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NLRP3 SIGNALING AND ITS ROLE IN ATHEROSCLEROSIS: REVIEW AND NEW INSIGHTS

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ABSTRACT

Atherosclerosis is associated with high morbidity and mortality. Recently, the role of inflammation in the cascade of events leading to the development of atherosclerosis has been the center of attention. Inflammasomes are protein complexes that are required for the secretion and maturation of proinflammatory cytokines as well as activation of inflammatory caspases. Among these inflammasomes, the NLRP3 inflammasome has gained more importance due to its implication in a number of metabolic diseases. The relation of NLRP3 inflammasome to atherosclerosis is an emerging and exciting area of ongoing research. Despite major leaps in our research of the signaling pathways of NLRP3 inflammasome, the entire assembly and activation process and its clinical implications are still eluding our understanding. This article will review some major aspects of the inflammasome signaling pathways. It will also review past studies and highlight recent developments in understanding the role of NLRP3 inflammasomes in relation to atherosclerosis.

Key Words: Atherosclerosis, Innate Immunity, Inflammasome, Nucleotide-binding Oligomerization Domain like Receptors, NLRP3

INTRODUCTION

Atherosclerosis is one of the world's most debilitating health condition which has serious physical and psychological implications. The basic process of atherosclerosis involves lipid deposition and plague formation. It is a multifactorial problem which requires a multi-faceted approach. A diverse group of risk factors has been identified of which inflammation has gained a lot of importance recently.

The survival of human beings without its defense system, consisting of innate and adaptive immunity, is almost impossible to imagine. One of the important components of our defense system are pattern-recognition receptors (PRRs). Pattern-recognition receptors (PRRs) are vital for recognition of pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs).[1] Three different classes of PRRs have been identified: cytosolic, trans-membrane and secreted. The cytosolic PRRs include the nucleotide-binding oligomerization domain like receptors (NLRs), the RIG-I-like receptors (RLRs) including RIG1 and MDA5 and contain C-terminal RNA recognizing domain (CTD), IFI16 (interferon-inducible protein16) and DNA sensors called AIM2 (absent in melanoma 2).[2] The trans-membrane PRRs include Toll-like receptors (TLRs) and the C-type lectins. These consist of some members being associated with intracellular compartment such as endosome and endoplasmic reticulum (TLR3/TLR7/TLR9) and some associated with cell surface (such as TLR2/TLR4/TLR5/TLR6/TLR11 and Dectin1/2).[3] Secreted PRRs include ficolins, pentraxins and collectins and can bind microbes leading to activation of the complement system. TLRs sense intracellular and extracellular stimuli while NLRs only sense intracellular (cytosolic) stimuli.[4] Upon detection of a PAMP or DAMP, NLRs in combination with ASC (apoptosis-associated speck-like protein containing a CARD) forms a complex called inflammasome, which was first identified by Tschopp group in 2002, to activate caspase-1.[5] Its these NLRs, and in particular the NLRP3 inflammasome, that are thought to play a vital role in the development of cholesterol induced atherosclerosis.

1. NLR Family Members

The Nod-like receptors (NLRs) consist of 22 proteins and are structurally composed of an N-terminal effector domain, a central nucleotide binding and oligomerisation (NACHT) domain and a C-terminal leucine-rich repeat (LRR) domain. The central NACHT domain has an important role in ligand sensing through the LRR and allows oligomerisation of NLRs. The N-terminal effector domain having a role in signaling can be either a caspase recruitment domain (CARD), a pyrin domain (PYD) or a Bir domain. Hence the NLR family can be further divided into subfamilies as the PYD containing NLRP subfamily (NLRP1-14), the CARD containing NLRC subfamily (NOD1, NOD2, NLRC3 and NLRC5), a Bir domain containing NAIP and NLRC4 which contains a CARD.[6,7]

2. The NLRP3 Inflammasome

NLRP3 (NOD-like receptor family, pyrin domain containing 3), is also called CIAS1, PYPAF1, Cryopyrin, CLR1.1 (CATERPILLAR 1.1) or NALP3 (NACHT, LRR and PYD domains-containing protein 3). NLRP3 in combination with ASC and caspase-1 forms the NLRP3 inflammasome. NLRP3 tissue expression in mice is mainly restricted to organs like kidney, colon, lung, liver, ovary, eye and skin.[8] At cellular level in mice, peripheral blood mononuclear cells (PBMCs), neutrophils, bone marrow derived macrophages (BMDMs), bone marrow-derived dendritic cells (BMDCs) and Th2 cells express this molecule at moderate to high level.[1]

In humans, primary mast cells (MS), primary human PBMCs, the monocyte-derived THP-1 cell line, primary human keratinocytes (PK), keratinocyte-derived HaCaT cells, granulocytes and B cells and osteoblasts all express NLRP3. Tissue distribution of NLRP3 is mainly in the skin cells mentioned above, in epithelial cells lining the oral and genital tracts and urothelial layer in the bladder.[9]

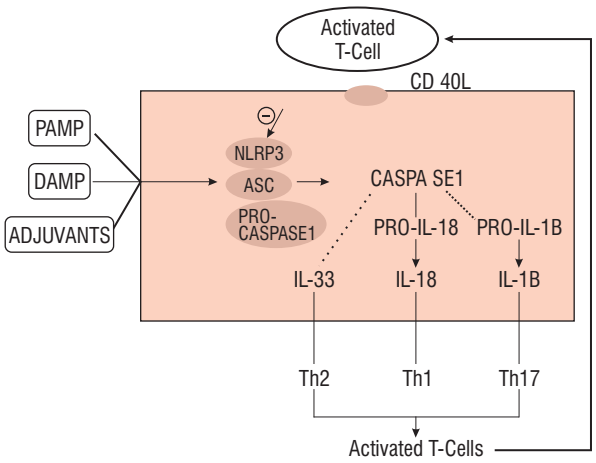
2.1 Adaptive Immunity and the NLRP3 Inflammasome

Cytokine signals from antigen presenting cells (APCs) are required for the differentiation of specific lineages of helper T cells.[10] Some of these cytokines arise from the NLRP3 inflammasome activation. Thus NLRP3 helps in the modulation of T cell response by activating caspase-1. Caspase-1 in turn helps in production of mature IL-18, IL-1ß and IL-33.

The Th1 response enhances the proliferation of cytotoxic Cd8 + T cells as well as the killing function of the macrophages. IL-18 (IFN-γ-inducing factor) is an important mediator in the Th1 response. It induces secretion of IFN-γ secretion from T cells and natural killer (NK) cells.[11] The Th2 response on the other hand is mainly associated with the humoral immune system. Cytokines like IL-33, an IL-1, are known to be processed by the NLRP3 inflammasome. IL-33 is responsible for chemo-attraction of Th2 cells and also causes production of Th2 cell-associated cytokines (Figure 1).[12] However, recent studies provided evidence that IL-33 function can be independent of NLRP3 inflammasome activity. Hence, the role of NLRP3 in Th2 response is not entirely clear.[13]

According to recent studies, IL-1ß is also involved in Th17 cell differentiation which in turn can produce proinflammatory cytokines such as IL-17A, IL-17F, IL-21 and IL-22.[14, 15] Hence, Th17 cell-mediated autoimmunity is dependant on the cascade of IL-1 signaling on T cells.

Figure 1: NLRP3 and Adaptive Immunity



Cd4+ T cells activated by TCR ligand can inhibit T cells. This in turn inhibits the IL-16 production, caspase-1 activation as well as NLRP1 and NLRP3 activation. Two conditions are required for T cell-mediated suppression of the NLRP3 inflammasome. (1) cell–cell contact and (2) the TNF family ligands such as RANKL, CD40L and OX40L which are expressed on the surface of effector T cells. These are factors that can inhibit NLRP3 activity in APCs and hence provide a mechanism for feedback regulation.[1,16]

2.2 Adjuvants Causing NLRP3 Inflammasome Activation

NLRP3 can be activated by a variety of exogenous and endogenous molecules and pathogens. Adjuvants are mainly of two types: (1) Particulate adjuvants, including mineral salts and oil-in-water emulsions, microparticles, monosodium urate crystals, alum, silica and polystyrene microparticles, Chitosan, Quil-A, poly(lactideco- glycolide) (PLG) , fibrils such as asbestos and (2) Derived from microbial components, such as ATP, biglycan, Tolllike receptor (TLR) agonists, hyaluronan and necrotic cell lysates, bacterial MDP, viral ds and ssRNA, fungi, and pore-forming toxins such as nigericin, maitotoxin and listeriolysin.[1,17]

2.3 Activation Mechanism of NLRP3

Three mechanisms are thought to activate NLRP3. (1) Activation of NLRP3 by reactive oxygen species (ROS). (2) NLRP3 activation by phagocytosis of particulates and fibrils. It can rupture or disrupt the lysosomal membrane, causing release of cathepsin B which activates NLRP3. (3) ATP stimulates P2X7 receptor which in turn causes pore formation by activation of the channel protein pannexin-1. This ultimately leads to entry of activating molecules and the release of K + from the cell leading to inflammasome activation.[6] A recent study[18] recently analyzed the relationship of thioredoxin interacting protein (TXNIP) to NLRP3 and reported that TXNIP could bind and activate NLRP3. In a study, Meissner et al. proposed the role of superoxide dismutase 1 (SOD1) in the regulation of caspase-1 activity. However, the common pathway by which all these diverse activation mechanisms converge still remains unclear. Upon activation of NLRP3, PYD of NLRP3 interacts with PYD of ASC which brings about interaction of CARD of ASC and CARD of Caspase-1. This brings about the complete assembly of NLRP3 inflammasome (Figure 2).

Inflammasome activation requires a primary and a secondary signal. A primary signal activates NFκ B which induces pro- IL-1ß mRNA synthesis. Then a secondary signal is responsible for activation of the inflammasome and as well as release of IL-1ß. In the case of NLRP3 inflammasome, priming for action is also required before activation by a second signal. Recently, Bauernfeind et al, showed that TLR, NOD2 or TNF∝ stimulation can bring about NLRP3 priming by NFkB-dependent process, in which NLRP3 protein expression levels are a limiting step.[19] Franchi et al, proposed that TNF∝ stimulation and IL-1ß and IL-1ß can prime the NLRP3 inflammasome.[20]

3. Role for mitochondrial function

Mitochondria regulate the innate immunity by regulating inflammasome-mediated generation of proinflammatory cytokines.[21] ROS has an important role in NLRP3 activation. One of the main sources of cellular ROS is mitochondria. Normally ROS generating mitochondria are cleared and removed by Autophagy/Mitohagy to prevent cellular damage and which if blocked can lead to increase in these ROS generating mitochondria. This can cause NLRP3 activation as well as its relocation near to ribosomes and mitochondria in the perinuclear region. Blockage of mitochondrial function can decrease NLRP3 activation and ROS production.[22]

NLRP3 inflammasome can be regulated by mitochondrial apoptotic signals. Release of oxidized mitochondrial DNA (mtDNA) into the cytosol and subsequent binding can lead to

Figure 2: NLRP3 Activation and its Regulation

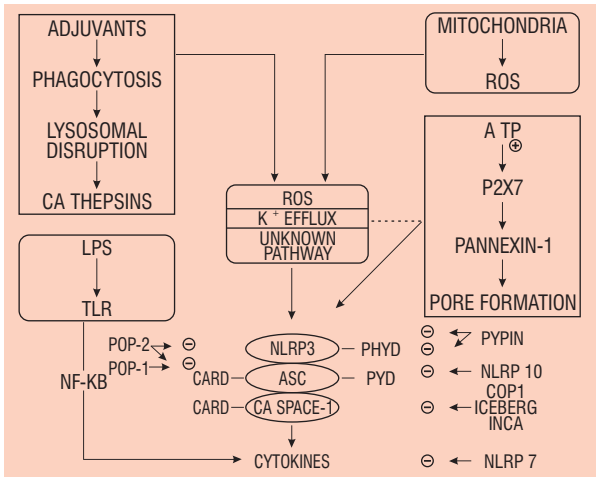
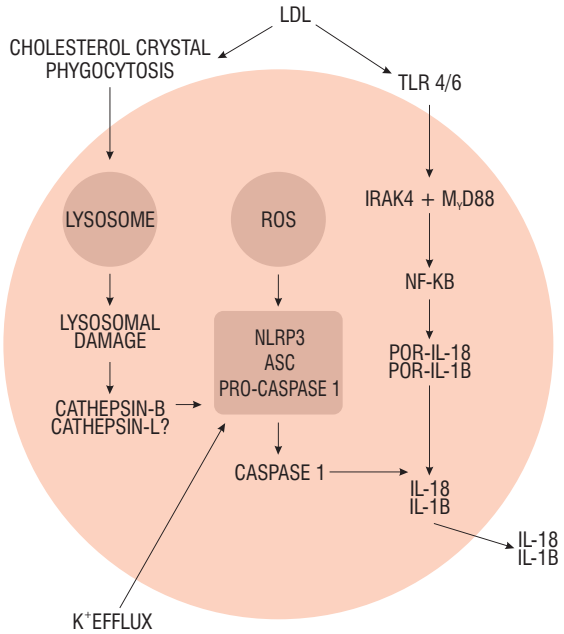


Fig. 3: Cholesterol İnduced NLRP3 Activation Leading to Activation of İnflammatory Cytokines



activation of the NLRP3 inflammasome. Bcl-2 and related antiapoptotic bodies inversely regulates mitochondrial dysfunction and NLRP3 inflammasome activation.[23] This clearly indicates the linkage of NLRP3 inflammasome to mitochondrial dysfunction. It also explains the frequent association of mitochondrial damage with inflammatory diseases.

4. Role for Endoplasmic Reticulum Function

In a recent study it was shown that endoplasmic reticulum (ER) stress can lead to activation of the NLRP3 inflammasome, which in turn causes the release of the pro-inflammatory cytokine interleukin-1b. This ER-triggered proinflammatory signal although requiring reactive oxygen species production and potassium efflux for NLRP3 inflammasome activation, is independent of the unfolded protein response (UPR). In order to repair a protein-folding defect, UPR causes a change in the cellular transcriptional and translational programs, but if the problem persists it causes apoptosis. Thus, drugs targeting NLRP3 targeting ER stress signals may prove vital in combating chronic inflammatory disease.[24]

5. Role for Ribosomal Function

In a study the role and importance of ribosomal function was analyzed and was found that molecules having different mechanisms of suppression of ribosomal function can lead to activation of NLRP3 by the process of inhibition of translation. A decrease in translation rate can cause a decrease in cellular levels of protein(s), thereby leading to formation of active NLRP3 inflammasomes. Although the mechanism by which intracellular potassium is linked to NLRP3 activation is still not entirely clear, it has been proposed that efflux of potassium plays a role. It is thought that decreased intracellular potassium suppresses protein synthesis thereby proving the vitality of potassium level for ribosomal function and NLRP3 activation.[25]

6. Negative regulation of NLRP3 Inflammasome

Negative regulators of inflammasome activity include CARD-only proteins, PYD-only proteins, NLRP proteins and pyrin. CARD-Only Proteins: Many CARD-only proteins (COPs) including negative regulators like Iceberg and INCA (inhibitory CARD upregulated by IFN) interact with caspase-1 and procaspase-1 and inhibit LPS induced IL-1ß secretion.[26, 27] PYD-Only Proteins: There are two PYD-only proteins (POPs), POP1 and POP2. POP1 is also known as ASC2, ASCI, ASCL and PYDC1. It binds to the H1 and H4 helices of the PYD of ASC and may disrupt the interaction of ASC with other proteins. POP2 was demonstrated to inhibit the recruitment of ASC by NLRP3.[28] NLRP Proteins: Some of NLRP proteins such as NLRP7 expression in THP-1s inhibits LPS-induced IL-1ß secretion. NLRP10 interacts with ASC and inhibit caspase-1 and IL-1ß maturation.[29] Pyrin: It inhibits or potentiates activation of the inflammasome.[6] In an animal study it was found that mice with disruption of pyrin were sensitive to LPS and had increased caspase-1 activation and IL-1ß secretion.[30] Pyrin's interaction with ASC can also activate the inflammasome. On the other hand, C-terminal interacts with procaspase-1, active caspase-1, NLRP1, NLRP2, NLRP3, caspase-5 and pro-IL-1ß. These interactions inhibit caspase-1 activation and IL-1ß secretion.[31] Pyrin although playing a important role in regulation of IL-1 production, its role in caspase 1 activation is not well understood.

7. Cholesterol and Atherosclerosis

Cholesterol crystals act as a major trigger as well as a sustaining actor for atherogenic inflammation through the NLRP3 inflammasome and induction of IL-1. In coronary arteries with atherosclerosis, disease severity and IL-1b levels are interlinked. Moreover, IL-1b is responsible for a number of steps in sustaining atherogenic inflammation, such as, enhancing the secretion of many other cytokines and chemokines, endothelin-1 and causing the expression of adhesion molecules and inducible nitric oxide synthase in endothelial cells.[32]

Nrf2 also plays an important role in promotion of cholesterol crystal induced atherosclerosis. Oxidative stress and inflammation leading to chronic inflammatory diseases may be linked by Nrf2 pathway to cholesterol crystals and could contribute to atherosclerosis.[33]

Drug therapies like statins have long been used in treatment of hyperlipedemic states and are essential part of regimens used in cases of atherosclerotic plaques. Some of the beneficial aspects of statin use in atherosclerosis include;

loss of pointed tip structures of cholesterol crystals and hence decreased fibrous membrane damage, dissolving cholesterol crystals and cholesterol density.[34]

8. NLRP3 Inflammasome and Vascular Inflammation

Cholesterol crystals can be detected by standard histology in advanced atherosclerotic lesions as cholesterol crystal clefts. Recently, with the help of laser reflection microscopy, large crystals that leave clefts in tissues can be seen along with a number of much smaller cholesterol crystals which are present in the extracellular space, as well as inside immune cells. In human and mice cells, IL-1b secretion was demonstrated from primed macrophages in response to cholesterol crystals in an NLRP3 inflammasome-dependent manner.[35] Inflammasome activation in bone-marrow-derived myeloid cells was shown to contribute to murine atherosclerosis. Mice deficient in either NLRP3, ASC or IL-1a/b after feeding of western high cholesterol diet showed decreased aortic lesion size, as well as decreased levels of circulating IL-18.

NLRP3 inflammasome activation in atherosclerosis starts with priming via recognition of modified LDL by TLRs and scavenger receptors on macrophages. Pro-IL-1b, caspase-1 and NLRP3 are upregulated by this priming step. The macrophage phagocytosed modified LDL-induced cholesterol crystals are stored as cellular cholesteryl esters. They cause lysosomal rupture, allowing release of lysosomal proteases (cathepsins). Cathepsin-B is required for the release of IL-1ß and it also activates caspase-II, which acts as an upstream activator of caspase-1 (Figure 3).[36] Cathepsins, in combination with potassium efflux and ROS production, mediate the activation of the NLRP3 inflammasome. This brings about cleavage of caspase-1 and production of mature IL-1ß cytokine.

Cholesterol dependant cytolysins induce the release of IL-1ß and IL-1α. It has been demonstrated that within atherosclerotic plaque, intimal and adventitial macrophages and free luminal leukocytes express mRNA for both IL-1ϐ and IL-1α. However, adherent leukocytes and vascular smooth muscle cells (VSMC) express mRNA for IL-1α only. So, although the role of IL-1α in atherosclerosis is more or less well known, its link to NLRP3 is still not quite established (Figure 3).[37]

IL-1ß released from cells causes continued upregulation of inflammasome components as well as mediates an inflammatory response that results in an influx of immune cells and progression of atherosclerotic plaque formation. The expression of ASC and caspase-1 at sites of myocardial I/R injury has been demonstrated and deficiency of ASC and caspase-1 decreased the inflammatory responses such as inflammatory cell infiltration and cytokine expression along with injuries such as infarct development, myocardial fibrosis. The study also stressed that ROS production and potassium efflux played important roles in inflammasome activation and myocardial injury.[38]

With progress of time, atherosclerosis advances and leads to narrowing of vascular lumen. The myocardial cells become more and more ischemic leading to anaerobic metabolism and acidosis. This brings about a change in the myocardial cell membrane permeability leading to electrolyte imbalances particularly of potassium. A drop in potassium levels along with production of toxic metabolites as well as ROS, can activate NLRP3 and hence further aggravate atherosclerosis.[39]

9. Need for Further Research

Despite the fact that NLRP3 inflammasome has been implicated in a variety of diseases such as atherosclerosis, diabetes, obesity, gout etc. its mechanism of activation and the signaling pathways have a lot of vague areas.[38] Some of these are, a common pathway of activation linking ROS and potassium efflux, relation of cathepsins and IL-1α to NLRP3 and secretion of IL-1ß.

Moreover, nlrp3 studies in atherosclerotic patients are almost negligible. Although a number of animal studies have been undertaken to target the downstream products of NLRP3 inflammasome activation, the entire regulatory process and its effects still remains to be understood clearly. Till date, no drugs that can act on and neutralize NLRP3 inflammasome have been developed. Hence, there is need to undertake more research to better understand the entire NLRP3 activation as well as the downstream pathways which will lay the foundation to make new drugs that could prove vital in the treatment strategies of some of the most debilitating diseases of human kind like atherosclerosis.

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