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REVIEW

NLRP3 Inflammasome in Cardiovascular Disease: David`s Stone against Goliath?

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ABSTRACT
Inflammation is involved in initiation, development and complications of the vast majority of non-communicable diseases. Recent research demonstrated that inflammation is involved in pathogenesis of all major cardiovascular diseases. Different endogenous factors (LDL, nucleic acid strands, uric acid – collectively called „Damage Associated Molecular Patterns – DAMPs") activate dedicated receptors („Pattern Recognition Receptors – PRR") on monocytes, macrophages or dendritic cells responsible for the innate immunologic response. They have a major role in natural defense mechanisms against different pathogens and in normal conditions have a protective role. Among PRRs „NOD-like, leucin rich, pyrin containing (NLRP)" receptors are a 14-member family located in the cytoplasm. One of these is the NLRP3 resulting from nuclear transcription under the influence of NF-kB, a second messenger from membrane PRRs to the nucleus. Mostly the same factors responsible for NLRP3 intracellular expression stimulate its oligomerization resulting in a large protein complex, the NLRP3 inflammasome. This activates caspase-1 responsible for IL-1b and IL-18 production and initiates an inflammatory reaction leading to various pathologic processes, such as atherosclerosis, hypertension, diabetes and heart failure. This is the current story as we know it of the NLRP3 inflammasome, a small intracellular component that when inappropriately activated may does more harm than good.
Keywords: NLRP3 inflammasome, inflammation, outcome, CV disease.

Rezumat
Inflamația este implicată în inițierea, progresia și complicațiile majorității bolilor cronice netransmisibile Studii recente arată că inflamația este implicată în patogenia tuturor bolilor cardiovasculare importante. Diferiți factori interni (LDL, fragmente de acizi nucleici, acidul uric, numiți „Damage Associated Molecular Patterns – DAMPs") activează receptori specifici („Pattern Recognition Receptors – PRR") pe monocite, macrofage și celule dendritice responsabile pentru răspunsul imun înnăscut. Ei au rol major în mecanismele de apărare împotriva diverșilor agenți patogeni și în condiții normale au rol protector. Dintre PRRs fac parte receptorii „NOD-like, leucin rich, pyrin containing (NLRP)", o familie de 14 receptori localizați în citoplasmă. Unul dintre aceștia este NLRP3 produs prin activarea transcripției în nucleul celular de către NF-KB, care acționează ca mesager secund între PRR de pe membrană și nucleu. Aceiași factori responsabili pentru sinteza NLRP3 îi stimulează polimerizarea cu apariția inflamazomului NLRP3, o moleculă multimerică voluminoasă. Acesta activează caspaza-1 responsabilă pentru producerea IL-1b și IL-18 cu inițierea reacției inflamatorii responsabile de progresiunea aterosclerozei, hipertensiunii, diabetului sau insuficienței cardiace. Aceasta este istoria curentă a inflamazomului NLRP3, o componentă intracelulară care, atunci când este activată, determină efecte nefavorabile care le surclasează pe cele favorabile.
Cuvinte cheie: inflamazomul NLRP3, inflamație, prognostic, boli CV.

INTRODUCTION

The human body is continuously reacting to various environmental offenders, both external and internal. We recognized long time ago that inflammation is the main reaction against pathogens entering the body to allow healing. It is specifically activated when the natural defense mechanisms recognize a large variety of factors as potentially dangerous. The most easily understood so far are the immune mechanisms that generate inflammatory reactions against foreign microorganisms, such as bacteria and viruses. Different antigens are recognized as non-self and initiate an immune response with inflammation (both humoral and cellular) to allow the body to safely dispose of them.

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We have progressively learned that immune reactions and inflammation may be also initiated when endogenous factors inappropriately activate our normal defense mechanisms. A disproportionate inflammatory reaction directed against self-antigens is responsible for different so-called „auto-inflammatory disease" (gout, familial Mediterranean fever) or "auto-immune disease" (systemic lupus erythematosus, vasculitis, rheumatoid arthritis). To cover them all a new term "inflammatory immune-mediated disease" was introduced.

Irrespective of the original pro-inflammatory stimulus, external or endogenous, a common inflammatory platform sends messages towards key cells via specific cytokines resulting in „the fire within"[1,2]. This „Jack of all trades" is represented by the pattern recognition receptors (PRR) distributed on most inflammatory cells.

Thus, a natural defense mechanism changes its biological purpose from protective to pathogenic. We also found out that most of cardiovascular disease (CVD) are produced by or due to inflammation to worsen outcome of CV patients. How this tiny molecular system gets to play the role of the small stone of David to dominate the mighty Goliath, is the purpose of this review.

INFLAMMASOMES AND THEIR FUNCTION

Inflammasomes are complex cytoplasmic receptors acting as turning points to initiate and amplify the effect of proinflammatory molecules. They are part of the innate immune response and nature has designed them as protective mechanisms against different external or internal pathogens. The first inflammasome was described in 2002[3]. Inflammasomes are widely expressed by monocytes, macrophages, neutrophils and other inflammatory cells. They are responsible to react to danger signals identified during continuous internal scanning performed by macrophages. When inappropriately stimulated they demonstrate how bad is too much of a good thing.

Inflammasomes are part of wider receptor family known as Pattern Recognition Receptors or PRR[4]. Five classes of PRR have been identified: toll-like receptors (TLR), nucleotide-binding domain (NOD) – like receptors (NLR), C-type lectins (CTL), RIG-I-like receptors (RLR) and absent-in-melanoma (AIM) like receptors (ALR)[5].

TLR family were the first identified PRR and are located on the cell membrane.

The NLRP receptor family („nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing") is similar to TLR, but is located in the cytoplasm. It is known as an „intracellular sensing protein". It consists of the 14 different members, NLRP1 to NLRP14[6]. NLRPs act as a molecular platform that can activate caspase-1[4]. In turn caspase-1 unlocks the inflammatory power of the 11 interleukin family members, mainly of IL-1b and IL-18[7]. The most widely studied of all inflammasomes is the NLRP3 complex, which is the largest multimeric protein complex identified[8].

All NOD-containing molecules are intracellular scaffolding proteins that consist of three parts (Figure 1):

- a central NOD (also known as NACHT), acting as an immune sensor;
- an N-terminal effector domain; this is the effector part of the molecule (*a Caspase Activation and Recruiting Domain* - CARD), interacting with other proteins and
- a C-terminal leucine-rich repeats (LRR); this domain acts as an activation sensor[6].

They exist as inactive monomers in the cytoplasm and upon ligand sensing undergo structural changes allowing activation by aggregation by CARD-CARD interaction with other intracellular components.

The oligomerization of NLRP3 monomers is mediated by an adaptor protein called ASC (Apoptosis-associated Speck-like protein containing CARD)[9].

Caspase-1 is activated either by a pyrin molecule contained on the N-terminal domain of the NLRP or a separate CARD (caspase activation and recruiting domain)-containing sensor, such as that on ASC. They interact with the CARD domain of pro-caspase-1 to transform it in active caspase-1[10]. Caspase-1 cleaves pro-IL-1β in its active form, IL-1β, a potent inflammatory molecule generated by tissue macrophages and circulating monocytes 11 (Figure 1).

Two NLRPs are structurally different. NLRP1 has a supplemental caspase recruiting domain (CARD) molecule allowing it to interact directly with caspase-1. NLRP10 lacks the LRR domain and probably acts as a signaling molecule and not in sensing[6].

NLRP3 was identified in peripheral circulating mononuclear cells, foam cells, myocardial cells, in the dysfunctional endothelium of myocardial microcirculation and in fibroblasts in myocardial interstitial space after ischemia-reperfusion[12]. It has been recently involved in a wide panel of major human disease, from atherogenesis to neurodegenerative disorders (Table 1).

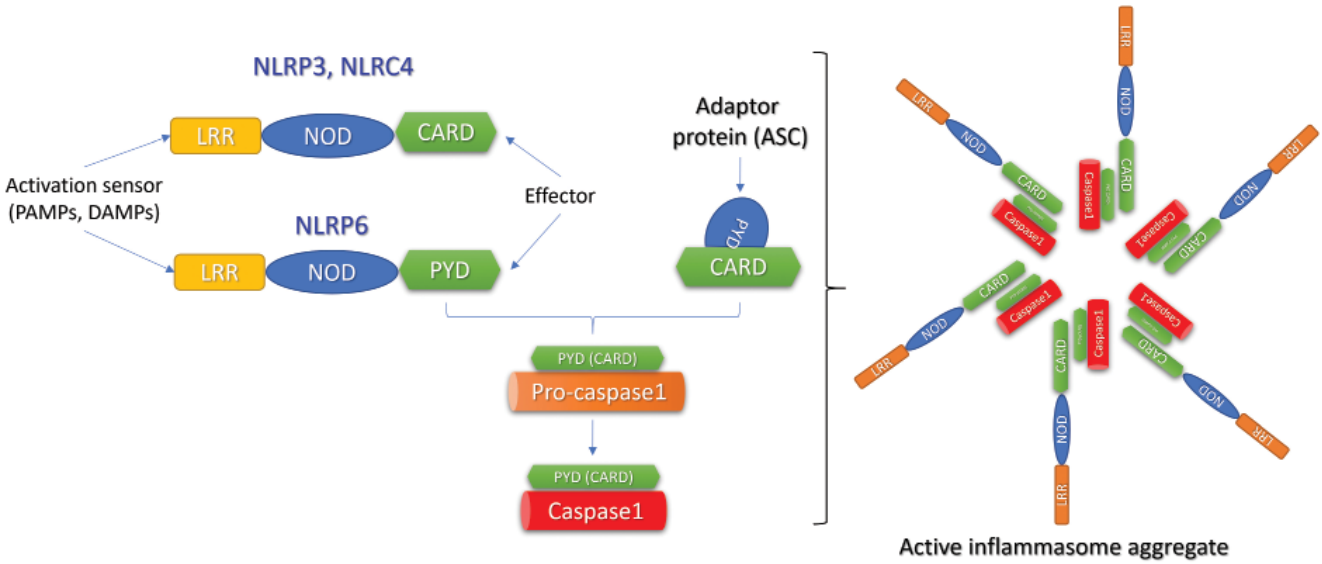

Figure 1. The molecular structure of the inflammasome. This is a large complex multimeric protein consisting of oligomerized NLRP. This consists of a central NOD immune receptor, a leucine-rich repeat (LRR) domain acting like an activation sensor and an effector domain, either a pyrin-containing (PYD) or a caspase activation and recruiting domain – CARD. When activated, the effector domain of NLRP interacts with an adaptor protein (ASC) via a CARD molecule and activate pro-caspase1 into mature caspase1. Pro-caspase1 also has a CARD molecule allowing interaction with the inflammasome.

POTENTIATED INFLAMMASOMES OR THE START ON THE „HIGHWAY TO HELL"

The activation of NLRP3 inflammasome as an innate, dormant, immune mechanism is determined by molecules, either exogenous or endogenous considered as danger signals[9]. The external activation factors are mostly infectious (*Pathogen-Associated Molecular Patterns,* PAMPs) while endogenous activation structures are molecular residues or deleterious molecules resulting from different metabolic pathways (*Damage-Associated Molecular Patterns,* DAMPs)[13].

Different PAMPs have been described acting on specific inflammasomes. NLRP1 responds to different highly pathogenic germs such as Shigella, Listeria or Bacillus anthracis. NLRP2 responds to bacterial lipopolysaccharides. NLRP6 is expressed mainly in intestinal epithelium where is activated by Salmonella, Staphylococcus or Listeria species. NLRP4 and NLRC4 may be activated by bacterial proteins such as flagellin[14] or viral RNA[4]. Recently the excessive inflammatory response due to SARS-CoV2 infection was found to be related with NLRP3 activation[15].

DAMPs interaction with PRR initiate the immune, inflammatory response. DAMPs that can activate the NLRP3 inflammasome are crystalline molecules (such as LDL), endogenous antigens (such as DNA or RNA degenerated strands), germs and/or ATP[16].

Table 1. Available proof for inflammatory pathways NLRP3-mediated in different conditions 8

Experimental		In humans "*in vivo*"	
Obesity	Involved in pathogenesis of metabolic syndrome sdr	Crohn's disease	NLRP3 gene mutations involved in pathogenesis
Diabetes mellitus	Acts as a sensor for metabolic imbalance, inducing DM	Asthma, COPD	Found activated in bronchial tree, leads to clinically relevant disease
Gout	Activated by urate crystals deposition in synovium	Neurodegenerative disorders	Involved in pathogenesis of Alzheimer disease; chronic neuro-inflammation
Rheumatoid arthritis	Downregulation protects against synovial damage	Cancer	NLRP3 involved in treatment-resistant leukemia. Activated in bronchogenic carcinoma (non-small cell type).
Haemorrhagic stroke	NLRP3 inhibition reduces brain damage	Cryopyrin-associated periodic syndromes*	Main pathogenic mechanism

* Cryopyrin-associated periodic syndromes (CAPS): Muckle-Wells syndrome; familial cold autoinflammatory sdr (FCAS); neonatal-onset multisystem inflammatory disease (NOMID).

The activation of NLRP3 inflammasome is completed in two different steps, priming and triggering or activation, due to the same initiating factors, DAMPs or PAMPs[4]. Priming is the first part of the process by which PAMPs and DAMPs connect to the membrane TLR; circulating Tumor Necrosis Factor (TNF) may also activate intracellular NF-kB by dedicated TNF receptors. Recently extruded depolymerized DNA strands exposed by activated or dying neutrophils were recognized not only as DAMPs but also as an activator for thrombotic complications in atherosclerosis[16]. A second messenger mechanism is activated by nuclear factor – kappaB (NF-kB) that acts in the cell nucleus to activate NLRP3, pro-IL-1β and pro-IL-18[17]. Most interestingly is that macrophages exposed to PAMPs do not overexpress NLRP3 activity, while preexposure to bacterial ligands markedly increase NLRP3 activity[18]. Thus, a combination between external and endogenous activation of inflammasome activity appears as most dangerous.

Triggering is the following process through which inactive NLRP3 and procaspase-1 molecules previously generated are oligomerized resulting in autocatalytic activation of caspase-1 and IL-1β[19]. Due to structural chemical diversity of DAMPs and PAMPs that activate NLRP3, it is highly unlikely all these directly interact with the inflammasome. Consequently, NLRP3 as a cytoplasmic molecular complex is activated by a common cytoplasmic signal, incompletely characterized so far. Included in this common intracellular pathway are potassium and chloride efflux, calcium and sodium influx, mitochondrial dysfunction and reactive oxygen species generation and finally, lysosomal degranulation[19-21].

Consequently caspase-1 activates the potent pro-interleukin (IL) -1b and pro-IL18 into their inflammatory counterparts, IL-1b and IL-18[13,22]. Both these interleukins may induce a particular form of cell death called pyroptosis, leading to inflammation[23]. It differs from apoptosis, a type of so-called programmed cell death that does not induce inflammatory changes. IL-1b suffers a self-amplification process in the macrophage and leads to synthesis of IL-6 (Figure Y). Circulating IL-6 is responsible the production of acute phase proteins in the hepatocyte (fibrinogen, PAI-1, SAA, CRP), while activating endothelial cells and smooth muscle cells in the vessel wall (Figure 1)[24].

Activated NLRP3 induces also inflammatory cell amplification (macrophages, Th1 and Th17 proinflammatory lymphocytes, while Treg lymphocytes are suppressed) and increases production of TNF (Figure 2)[13,22]. Inflammasome activation may generate other DAMPs amplifying inflammation in a positive feed-back loop[25].

Persistent NLRP3 inflammasome activation due to an inherited genetic defect („gain of function" pattern) has been recently described and linked to coronary atherosclerosis and cardio-vascular death[26]. These data provide another proof for the importance of genetically-determined inflammation for both cardiovascular morbidity and mortality and support investigation for identifying efficient NLRP3 inhibitors.

ATHEROSCLEROSIS, AN „INFLAMMATORY IMMUNE-MEDIATED DISEASE"

The role of inflammation in initiation, progression and complications of atherosclerosis is widely accepted. Oxidized LDL, the main lipoprotein involved in atherosclerosis, has its own PRR, the CD36 receptor. CD36-LDL activated receptor induces NLRP3 inflammasome activation through cholesterol endocytosis and lysosomal damage[27,28]. NLRP3 inflammasome is involved in cytoplasmic LDL crystals cleavage in the foam cells[29].

NLRP3 expression in blood mononuclear cells is increased in patients with coronary artery disease and mainly in those with an acute coronary syndrome[30]. NLRP3 is correlated with the severity of atherosclerosis in coronary arteries[31] and aorta[32]. It was also an independent predictor of major adverse cardio-vascular events. In ischemic myocardial necrosis various myocyte debris act as DAMPs to activate inflammasomes. Mainly the reperfusion injury leading to mitochondrial dysfunction that generates ROS and release of pro-apoptotic proteins also activate inflammasomes[33]. Thus, it has been hypothesized that initial ischemic phase is responsible for priming the NLRP3 and pro-IL-1b through locally released PAMPs, while reperfusion activates the inflammasome by mitochondrial damage and ROS generation[12].

A major recently published genetic trial performed on 538.167 subjects demonstrated that carriers of intronic NLRP3 variant rs10754555 have significantly higher serum levels of CRP and serum amyloid A and show a higher prevalence of cardiovascular disease and mortality[26].

Meanwhile, atorvastatin reduces the expression of NLRP3 in circulating mononuclear cells in patients with coronary artery disease[34,35]; it contributes to be-

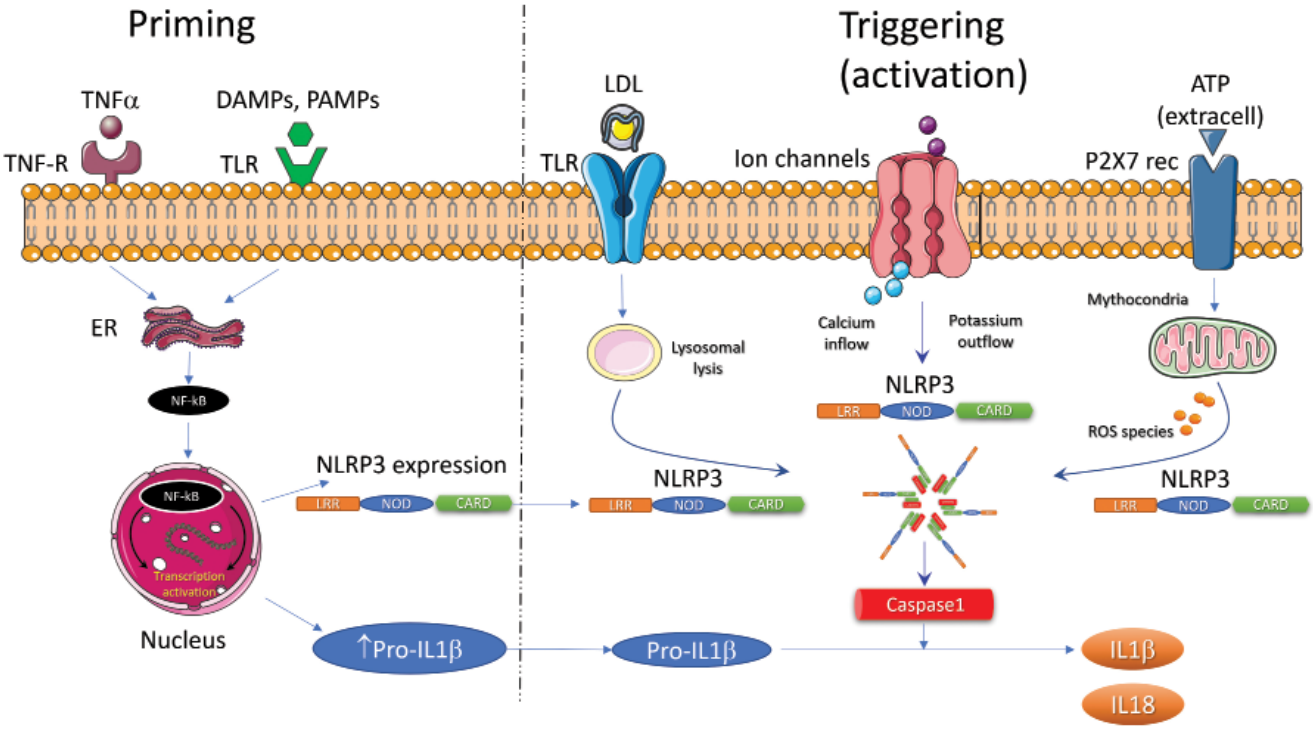

Figure 2. The two main steps for full inflammasome activation. Initiation of the process is priming which leads to increased expression of NLRP3 and pro-IL-1b via nuclear factor-kappa B. This acts as a second messenger for toll-like receptor activation via DAMPs or PAMPs; an alternative pathway is NF-kB stimulation by TNF receptor and TNFa. Triggering or full activation of the inflammasome is the final oligomerization of NLRP3 with pro-caspase-1 cleaving properties. It can be induced by intracellular signaling due to increased ROS generation by dysfunctional mitochondria, potassium efflux or calcium influx, or lysosomal lysis after LDL crystal endocytosis.

neficial effects of statins due to reduction of serum LDL. Colchicine also lowers NLRP3 expression, IL-1b and IL18 in patients with acute coronary syndromes[36].

There is consistent proof that NLRP3 is excessively and inappropriately activated in atherosclerosis and is independently associated to cardiovascular outcome[37]. Recent clinical data suggests that therapeutic NLRP3 – IL1b pathway inhibition may provide benefit in atherothrombosis. Therapeutic inhibition of NLRP3 may limit the volume of necrosis and preserves cardiac function irrespective of reperfusion[38]. Small molecule inhibitors of NLRP3 may limit infarct size in ischemia-reperfusion models[39]. Canakinumab, a human monoclonal antibody directed against IL-1b, tested in patients with recent acute coronary syndromes in the CANTOS trial, demonstrated significant reduction of CRP and improved CV outcome[40]. Clinical trials with different agents targeting NLRP3 in atherothrombosis are underway[41].

HYPERTENSION AND NLRP3 INFLAMMASOME?

Primary or essential hypertension is the multifactorial disease that has no clear culprit involved in its pathophysiology. Different mechanisms such as increased sympathetic activity, activation of the renin-angiotensin system or increased salt ingestion superimposed on genetic predisposition are involved in the pathophysiology of idiopathic hypertension. In the last decade, basic research and translational medicine confirmed that hypertension may develop starting from inflammatory changes induced by increased oxidative stress in both vessels and kidneys[42,43]. Different DAMPs were identified in the serum of hypertensive patients[44]. Increased TNFa, IL-6 and IL-17 produced by macrophages, Th-17 cells and dendritic cells are long-time proven offenders in the pathophysiology of the disease[45].

NLRP3-induced pyroptosis is currently investigated in the pathogenesis of hypertension. First, Il-1b serum levels are high in spontaneous hypertensive rats[46], but also in humans[47]. Consequently, transcription RNA for procaspase-1 was identified in the renal arteries and in the aorta of spontaneous hypertensive rats[48]; NLRP3 mRNA is overexpressed in renal biopsies of patients with hypertensive nephrosclerosis[49]. Furthermore, an intronic 42 base pair polymorphism in the CIASI gene that codes the NLRP3 protein complex was correla-

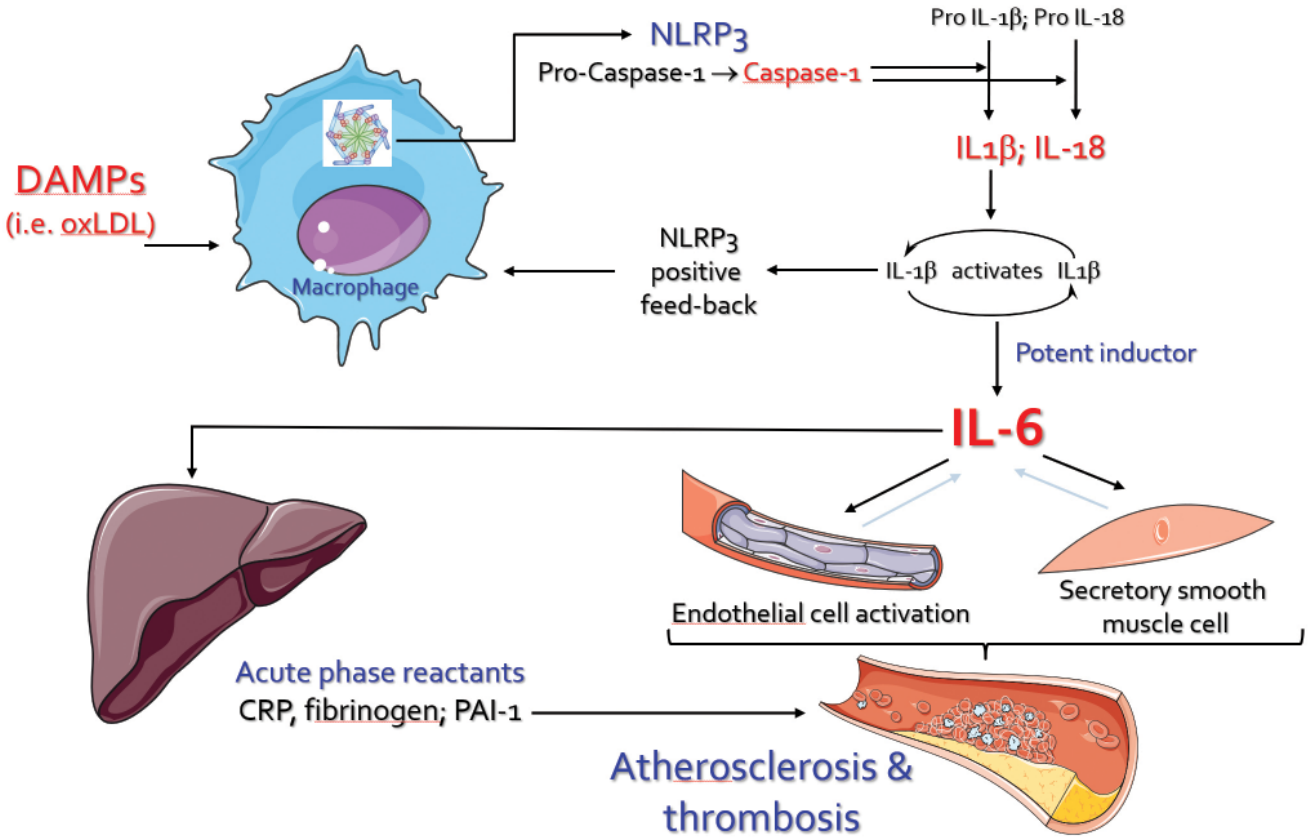

Figure 3. Initiating factors and activation pathways of inflammasomes and consecutive inflammation. Either PAMPs or DAMPs act on pattern recognition receptors, inflammasomes included. NLRP3 caspase-1 activation leads to IL-1b and IL-18 production and triggers the inflammatory response. CARD – caspase recruitment domain (NLRC4); TNF – Tumor Necrosis Factor; Th – T helper lymphocytes; Treg – T regulatory (suppressor) lymphocytes.

ted with predisposition to essential hypertension[50]. A sodium-rich diet increases the expression of NLRP3, ASC and also of procaspase-1, leading to increased levels of IL-1b[46]. Knockdown of NF-kB, the intracellular messenger of activated PRR, reduces transcription of NLRP3 components and delays progression of hypertension[51]. Finally, drugs such as ellagic acid inhibits NLRP3 as an anti-inflammatory effect to lower pulmonary hypertension[52].

Activated NLRP3 is also related to pregnancy-associated hypertension, pre-eclampsia, placental disruption and HELLP syndrome[53].

Experimental studies performed with the selective NLRP3 inhibitor MCC950 demonstrated that the drug reduced blood pressure, renal inflammation and dysfunction in hypertensive rats with no effect in normotensive animals[54].

All these data strongly support the pathogenic role of NLRP3 inflammasome into progression to full-blown hypertension and may suggest the significance for therapeutic inhibition.

DIABETES MELLITUS

NLRP3 inflammasome, IL-1b and consecutive pyroptosis are also involved in the pathogenesis of diabetes[55]. Development of both type 1[56] and type 2[57] diabetes mellitus is regulated by NLRP3 inflammasome. However, NLRP3 inflammasome has a major role in the progression of insulin resistance in type 2 diabetes, while in type 1 diabetes (a pure auto-immune disease) its role is less characterized. Obese subjects with insulin resistance have particular gut microbiota associated with bacterial (endotoxin) translocation and PAMPs-dependent systemic inflammation[58].

In type 2 diabetes the NLRP3 complex determines glucose intolerance, impaired insulin sensitivity and deleterious effects on of intestinal microbiome. It is activated by high glucose levels, saturated fatty acids and uric acid, all associated to the metabolic disturbances of diabetes. NLRP3 induces IL-1b production in the b-cells of the pancreas[59]. Increased levels of IL-1b causes an increased insulin secretion and leads to insulin-mediated macrophage activation[60]. IL-18, the

second proinflammatory cytokine NLRP3-derived, is significantly increased in diabetics[61] and induces obesity, insulin resistance and dyslipidemia.

The adipocytes and associated macrophages of obese patients show an increased expression of NLRP3, of caspase-1 activity and IL-1b concentration[62]. They were correlated with the severity of diabetes and all anomalies of metabolic syndrome[63].

There is a clear relationship between abnormalities of glucose metabolism, diabetes and NLRP3 activation, mediated by IL-1b. A significant research effort should be carried out to clarify the effect of NLRP3 pathway inhibition in diabetes.

INFLAMMATION AND HEART FAILURE: OLD NEWS?

Inflammation and heart failure (HF) may look like old news. Chronic reduction of cardiac output is associated with increased levels of serum TNF and an increased catabolic state, leading to body waste.

Multiple studies demonstrated that both IL-1b and IL-18 are upregulated in the myocardium of patients with chronic HF[64]. It was demonstrated that activated NLRP3 inflammation cascade via IL-1b and IL-18 are involved cardiac remodeling that characterizes HF[65]. Dysfunctional calcium homeostasis and signaling is suggested as activator of NLRP3 pathway[66]. An increased cytoplasmic calcium levels, released from endoplasmic reticulum or due to positive inotropic medication, is responsible for NLRP3 activation[67]. This may partly explain the lack of long-term benefit of chronic positive inotropic treatment in HF.

It was also found that genetic deletion of NLRP3 may reduce the prevalence of atrial fibrillation in cardiomyopathies[68].

The research on NLRP3 inflammatory pathway in HF is underway[69].

THERAPEUTIC INHIBITION OF NLRP3 INFLAMMASOME

Due to multiple proof of clinical significance of pathogenic NLRP3 effects and reduction of adverse events with NLRP3 down-regulation, there is a wide interest to develop specific inflammasome inhibitors (Table 2). The complex inflammatory pathway of NLRP3 inflammasome may be intercepted at different levels:

- first would be the block of upstream signals from PRR: reduction of DAMPs or PAMPs production, specific block of membrane PRR;
- interruption of NF-KB – induced transcriptional upregulation of NLRP3 generation;
- inhibition of NLRP3 assembly (activation); inhibition NLRP3-NLRP3 or NLRP3-ASC interaction;
- ROS production inhibitors;

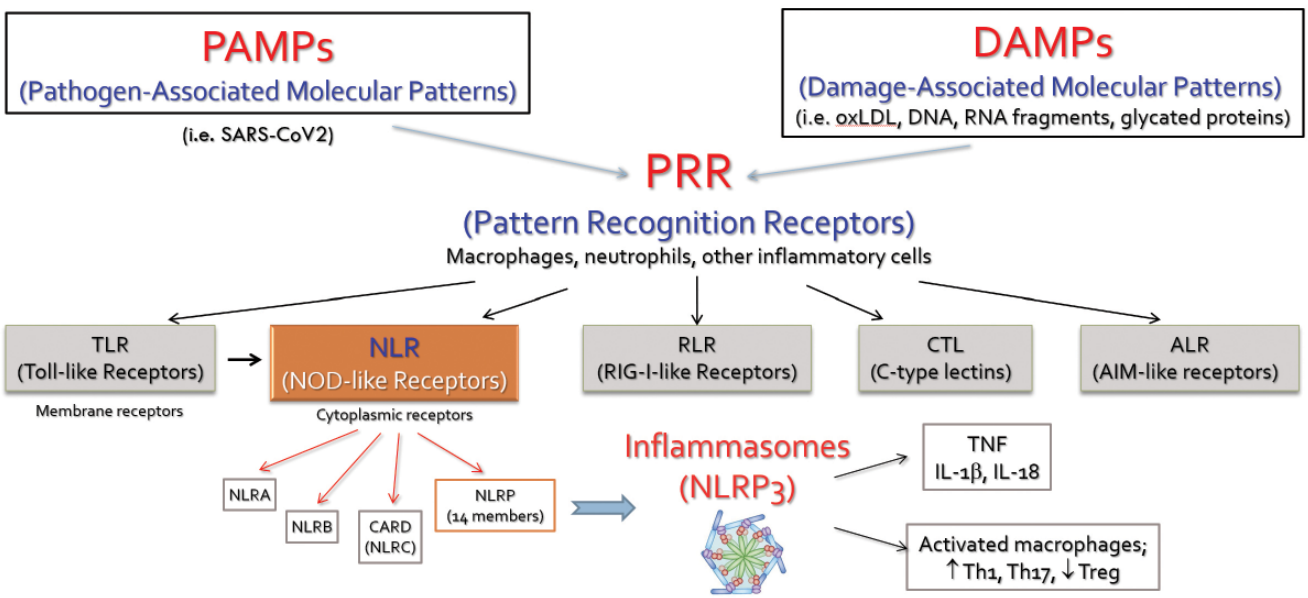

Figure 4. The systemic effects of NLRP3 caspase-1 mediated interleukin activation. Interleukin-6 generated by the potent stimulus of IL-1b leads to secretion of acute phase proteins in the liver, induces endothelial dysfunction and activates smooth muscle cells in arterial media. IL – interleukin; oxLDL – oxidated LDL molecule; CRP – C-reactive protein; PAI-1 – plasminogen activator inhibitor-1. (Adapted from (24).

Table 2. Overview of NLRP3 inhibitors, either specific or as „pleiotropic effects" of other therapeutic agents

Drug	Main therapeutic effect	NLRP3 inhibition
Atorvastatin	LDL reduction by hepatocyte HMG-CoA inhibition	Reduction of NLRP3 expression in mononuclear cells; lowers IL-1b, IL-18
Colchicine	Tubulin disruption with down-regulation of inflammatory pathways	Prevents activation of NLRP3 by inhibiting oligomerization depending on cytoplasmic microtubules; inhibits caspase-1 gene transcription
Canakinumab	Monoclonal-antibody against IL-1b	Downregulates NLRP3 and IL-1 gene expression
Anakinra	Recombinant IL-1b receptor antagonist	Downstream inhibition of activated NLRP3 cascade
MCC950	Small molecule inhibitor of NLRP3	Specific for NLRP3; no effect on other inflammasomes
CY-09	Small molecule inhibitor of NLRP3	Specific for NLRP3, binds to the NOD (NACHT) immune receptor
Flavonoids	Inhibit expression of NLRP3 and ASC; blocks inflammasome assembly	Idem
PEDF	Inhibits mitochondrial division	Mitochondrial stabilization; reduces ROS production; promotes mitophagy in hypoxic cardiomyocytes
Tranilast	Anthranilic acid anti-allergic, anti-asthmatic drug	Binds to the NOD (NACHT) domain, to block NLRP3 oligomerization
Parthenolide	Natural (sesquiterpene lactone) anti-inflammatory agent, COX inhibitor	Blocks NLRP3 ATP-ase capacity and inactivates caspase-1
Oridonin	Anti-neoplastic drug, inhibits angiogenesis and induces apoptosis	Blocks NLRP3 activation by selective binding to cysteine
Pralnacasan (VX-740), Belnacasan (VX-765)	Caspase-1 inhibitors	Specific downstream inhibition of NLRP3 activation by caspase-1 blockage
Cilostazol	Phosphodiesterase-3 inhibitor	Reduces NLRP3 activation

- caspase-1 inhibition;
- neutralization of IL-1 and IL-18 or their derived cytokines (such as IL-6).

Different drugs act by one or a combination of these postulated mechanisms.

Statins, mainly atorvastatin, reduce NLRP3 activity, IL1 and IL-18 levels in coronary artery disease[70]. In acute myocardial infarction the demonstrated inhibition of NLRP3 exerts protective effects after reperfusion[71].

Multiple antidiabetic drug classes have anti-inflammatory effects by inhibiting NLRP3 pathway. They improve endothelial dysfunction and some of the cardio-vascular protection exerted by them could be attributed to their anti-inflammatory action[9].

- **Glyburide,** a sulfonylurea anti-diabetic drug, blocks ATP-sensitive potassium and chloride channels of b cells in pancreatic islets. However, the inhibitory effect on NLRP3 activation in DAMP-exposed macrophage is not due to ionic channel block[72]. It inhibits ASC aggregation. Doses necessary to inhibit inflammasome activation are high and drug use is limited to diabetic patients.
- Dipeptidyl-peptidase-4 (DPP-4) inhibitors[73], glucagon-like peptide-1 receptor agonists (GLP-1 RA)[74,75], acarbose[76] or fenofibrate[77] have all different inhibitory effects over NLRP3 activation. The SGLT2 inhibitor empagliflozin inhibits NLRP3 and IL-1b secretion via increased plasma b-hydroxybutyrate and decreased serum insulin[78].

Recently **colchicine** demonstrated improved CV outcome either in patients with stable coronary artery disease in LoDoCo2[79] or after acute coronary syndromes in COLCOT[80]. Colchicine inhibits NLRP3-caspase-1 pathway besides metalloproteinase expression that are responsible for atherosclerotic plaque vulnerability[81].

Canakinumab, tested in CANTOS trial, reduced expression of genes coding IL-1 and improved outcome in the experimental arm did not depend on LDL levels[82]. CANTOS demonstrated that improved cardio-vascular outcome with canakinumab was not due to LDL reduction, with similar levels in the statin-treated control arm.

Anakinra, a recombinant IL-1b antagonist, was tested in a phase-2 trial in patients with acute myo-

cardial infarction in order to block the inflammasome-dependent inflammatory cascade[83]. The primary endpoint was the surrogate CRP levels 14 days after randomization. The results of the completed trial are not published yet. Considering the cost-to-benefit ratio of biological agents, small molecule inhibitors appear much more attractive.

MCC950 (or cytokine release inhibitor drug 3 – CRID3) is a small molecule inhibitor orally administered that selectively blocks NLRP3 with no effect on other inflammasomes or immune reactions[84]. It is a sulfonylurea chemical compound, the most specific and potent direct inhibitor of NLRP3[85]. It was shown to improve microvascular disease in diabetes[86] and to protect against cardio-vascular disease[87].

Another small molecule NLRP3-inhibitor is **CY-09**. It binds to the central NOD (NACHT) domain immune receptor of the molecule and prevents oligomerization and consequently the formation of the active inflammasome[88]. It also inhibits platelet aggregation that may prove useful in atherosclerotic CV disease[89].

PIGMENT EPITHELIUM-DERIVED FACTOR (PEDF) INHIBITS INFLAMMASOME ACTIVATION BY STABILIZING MITOCHONDRIA IN EXPERIMENTAL MODELS OF ISCHEMIA-REPERFUSION[90]

Different **flavones** prevent apoptosis and reduce oxidative stress. Total flavones inhibit NLRP3 activation in a rat ischemia-reperfusion model[91]. Another flavone, triptolide, inhibits a NLRP3-TGFb and reduces myocardial fibrosis induced by isoproterenol and angiotensin II[92].

Many other molecules such as sulfonamides, glitazones, curcumin analogues, benzimidazoles, anthranilic acid analogues are under scrutiny for beneficial effects on inflammasome pathway blockage[84,93].

Noncoding RNA (ncRNA) are a large nucleic acid family with high transcriptional activity but do not encode proteins. This wide group consists of microRNAs (miRNAs), circular RNAs (circRNAs) and long noncoding RNAs (lncRNAs)[9]. They have structural and cell regulatory functions and control gene expression and regulate immunity and inflammatory reactions[94]. Some ncRNAs act as direct or indirect activators of the NLRP3 inflammasome pathway[95], while others down-regulate it[96]. Other ncRNA, such as miRNAs are used for diagnostic purposes in CV disease[97]. Despite the large therapeutic potential of targeting ncRNA to reduce inflammation, there are no current clinical applications[98].

CONCLUSIONS

Inflammasomes and mainly NLRP3 widened our knowledge about the inflammatory mechanisms of cardiovascular disease, known as "response to injury". Pyroptosis due to NLRP3 activation and IL-1b -IL18 secretion contribute to all major CV disease, starting from atherosclerosis to heart failure. Surprising correlates were identified in hypertension, diabetes, obesity, ischemia-reperfusion injury, a.s.o. in the last decade.

A small molecular complex, the inflammasome, acts like the legendary David, armed with a stone (DAMPs and PAMPs) and a sling (interleukins 1b and 18) to hit Goliath (the patient) in an unexpected and deathly way. There is hope that direct and indirect inhibitors of inflammasome cascade, under clinical testing in different trials, will provide useful approaches to limit cardiovascular risk.

A word of caution must be mentioned here: since inflammasomes have been designed by nature to be protective against potential danger signals, NLRP3 inhibition may have adverse physiological effects that have to be properly assessed prior to wide therapeutic use.

Compliance with ethics requirements:

The authors declare no conflict of interest regarding this article. The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

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