarction based on evidence of its increased secretion in human *ex vivo* cardiac I/R models [98], though it remains unclear what mechanisms may trigger IL-1β in myocardial I/R injury. In healthy animal models of I/R, PAMPs are unlikely to stimulate clinical and laboratory indicators of acute inflammation, as cytokine release occurs only after occlusion and induction of ischemia. An alternative explanation for the secretion of IL-1β and other cytokines in I/R could be the activation of inflammasomes in response to endogenous molecules, e.g. host-derived DNA, RNA, or particles (e.g. uric acid crystals) that are released upon I/R-mediated cellular injury. Indeed, a significant amount of literature supports the formation of uric acid and calcium pyrophosphate crystals (CaPP) during I/R conditions [99-100]. Further, due to reduced perfusion and oxygen deprivation during ischemia, tissues shift to anaerobic respiration. The resultant intracellular metabolic acidosis and disruption of mitochondrial ATP production creates alterations in ion channels, an influx of Na^+ and $Ca^{+,}$ and an efflux of K^+ ions [101]. Thus, conditions conducive to NLRP3 activation and IL-1β secretion are generated during ischemia. The end result of an ischemia-induced, IL-1β-mediated inflammatory cascade is cell membrane damage, edema and cell death that can be further enhanced during a reperfusion phase which is associated with the generation of free radicals [102]. This, in turn, can lead to more intracellular danger signal formation and create a self-perpetuating cycle of inflammation overwhelming repair mechanisms. A recent study in mice demonstrated that the occlusion of the left anterior descending artery followed by reperfusion resulted in a significant increase in ASC expression primarily detected on infiltrating neutrophils and mononuclear cells, and weakly on cardiac resident fibroblasts. *ASC*$^{-/-}$ and *Caspase-1*$^{-/-}$ mice, as compared to wild-type controls, exhibited a significant decline in cardiac infiltration of phagocytes, inflammatory cytokine (IL-1β, TNF-α) levels, I/R-induced infarct size, and myocardial fibrosis and dysfunction. Using a transplantation model whereby bone marrow cells from wild type mice were transplanted into *Asc*$^{-/-}$ mice (and vice versa), the authors showed that infiltrating bone marrow cells and myocardial resident fibroblasts (not cardiomyocytes) are the primary site of ASC, caspase-1, and IL-1β expression in response to I/R injury, and this activation of inflammasomes was dependent upon ROS and K^+ efflux [103]. A similar up regulation of ASC and inflammasomes closely associated with inflammation in cardiac allograft has been noted, and it is suggested that ASC could be a new target to inhibit the rejection of transplanted hearts [104].

Conclusions

The studies discussed in this review point to key role of inflammasomes in inducing PAMP or DAMP-mediated inflammation in the vascular system and heart. Thus, development of antagonists of the inflammasomes and their use alone or in combination with the currently used therapies to achieve synergistic effects for control of atherosclerosis, I/R injury, and possibly other cardiovascular diseases, is a promising avenue of research.

Abbreviations

ASC, Apoptotic speck *protein*; BIR, Baculovirus inhibitor of apoptosis protein repeat; CARD, Caspase recruitment domain; DAMPs, Danger-

associated molecular patterns; IL-1β, Interleukin 1 beta; IL-18, Interleukin 18; LRR, Leucine-rich repeats; NLR, Nod-like receptors; NOD, Nucleotide-binding oligomerization domain; PAMPs, Pathogen-associated molecular patterns; PRR, Pattern recognition receptors; PYD, Pyrin domain.

Acknowledgements

The research in NJG lab is currently supported by grants from the National Heart Lung Blood Institute (HL088230, HL094802) and the National Institutes of Allergy and Infectious Diseases (AI054578) of the National Institutes of Health.

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References

[1] Abdelaziz DH, Gavrilin MA, Akhter A, Caution K, Kotrange S, Khweek AA, Abdulrahman BA, Grandhi J, Hassan ZA, Marsh C, Wewers MD, Amer AO. Apoptosis-associated speck-like protein (ASC) controls Legionella pneumophila infection in human monocytes. J Biol Chem. 2011; 286: 3203-8.

[2] Burman C, Ktistakis NT. Autophagosome formation in mammalian cells. Semin Immunopathol. 2010; 32: 397-413.

[3] Lorentzen E, Conti E. The exosome and the proteasome: nano-compartments for degradation. Cell. 2006; 125: 651-4.

[4] Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell. 2002; 10: 417-26.

[5] Kanneganti TD. Central roles of NLRs and inflammasomes in viral infection. Nat Rev Immunol. 2010; 10: 688-98.

[6] Ting JP, Lovering RC, Alnemri ES, Bertin J, Boss JM, Davis BK, Flavell RA, Girardin SE, Godzik A, Harton JA, Hoffman HM, Hugot JP, Inohara N, Mackenzie A, Maltais LJ, Nunez G, Ogura Y, Otten LA, Philpott D, Reed JC, Reith W, Schreiber S, Steimle V, Ward PA. The NLR gene family: a standard nomenclature. Immunity. 2008; 28: 285-7.

[7] Davis BK, Wen H, Ting JP. The inflammasome NLRs in immunity, inflammation, and associated diseases. Annu Rev Immunol. 2011; 29: 707-35.

[8] Guarda G, So A. Regulation of inflammasome activity. Immunology. 2010; 130: 329-36.

[9] Coussens NP, Mowers JC, McDonald C, Nunez G, Ramaswamy S. Crystal structure of the Nod1 caspase activation and recruitment domain. Biochem Biophys Res Commun. 2007; 353: 1-5.

[10] Netea MG, Azam T, Ferwerda G, Girardin SE, Walsh M, Park JS, Abraham E, Kim JM, Yoon DY, Dinarello CA, Kim SH. IL-32 synergizes with nucleotide oligomerization domain (NOD) 1 and NOD2 ligands for IL-1beta and IL-6 production through a caspase 1-dependent mechanism. Proc Natl Acad Sci U S A. 2005; 102: 16309-14.

[11] Inohara N, Koseki T, del Peso L, Hu Y, Yee C, Chen S, Carrio R, Merino J, Liu D, Ni J, Nunez G. Nod1, an Apaf-1-like activator of caspase-9 and nuclear factor-kappaB. J Biol Chem. 1999; 274: 14560-7.

[12] Steimle V, Otten LA, Zufferey M, Mach B. Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). Cell. 1993; 75: 135-46.

[13] van de Veerdonk FL, Netea MG, Dinarello CA, Joosten LA. Inflammasome activation and IL-1beta and IL-18 processing during infection. Trends Immunol. 2011; 32: 110-6.

[14] 14.Yin Y, Yan Y, Jiang X, Mai J, Chen NC, Wang H, Yang XF. Inflammasomes are differentially expressed in cardiovascular and other tissues. Int J Immunopathol Pharmacol. 2009; 22: 311-22.

[15] Kummer JA, Broekhuizen R, Everett H, Agostini L, Kuijk L, Martinon F, van Bruggen R, Tschopp J. Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response. J Histochem Cytochem. 2007; 55: 443-52.

[16] Sutterwala FS, Ogura Y, Zamboni DS, Roy CR, Flavell RA. NALP3: a key player in caspase-1 activation. J Endotoxin Res. 2006; 12: 251-6.

[17] Kanneganti TD, Body-Malapel M, Amer A, Park JH, Whitfield J, Franchi L, Taraporewala ZF, Miller D, Patton JT, Inohara N, Nunez G. Critical role for Cryopyrin/Nalp3 in activation of caspase-1 in response to viral infection and double -stranded RNA. J Biol Chem. 2006; 281: 36560 -8.

[18] Mariathasan S, Weiss DS, Newton K, McBride J, O'Rourke K, Roose-Girma M, Lee WP, Weinrauch Y, Monack DM, Dixit VM. Cryopyrin activates the inflammasome in response to toxins and ATP. Nature. 2006; 440: 228-32.

[19] Petrilli V, Dostert C, Muruve DA, Tschopp J. The inflammasome: a danger sensing complex triggering innate immunity. Curr Opin Immunol. 2007; 19: 615-22.

[20] Shio MT, Eisenbarth SC, Savaria M, Vinet AF, Bellemare MJ, Harder KW, Sutterwala FS, Bohle DS, Descoteaux A, Flavell RA, Olivier M. Malarial hemozoin activates the NLRP3 inflam-

masome through Lyn and Syk kinases. PLoS Pathog. 2009; 5: e1000559.
[21] Hasegawa M, Kawasaki A, Yang K, Fujimoto Y, Masumoto J, Breukink E, Nunez G, Fukase K, Inohara N. A role of lipophilic peptidoglycan-related molecules in induction of Nod1-mediated immune responses. J Biol Chem. 2007; 282: 11757-64.
[22] Welter-Stahl L, Ojcius DM, Viala J, Girardin S, Liu W, Delarbre C, Philpott D, Kelly KA, Darville T. Stimulation of the cytosolic receptor for peptidoglycan, Nod1, by infection with *Chlamydia trachomatis* or *Chlamydia muridarum*. Cell Microbiol. 2006; 8: 1047-57.
[23] Viala J, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP, Athman R, Memet S, Huerre MR, Coyle AJ, DiStefano PS, Sansonetti PJ, Labigne A, Bertin J, Philpott DJ, Ferrero RL. Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* cag pathogenicity island. Nat Immunol. 2004; 5: 1166-74.
[24] Silva GK, Gutierrez FR, Guedes PM, Horta CV, Cunha LD, Mineo TW, Santiago-Silva J, Kobayashi KS, Flavell RA, Silva JS, Zamboni DS. Cutting edge: nucleotide-binding oligomerization domain 1-dependent responses account for murine resistance against *Trypanosoma cruzi* infection. J Immunol. 2010; 184: 1148-52.
[25] Fritz JH, Le Bourhis L, Sellge G, Magalhaes JG, Fsihi H, Kufer TA, Collins C, Viala J, Ferrero RL, Girardin SE, Philpott DJ. Nod1-mediated innate immune recognition of peptidoglycan contributes to the onset of adaptive immunity. Immunity. 2007; 26: 445-59.
[26] Gurcel L, Abrami L, Girardin S, Tschopp J, van der Goot FG. Caspase-1 activation of lipid metabolic pathways in response to bacterial pore-forming toxins promotes cell survival. Cell. 2006; 126: 1135-45.
[27] Miao EA, Warren SE. Innate immune detection of bacterial virulence factors via the NLRC4 inflammasome. J Clin Immunol. 2010; 30: 502-6.
[28] Cook GP, Savic S, Wittmann M, McDermott MF. The NLRP3 inflammasome, a target for therapy in diverse disease states. Eur J Immunol. 2010; 40: 631-4.
[29] Cassel SL, Sutterwala FS. Sterile inflammatory responses mediated by the NLRP3 inflammasome. Eur J Immunol. 2010; 40: 607-11.
[30] Watanabe H, Gaide O, Petrilli V, Martinon F, Contassot E, Roques S, Kummer JA, Tschopp J, French LE. Activation of the IL-1beta-processing inflammasome is involved in contact hypersensitivity. J Invest Dermatol. 2007; 127: 1956-63.
[31] Feldmeyer L, Keller M, Niklaus G, Hohl D, Werner S, Beer HD. The inflammasome mediates UVB-induced activation and secretion of interleukin-1beta by keratinocytes. Curr Biol. 2007; 17: 1140-5.
[32] Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS, Flavell RA. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. Nature. 2008; 453: 1122-6.
[33] Hornung V, Bauernfeind F, Halle A, Samstad EO, Kono H, Rock KL, Fitzgerald KA, Latz E. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. Nat Immunol. 2008; 9: 847-56.
[34] Kool M, Petrilli V, De Smedt T, Rolaz A, Hammad H, van Nimwegen M, Bergen IM, Castillo R, Lambrecht BN, Tschopp J. Cutting edge: alum adjuvant stimulates inflammatory dendritic cells through activation of the NALP3 inflammasome. J Immunol. 2008; 181: 3755-9.
[35] Cassel SL, Eisenbarth SC, Iyer SS, Sadler JJ, Colegio OR, Tephly LA, Carter AB, Rothman PB, Flavell RA, Sutterwala FS. The Nalp3 inflammasome is essential for the development of silicosis. Proc Natl Acad Sci U S A. 2008; 105: 9035-40.
[36] Dostert C, Petrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. Science. 2008; 320: 674-7.
[37] Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature. 2006; 440: 237-41.
[38] Halle A, Hornung V, Petzold GC, Stewart CR, Monks BG, Reinheckel T, Fitzgerald KA, Latz E, Moore KJ, Golenbock DT. The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. Nat Immunol. 2008; 9: 857-65.
[39] Schroder K, Zhou R, Tschopp J. The NLRP3 inflammasome: a sensor for metabolic danger? Science. 2010; 327: 296-300.
[40] Hasegawa M, Yang K, Hashimoto M, Park JH, Kim YG, Fujimoto Y, Nunez G, Fukase K, Inohara N. Differential release and distribution of Nod1 and Nod2 immunostimulatory molecules among bacterial species and environments. J Biol Chem. 2006; 281: 29054-63.
[41] Chamaillard M, Hashimoto M, Horie Y, Masumoto J, Qiu S, Saab L, Ogura Y, Kawasaki A, Fukase K, Kusumoto S, Valvano MA, Foster SJ, Mak TW, Nunez G, Inohara N. An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. Nat Immunol. 2003; 4: 702-7.
[42] Yang Y, Yin C, Pandey A, Abbott D, Sassetti C, Kelliher MA. NOD2 pathway activation by MDP or *Mycobacterium tuberculosis* infection involves the stable polyubiquitination of Rip2. J Biol Chem. 2007; 282: 36223-9.
[43] Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ, Sansonetti PJ. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem. 2003; 278: 8869-72.
[44] Ogura Y, Inohara N, Benito A, Chen FF, Yama-

oka S, Nunez G. Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. J Biol Chem. 2001; 276: 4812-8.
[45] Hasegawa M, Fujimoto Y, Lucas PC, Nakano H, Fukase K, Nunez G, Inohara N. A critical role of RICK/RIP2 polyubiquitination in Nod-induced NF-kappaB activation. EMBO J. 2008; 27: 373-83.
[46] Hsu YM, Zhang Y, You Y, Wang D, Li H, Duramad O, Qin XF, Dong C, Lin X. The adaptor protein CARD9 is required for innate immune responses to intracellular pathogens. Nat Immunol. 2007; 8: 198-205.
[47] Martinon F, Tschopp J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. Cell. 2004; 117: 561-74.
[48] Dinarello CA. Interleukin-1 beta, interleukin-18, and the interleukin-1 beta converting enzyme. Ann N Y Acad Sci. 1998; 856: 1-11.
[49] Pan Q, Mathison J, Fearns C, Kravchenko VV, Da Silva Correia J, Hoffman HM, Kobayashi KS, Bertin J, Grant EP, Coyle AJ, Sutterwala FS, Ogura Y, Flavell RA, Ulevitch RJ. MDP-induced interleukin-1beta processing requires Nod2 and CIAS1/NALP3. J Leukoc Biol. 2007; 82: 177-83.
[50] Hsu LC, Ali SR, McGillivray S, Tseng PH, Mariathasan S, Humke EW, Eckmann L, Powell JJ, Nizet V, Dixit VM, Karin M. A NOD2-NALP1 complex mediates caspase-1-dependent IL-1beta secretion in response to Bacillus anthracis infection and muramyl dipeptide. Proc Natl Acad Sci U S A. 2008; 105: 7803-8.
[51] Lightfield KL, Persson J, Trinidad NJ, Brubaker SW, Kofoed EM, Sauer JD, Dunipace EA, Warren SE, Miao EA, Vance RE. Differential requirements for NAIP5 in activation of the NLRC4 inflammasome. Infect Immun. 2011; 79: 1606-14.
[52] Franchi L, Eigenbrod T, Nunez G. Cutting edge: TNF-alpha mediates sensitization to ATP and silica via the NLRP3 inflammasome in the absence of microbial stimulation. J Immunol. 2009; 183: 792-6.
[53] Petrilli V, Papin S, Dostert C, Mayor A, Martinon F, Tschopp J. Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. Cell Death Differ. 2007; 14: 1583-9.
[54] Kanneganti TD, Lamkanfi M, Kim YG, Chen G, Park JH, Franchi L, Vandenabeele P, Nunez G. Pannexin-1-mediated recognition of bacterial molecules activates the cryopyrin inflammasome independent of Toll-like receptor signaling. Immunity. 2007; 26: 433-43.
[55] Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. Annu Rev Immunol. 2009; 27: 229-65.
[56] Dostert C, Guarda G, Romero JF, Menu P, Gross O, Tardivel A, Suva ML, Stehle JC, Kopf M, Stamenkovic I, Corradin G, Tschopp J. Malarial hemozoin is a Nalp3 inflammasome activating danger signal. PLoS One. 2009; 4: e6510.
[57] Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol. 2003; 552: 335-44.
[58] Khaper N, Bryan S, Dhingra S, Singal R, Bajaj A, Pathak CM, Singal PK. Targeting the vicious inflammation-oxidative stress cycle for the management of heart failure. Antioxid Redox Signal. 2010; 13: 1033-49.
[59] Lakshmi SV, Padmaja G, Kuppusamy P, Kutala VK. Oxidative stress in cardiovascular disease. Indian J Biochem Biophys. 2009; 46: 421-40.
[60] Marczin N, El-Habashi N, Hoare GS, Bundy RE, Yacoub M. Antioxidants in myocardial ischemia-reperfusion injury: therapeutic potential and basic mechanisms. Arch Biochem Biophys. 2003; 420: 222-36.
[61] Niethammer P, Grabher C, Look AT, Mitchison TJ. A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish. Nature. 2009; 459: 996-9.
[62] Bryant C, Fitzgerald KA. Molecular mechanisms involved in inflammasome activation. Trends Cell Biol. 2009; 19: 455-64.
[63] Bauernfeind F, Bartok E, Rieger A, Franchi L, Nunez G, Hornung V. Cutting Edge: Reactive Oxygen Species Inhibitors Block Priming, but Not Activation, of the NLRP3 Inflammasome. J Immunol. 2011; 187: 613-7.
[64] Hewinson J, Moore SF, Glover C, Watts AG, MacKenzie AB. A key role for redox signaling in rapid P2X7 receptor-induced IL-1 beta processing in human monocytes. J Immunol. 2008; 180: 8410-20.
[65] Moore SF, MacKenzie AB. NADPH oxidase NOX2 mediates rapid cellular oxidation following ATP stimulation of endotoxin-primed macrophages. J Immunol. 2009; 183: 3302-8.
[66] van de Veerdonk FL, Smeekens SP, Joosten LA, Kullberg BJ, Dinarello CA, van der Meer JW, Netea MG. Reactive oxygen species-independent activation of the IL-1beta inflammasome in cells from patients with chronic granulomatous disease. Proc Natl Acad Sci U S A. 2010; 107: 3030-3.
[67] van Bruggen R, Koker MY, Jansen M, van Houdt M, Roos D, Kuijpers TW, van den Berg TK. Human NLRP3 inflammasome activation is Nox1-4 independent. Blood. 2010; 115: 5398-400.
[68] Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. Nature. 2011; 469: 221-5.
[69] Fay AJ, Qian X, Jan YN, Jan LY. SK channels mediate NADPH oxidase-independent reactive oxygen species production and apoptosis in granulocytes. Proc Natl Acad Sci U S A. 2006; 103: 17548-53.
[70] Bergstrand H. The generation of reactive oxygen-derived species by phagocytes. Agents Actions Suppl. 1990; 30: 199-211.
[71] Windelborn JA, Lipton P. Lysosomal release of

cathepsins causes ischemic damage in the rat hippocampal slice and depends on NMDA-mediated calcium influx, arachidonic acid metabolism, and free radical production. J Neurochem. 2008; 106: 56-69.
[72] Cruz CM, Rinna A, Forman HJ, Ventura AL, Persechini PM, Ojcius DM. ATP activates a reactive oxygen species-dependent oxidative stress response and secretion of proinflammatory cytokines in macrophages. J Biol Chem. 2007; 282: 2871-9.
[73] Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol. 2010; 11: 136-40.
[74] Coeshott C, Ohnemus C, Pilyavskaya A, Ross S, Wieczorek M, Kroona H, Leimer AH, Cheronis J. Converting enzyme-independent release of tumor necrosis factor alpha and IL-1beta from a stimulated human monocytic cell line in the presence of activated neutrophils or purified proteinase 3. Proc Natl Acad Sci U S A. 1999; 96: 6261-6.
[75] Schonbeck U, Mach F, Libby P. Generation of biologically active IL-1 beta by matrix metalloproteinases: a novel caspase-1-independent pathway of IL-1 beta processing. J Immunol. 1998; 161: 3340-6.
[76] Ikawa K, Nishioka T, Yu Z, Sugawara Y, Kawagoe J, Takizawa T, Primo V, Nikolic B, Kuroishi T, Sasano T, Shimauchi H, Takada H, Endo Y, Sugawara S. Involvement of neutrophil recruitment and protease-activated receptor 2 activation in the induction of IL-18 in mice. J Leukoc Biol. 2005; 78: 1118-26.
[77] Sugawara S, Uehara A, Nochi T, Yamaguchi T, Ueda H, Sugiyama A, Hanzawa K, Kumagai K, Okamura H, Takada H. Neutrophil proteinase 3-mediated induction of bioactive IL-18 secretion by human oral epithelial cells. J Immunol. 2001; 167: 6568-75.
[78] Omoto Y, Yamanaka K, Tokime K, Kitano S, Kakeda M, Akeda T, Kurokawa I, Gabazza EC, Tsutsui H, Katayama N, Yamanishi K, Nakanishi K, Mizutani H. Granzyme B is a novel interleukin-18 converting enzyme. J Dermatol Sci. 2010; 59: 129-35.
[79] Foldes G, Liu A, Badiger R, Paul-Clark M, Moreno L, Lendvai Z, Wright JS, Ali NN, Harding SE, Mitchell JA. Innate immunity in human embryonic stem cells: comparison with adult human endothelial cells. PLoS One. 2010; 5: e10501.
[80] Tousoulis D, Andreou I, Antoniades C, Tentolouris C, Stefanadis C. Role of inflammation and oxidative stress in endothelial progenitor cell function and mobilization: therapeutic implications for cardiovascular diseases. Atherosclerosis. 2008; 201: 236-47.
[81] Schultz K, Murthy V, Tatro JB, Beasley D. Endogenous interleukin-1 alpha promotes a proliferative and proinflammatory phenotype in human vascular smooth muscle cells. Am J Physiol Heart Circ Physiol. 2007; 292: H2927-34.
[82] Hirao K, Yumoto H, Takahashi K, Mukai K, Nakanishi T, Matsuo T. Roles of TLR2, TLR4, NOD2, and NOD1 in pulp fibroblasts. J Dent Res. 2009; 88: 762-7.
[83] Boyd JH, Mathur S, Wang Y, Bateman RM, Walley KR. Toll-like receptor stimulation in cardiomyoctes decreases contractility and initiates an NF-kappaB dependent inflammatory response. Cardiovasc Res. 2006; 72: 384-93.
[84] Grundmann S, Bode C, Moser M. Inflammasome activation in reperfusion injury: friendly fire on myocardial infarction? Circulation. 2011; 123: 574-6.
[85] Kirii H, Niwa T, Yamada Y, Wada H, Saito K, Iwakura Y, Asano M, Moriwaki H, Seishima M. Lack of interleukin-1beta decreases the severity of atherosclerosis in ApoE-deficient mice. Arterioscler Thromb Vasc Biol. 2003; 23: 656-60.
[86] Isoda K, Sawada S, Ishigami N, Matsuki T, Miyazaki K, Kusuhara M, Iwakura Y, Ohsuzu F. Lack of interleukin-1 receptor antagonist modulates plaque composition in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol. 2004; 24: 1068-73.
[87] Merhi-Soussi F, Kwak BR, Magne D, Chadjichristos C, Berti M, Pelli G, James RW, Mach F, Gabay C. Interleukin-1 plays a major role in vascular inflammation and atherosclerosis in male apolipoprotein E-knockout mice. Cardiovasc Res. 2005; 66: 583-93.
[88] Elhage R, Jawien J, Rudling M, Ljunggren HG, Takeda K, Akira S, Bayard F, Hansson GK. Reduced atherosclerosis in interleukin-18 deficient apolipoprotein E-knockout mice. Cardiovasc Res. 2003; 59: 234-40.
[89] Mallat Z, Corbaz A, Scoazec A, Graber P, Alouani S, Esposito B, Humbert Y, Chvatchko Y, Tedgui A. Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. Circ Res. 2001; 89: E41-5.
[90] Whitman SC, Ravisankar P, Daugherty A. Interleukin-18 enhances atherosclerosis in apolipoprotein E(-/-) mice through release of interferon -gamma. Circ Res. 2002; 90: E34-8.
[91] Menu P, Pellegrin M, Aubert JF, Bouzourene K, Tardivel A, Mazzolai L, Tschopp J. Atherosclerosis in ApoE-deficient mice progresses independently of the NLRP3 inflammasome. Cell Death Dis. 2011; 2: e137.
[92] Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, Abela GS, Franchi L, Nunez G, Schnurr M, Espevik T, Lien E, Fitzgerald KA, Rock KL, Moore KJ, Wright SD, Hornung V, Latz E. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Nature. 2010; 464: 1357-61.
[93] Roth GA, Moser B, Roth-Walter F, Giacona MB,

Harja E, Papapanou PN, Schmidt AM, Lalla E. Infection with a periodontal pathogen increases mononuclear cell adhesion to human aortic endothelial cells. Atherosclerosis. 2007; 190: 271-81.
[94] Goodie DB, Geiser JF. Atrial thrombosis induced by percutaneous central venous catheter: a potential hazard during cardiac surgery diagnosed by intra-operative transoesophageal echo. Anaesth Intensive Care. 1995; 23: 725-7.
[95] Inaba H, Amano A. Roles of oral bacteria in cardiovascular diseases-from molecular mechanisms to clinical cases: Implication of periodontal diseases in development of systemic diseases. J Pharmacol Sci. 2010; 113: 103-9.
[96] Bernardini C, Gaibani P, Zannoni A, Vocale C, Bacci ML, Piana G, Forni M, Sambri V. *Treponema denticola* alters cell vitality and induces HO-1 and Hsp70 expression in porcine aortic endothelial cells. Cell Stress Chaperones. 2010; 15: 509-16.
[97] Babolin C, Amedei A, Ozolins D, Zilevica A, D'Elios MM, de Bernard M. TpF1 from *Treponema pallidum* activates inflammasome and promotes the development of regulatory T cells. J Immunol. 2011.
[98] Pomerantz BJ, Reznikov LL, Harken AH, Dinarello CA. Inhibition of caspase 1 reduces human myocardial ischemic dysfunction via inhibition of IL-18 and IL-1beta. Proc Natl Acad Sci U S A. 2001; 98: 2871-6.
[99] Carr EA, Jr., Carroll M, Gona JM, Balderman SC, Montes M, Steinbach JJ. Effect of vitamin D3 on imaging of experimental myocardial infarcts with technetium-99m pyrophosphate: further studies of the role of calcium. J Nucl Med. 1985; 26: 157-64.
[100] Bos MJ, Koudstaal PJ, Hofman A, Witteman JC, Breteler MM. Uric acid is a risk factor for myocardial infarction and stroke: the Rotterdam study. Stroke. 2006; 37: 1503-7.
[101] Weinberg JM. The cell biology of ischemic renal injury. Kidney Int. 1991; 39: 476-500.
[102] Lefer AM, Lefer DJ. Pharmacology of the endothelium in ischemia-reperfusion and circulatory shock. Annu Rev Pharmacol Toxicol. 1993; 33: 71-90.
[103] Kawaguchi M, Takahashi M, Hata T, Kashima Y, Usui F, Morimoto H, Izawa A, Takahashi Y, Masumoto J, Koyama J, Hongo M, Noda T, Nakayama J, Sagara J, Taniguchi S, Ikeda U. Inflammasome activation of cardiac fibroblasts is essential for myocardial ischemia/reperfusion injury. Circulation. 2011; 123: 594-604.
[104] Seto T, Kamijo S, Wada Y, Yamaura K, Takahashi K, Komatsu K, Otsu Y, Terasaki T, Fukui D, Amano J, Taniguchi S, Sagara J, Ito K. Upregulation of the apoptosis-related inflammasome in cardiac allograft rejection. J Heart Lung Transplant. 2010; 29: 352-9.