Inflammasome - A Novel Drug Target for Inflammatory Diseases

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Abstract: Inflammasomes are cytoplasmic caspase-1-activating protein complexes that promote maturation and secretion of the proinflammatory cytokines. The inflammasomes are increasingly reported to play a major role in human pathologies associated with various diseases such as cancer, lung diseases, diabetes, cardiovascular and metabolic disorders. In inflammatory diseases, the inflammasome complex is widely expressed in a variety of cell types, which can be activated by environmental pollutants, infectious or non-infectious triggers, in which innate immunity play a key role in activation inflammasome complex through toll like receptors (TLRs). Therefore reviewing of molecular pathological events in inflammasome complex activation provides important information in the pathogenesis of various diseases associated with inflammation. Thus, understanding inflammasome pathways may provide insight into inflammatory disease pathogenesis that might identify potential drug targets for therapeutic interventions.

Key words: Inflammasome • Immunity • Drugs • Inflammatory diseases • Cytokines

INTRODUCTION

The immune system is capable of detecting a wide range of exogenous and endogenous insults against the host including tissue damage, metabolic stress, and infections. Growing evidence indicates that the inflammasome plays a key role in the pathogenesis of inflammatory diseases [1]. A key event for the pathogenesis of inflammatory disorders is the recruitment of leukocytes and other immune cells to the infected or damaged cells to restore homeostasis. In the absence of microbial stimuli, sterile injury can trigger an acute inflammatory response which might be responsible for the pathogenesis of several diseases, including rheumatoid arthritis, lung fibrosis and acute liver failure.

Interleukin (IL)-1 was first cloned in the 1980s and rapidly emerged as a key player in the regulation of inflammatory processes. IL1b plays a crucial role in systemic inflammation due to its ability to induce the expression of a large panel of pro-inflammatory genes and to act on various target organs. The activation of inflammasome by different stimuli triggers the proteolytic cleavage of pro-caspase 1 into active caspase 1, which, in turn converts pro-interleukin 1b (pro-IL1b) into the mature IL1b. The nucleotide-binding domain leucine-rich repeat containing (NLR) family of receptors are members of the innate immune system, and have a critical role in host defense [1,2,3]. These molecules are key to driving inflammatory responses to abnormal cellular conditions. Many NLRs serve this role on activation by forming a multiprotein complex called an inflammasome (Fig 1). The nucleotide-binding domain leucine-rich repeat (LRR)-containing receptors (NLRs) are pattern recognition receptors (PRRs) that initiate inflammatory responses to a wide range of stimuli. NLR are found intracellularly and share a unique domain architecture consisting of a central nucleotidedebinding and oligomerization domain called the NACHT domain that is located between an N-terminal effector domain and a C-terminal LRR domain.

The NLPR3 inflammasome is the best characterized and participates in immune responses to infectious and noninfectious agents. It consists of the aforementioned NLPR3 receptor, the adaptor protein ASC and caspases. In 2002, Martinon et al., described, for the first time, an

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inducible high-molecular-weight complex containing NLRP3, an adaptor protein [apoptosis-associated speck-like protein containing a CARD domain (ASC)], and proinflammatory caspases, which they called the inflammasome [4]. The activators of NLRP3 are quite varied and include environmental irritants, endogenous danger signals, pathogens, and distinct pathogen-associated molecular patterns (PAMPs) and has been associated with a wide range of diseases including infectious, autoinflammatory, and autoimmune disorders [3,5,6]. It was earlier documented that Caspase-1 activation by inflammasome complexes in response to PAMPs and damage-associated molecular patterns (DAMPs) induces pro-inflammatory cytokines [2].

In this regard specific therapeutic interventions currently under investigation include anti-inflammatory agents (e.g., steroids), antioxidants (e.g., tocopherols, melatonin, N-acetylcysteine, nitric oxide synthase inhibitors), protease inhibitors (e.g., doxycycline, aprotinin, ilomastat), surfactant replacement, and bronchodilators [7,8,9]. Effective treatments may depend on the extent of lung injury and require a multi-faceted pharmacological approach. These studies possibly suggest that fibrotic process can be reversed via blockade of pathways associated with inflammasome activity which may provide hope for future drug strategies.

**Inflammasome Targets in Autoinflammatory Disorders:**
Autoinflammatory syndromes are disorders characterized by the hyperactivation of the innate immune system in the absence of microbial infection or autoantibody production [5,6]. Some autoinflammatory syndromes are caused by dysregulated activation of inflammasomes, molecular platforms responsible for the activation of caspase-1 and the production of interleukin (IL)-1b. Cryopyrin-associated periodic syndromes (CAPS) were the first autoinflammatory disorders found to be directly mediated by dysfunctional inflammasome activation. Understanding the molecular pathophysiology of these syndromes has further guided the successful development of targeted therapy against IL-1.

Both CAPS and familial Mediterranean fever (FMF) are characterized by increased inflammasome activity and overproduction of IL-1b which is ultimately responsible for disease manifestations. Importantly, understanding the molecular mechanisms of these syndromes has led to effective treatment for these rare diseases with biological drugs that target IL-1b-mediated signalling. The mechanisms leading to IL1b hypersecretion in other autoinflammatory disorders remain to be identified, as is the case for the role of each inflammasome *in vivo*. Better knowledge in this field should also contribute to the development of new anti-inflammatory treatments.

**Therapeutic Targets of Inflammasomes in Lung Disorders:** Airways are constantly exposed to microbial pathogens and detection of microbial products by host inflammasomes is an important mechanism of innate immune surveillance in which toll like receptors (TLR) and NLR act as central innate immune sensors [10]. Activation of NLR members, especially NLRC4 and NAIP5, leads to the infected cell death by pyroptosis, which is accompanied by secretion of the pro-inflammatory cytokines IL-1beta, IL-18, and IL-33 [11,12,13]. In airways diseases such as asthma, cystic fibrosis, chronic lung disease of infancy, tuberculosis, chronic obstructive pulmonary disease, bronchopulmonary dysplasia and lung cancers all have prominent inflammatory components...
and multiprotein interactions of inflammatory components, which play a major role during inflammatory conditions. In asthmatic conditions the production of IL-17 and IL-22 mediators by Th17 cells has been mediated by NLRP3 inflammasome activation [13, 14, 15]. Recent investigations revealed that airway exposure to allergen in sensitized individuals causes the release of ATP and uric acid, activating the NLRP3 inflammasome complex and cleaving pro-IL-1b to mature IL-1b through caspase-1. Recent progress has contributed to understanding that activation of proinflammatory cytokines (IL-1b and IL-18) and components of the inflammasome complex itself, such as the adaptor protein ASC have been associated with Pulmonary fibrosis development. Chronic Obstructive Pulmonary Disease (COPD) is a cigarette smoke (CS)-driven inflammatory airway disease with an increasing global prevalence. It has recently been shown that an activator of the P2X7/inflammasome pathway, ATP, and the resultant products (IL-1b/IL-18) are increased in COPD patients [16,17]. Collectively, these observations provided a convincing rationale for a novel therapeutic approach to these patients, namely through the inhibition of inflammasome activity. Strikingly, treating these patients with anakinra, a non-glycosylated recombinant form of the naturally occurring IL-1 receptor antagonist (IL-1Ra) which blocks inflammasome-dependent IL-1b signalling, resulted in a complete cessation of clinical symptoms and biochemical changes within hours of administration [18,19]. The clinical availability of IL-1 inhibitors has thus been critical to the identification of the NLRP3 inflammasome as a critical regulator of inflammation in vivo. The prototypic anti-IL-1b therapy has been based so far on the daily subcutaneous injection of anakinra [19,20].

**Inflammasome Drug Targets in Metabolic and Non-metabolic Disorders:** It has become clear over the past few years that IL-1b and IL-18 play a crucial role in the pathogenesis of diabetes and a recent clinical trial supports the notion that IL-1b is indeed a key player in type 2 diabetes patients receiving IL-1b antagonists featured improved glycaemic control and beta cell mass [20-24]. Notably, diabetic markers such as increased levels of saturated fatty acids and islet-derived amyloid polypeptide have been reported as capable of activating the NLRP3 inflammasome. Although it is known that increased levels of IL-1b are associated with an increased risk for developing diabetes, the mechanism behind this increase was unclear until recently. Further experimental data suggest that the NLRP3 inflammasome is an important regulator of adipocyte differentiation and insulin sensitivity. Adipocytes are rendered more metabolically active and insulin-sensitive upon NLRP3 inflammasome inhibition in murine models of obesity [23,24]. Strikingly, calorie restriction and exercise-mediated weight loss in obese type 2 diabetes patients is associated with a decreased NLRP3 expression in adipose tissue, coupled to decreased inflammation and improved insulin sensitivity. Sterile inflammation is a crucial event in the pathological process that underlies atherosclerosis; convincing evidence suggests NLRP3 is involved in this process and may function in inflammatory signalling during atherosclerosis [25,26,27].

Several groups have also examined the role of NLRP3 and caspase-1 in inflammatory bowel diseases using the dextran sulphate sodium (DSS) mouse model of colitis. Ulcerative colitis results from hyper-responsive inflammation, and in this context it has been demonstrated that excessive IL-1b and IL-18 production can also contribute to DSS induced colitis and possibly cancer [28-30].

The fibrillar peptide amyloid-b, which plays a key function in the development of Alzheimer’s disease, was also shown to activate the NLRP3 inflammasome [31]. Moreover, the NLRP3 inflammasome was suggested to be instrumental in the inflammatory component of the disease and its associated brain tissue damage. In the skin, NLRP3 inflammasome activation has been linked to UVB-induced damage and contact hypersensitivity. Recent studies have shown that haemozoin, a crystal produced by plasmodium species in the course of malarial infection, activates the NLRP3 inflammasome [32,33]. More surprising still, non-coding NLRP3 mutations were linked to essential hypertension susceptibility, possibly due to increased expression of the proteins.

It is established that inflammation plays a pivotal role in the pathogenesis of Ischemia-reperfusion injury (IRI) and a growing body of evidence demonstrates in particular the importance of innate immunity and pattern recognition receptors [34-36]. An overview of recent data suggest a role for NLRP3 inflammasome in IRI, supporting the possible strategy to inhibit NLRP3 inflammasome activation in order to improve clinical outcomes following IRI. The primary mechanism through which the IRI affected kidney initiates and regulates this complex inflammatory process has been a major question although emerging data demonstrate that pattern recognition receptors and in particular the NLRP3 inflammasome have a key role [35,36]. Myocardial IRI induces cardiomyocyte
damage and a subsequent profound inflammatory response. In vitro studies further revealed that cardiac fibroblasts, not cardiomyocytes, induce inflammasome activation during hypoxia/reoxygenation [37,38].

Gout is a sterile inflammatory disease caused by monosodium urate (MSU) crystal deposition in various tissues. MSU crystals were demonstrated to specifically activate the NLRP3 inflammasome, both in vitro and in vivo [39,40]. Taken together, this suggests that the NLRP3 inflammasome has evolved as a sensor of metabolic endogenous danger, in addition to its pathogen-detecting functions. Excitingly, preliminary clinical trials involving in vivo IL-1β blockade by anakinra or rilonacept in gout patients demonstrated high efficacy and the absence of adverse effects. These findings require confirmation in large-scale controlled studies, and it will be interesting to see whether long-acting therapies such as canakinumab are able to tame chronic gout flares over time.

In Alzheimer’s disease, progressive dementia is associated with cerebral accumulation of amyloid β plaques as well as neuronal cell death [41, 42]. Inflammation is believed to be a causative factor in the described neuronal cell death; in particular, inflammation triggered by the amyloid plaques has been suspected [41-44]. Given the important role of the NLRP3 inflammasome in non-infectious diseases, targeting the individual components and signalling pathway of this complex may offer a wide range of future therapeutics. Until now strategies for targeting the NLRP3 inflammasome pathway in the clinic have been focused on IL-1β blockade. Three agents targeting IL-1β are approved by the US Food and Drug Administration (FDA) and have been brought to the market: anakinra for rheumatoid arthritis, rilonacept and canakinumab for cryopyrin-associated periodic syndromes. Anakinra was the first IL-1β blocker to be FDA approved and was shown to be moderately effective for the treatment of patients with rheumatoid arthritis and osteoarthritis when compared to other biological agents. An alternative to blocking the NLRP3 inflammasome pathway through interference with IL-1β is to target caspase-1 directly. Two small caspase-1 inhibitors, VX-765 and pralnacasan (VX-740), were developed and used in clinical trials for the treatment of chronic plaque psoriasis, rheumatoid arthritis, and psoriasis [45,46]. This knowledge clearly opens new opportunities for therapeutic intervention.

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REFERENCES


