The inflammasome in lung diseases

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Submitted 17 July 2012; accepted in final form 13 August 2012

dos Santos G, Kutuzov MA, Ridge KM. The inflammasome in lung diseases. Am J Physiol Lung Cell Mol Physiol 303: L627–L633, 2012. First published August 17, 2012; doi:10.1152/ajplung.00225.2012.—Inflammation, the process aimed at restoring homeostasis after an insult, can be more damaging than the insult itself if uncontrolled, excessive, or prolonged. The inflammasome is an intracellular multimeric protein complex that regulates the maturation and release of proinflammatory cytokines of the IL-1 family in response to pathogens and endogenous danger signals. Growing evidence indicates that the inflammasome plays a key role in the pathogenesis of acute and chronic respiratory diseases. The inflammasome can be activated by the pathogens that account for the most prevalent infectious diseases of the respiratory tract, such as influenza A virus, Streptococcus pneumoniae, Pseudomonas aeruginosa, and Mycobacterium tuberculosis. The inflammasome also plays a role in the chronic inflammation of the airways of patients with asthma and chronic obstructive pulmonary disease, as well as in the initiation and progression of the inflammatory process in pulmonary fibrosis. The aim of this review is to summarize the most relevant points of inflammasome activation in lung diseases.

innate immunity; interleukin-1β; respiratory diseases

INFLAMMATION IS AN ADAPTIVE response to noxious stimuli (41). The innate immunity comprises a system of germline-encoded receptors that inspect the intracellular and extracellular compartments for signs of infection and recognize highly conserved microbial motifs or “pathogen-associated molecular patterns” (PAMPs). These pattern-recognition receptors (PRRs) are expressed by cells at the front line of host defense against infection, such as macrophages, monocytes, dendritic cells, and epithelial cells. PRRs are not clonally distributed; therefore, all cells expressing PRRs immediately identify PAMP-expressing microbes as a potential threat (34). Membrane-bound Toll-like receptors (TLRs) and C-type lectins are the PRRs that probe the extracellular milieu and the endosomal compartments for PAMPs, while the cytosol is constantly scanned by intracellular nucleic acid sensors, such as interferon-inducible protein (AIM2) and retinoic acid-inducible gene-like helicases. Activation of these receptors causes proinflammatory cytokine production and type I interferon-dependent antiviral responses via the transcription factor NF-κB (53).

Nucleotide oligomerization domain (NOD)-like receptors (NLRs) are a particular type of intracellular PRR that recognize PAMPs and the host-derived signals named DAMPs (danger-associated molecular patterns). NLRs are composed of a conserved central domain, which mediates nucleotide binding and oligomerization [NACHT, NOD, or nucleotide-binding site (NBS) domain], a COOH-terminal leucine-rich domain (LRR), which senses NLR agonists and has an autoinhibitory effect in their absence (14), and an NH2-terminal region, which is required for protein-protein interaction. The human NLR gene family is composed of 22 members, which, depending on their NH2-terminal domains, are classified into 4 subfamilies: NLRA, NLRB, NLRC, and NLRP. NLRA contains an acidic transactivation domain, NLRB a baculovirus inhibitory repeat domain, NLRC a caspase recruitment domain (CARD), and NLRP a pyrin domain (PYD). Activation of certain NLRs (NLRP1, NLRP3, and NLRC4) leads to assembly of the inflammasome, a high-molecular-weight platform for the activation of caspase-1, which is required for proteolytic maturation and release of the proinflammatory cytokines IL-1β and IL-18 (15, 28, 42, 51).

Historical Perspective

The link between mutations in NLR genes and inflammatory diseases was established by Hoffmann and colleagues (24) in 2001, when they described mutations in NLRP3 in individuals affected by Muckle-Wells syndrome, a rare autoinflammatory disease characterized by recurrent episodes of fever and rash associated with ocular and articular manifestations (46). In 2002, Martinon et al. (38) described, for the first time, an 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. Two years later, Agostini et al. (2) demonstrated that constitutive production of active IL-1β observed in Muckle-Wells syndrome was the molecular basis of the NLRP3 inflammasome-dependent disorders. In 2004, Mariathasan et al. (36) demonstrated the requirement of the adaptor ASC within the inflammasome, since macrophages from ATP-challenged ASC−/− mice were...
unable to produce mature IL-1β and IL-18. Moreover, ASC−/− mice also showed defective caspase-1-dependent cell death, establishing a molecular link between inflammation and cell death pathways.

The NLRP3 Inflammasome

The NLRP3 inflammasome is the best characterized (51) and participates in immune responses to infectious and noninfectious agents. It consists of the aforementioned NLRP3 receptor, the adaptor protein ASC, and caspase-1 (Fig. 1).

ASC, also known as Pycard, is an adaptor protein that bridges NLRP3 and caspase-1. In resting monocytes and macrophages, ASC is sequestered in the nucleus; inflammatory stimuli induce ASC redistribution to the cytosol (9), where it interacts with NLRP3 through its PYD and with caspase-1 through its CARD.

Caspases are cysteine proteases involved in inflammatory or apoptotic pathways (57). Caspase-1, the most fully characterized of the proinflammatory caspases, is synthesized as an inactive zymogen. Upon appropriate stimulation, NLRP3 recruits ASC and procaspase-1 to form the inflammasome, where procaspase-1 is cleaved into a p10 and a p35 fragment. The latter is subsequently processed into the p20 subunit and its CARD, and two molecules of p20 heterodimerize with two molecules of p10 to form the mature and active enzyme (38, 50). Active caspase-1, in turn, cleaves the precursors of two potent proinflammatory cytokines, IL-1β and IL-18, in the cytoplasm; IL-1β and IL-18 are then released to the extracellular milieu by a yet undefined mechanism (35, 47).

NLRP3 inflammasome can be activated by multiple stimuli: whole pathogens (bacteria, viruses, and fungi), PAMPs, DAMPs (extracellular ATP and monosodium urate crystals), and environmental irritants (silica, asbestos, and UVB radiation). Two signals are thought to be required for activation: a “priming signal,” which induces the transcription of pro-IL-1β and pro-IL-18 and the expression of NLRP3 after TLR stimulation (29), and a “second signal,” which activates the inflammasome. However, the molecular mechanism that triggers inflammasome assembly remains unclear (Fig. 2). One of the proposed models of activation is purinergic P2X7 receptor-dependent pore formation by pannexin-1 hemichannel in response to extracellular ATP and K+ efflux, which allows extracellular PAMPs and/or DAMPs to access the cytosol and directly activate NLRP3 (10, 27). Another model is lysosomal rupture. After engulfment of particulate or crystalline agonists, such as silica and asbestos, the phagosome destabilizes and releases its content into the cytosol, which is sensed by NLRP3 and causes inflammasome activation (25). Finally, production of reactive oxygen species (ROS) appears to be a crucial event upstream of inflammasome assembly (66). ROS are sensed by thioredoxin and the thioredoxin-interacting protein (TXNIP) complex, causing dissociation of the complex and subsequent binding of TXNIP to NLRP3. This leads to recruitment of ASC and procaspase-1 by NLRP3 and assembly of the inflammasome (65). A recent study showed that ROS act as priming signals required for transcriptional upregulation of NLRP3, rather than its oligomerization (5).

Other Inflammasomes

Other inflammasomes, such as NLRP1, NLRC4, and AIM2, have also been characterized (Fig. 3, Table 1). The NLRP1 inflammasome, the first to be described, is activated by anthrax lethal toxin (7). Unlike most NLRs, NLRP1 activates caspase-1 directly, and ASC is not required for production of mature IL-1β (20). The NLRC4 or interleukin-converting enzyme protease-activating factor (IPAF) inflammasome is activated by cytosolic flagellin or by the basal body rod component of the type 3 secretion system found in Salmonella typhimurium, Shigella flexneri, Legionella pneumophila, and Pseudomonas aeruginosa (55). Differences from the NLRP3 and NLRP1 inflammasomes include the ability to activate caspase-1 without the adaptor ASC (56) and the constitutive expression of NLRC4 without a TLR-mediated priming signal (29). Finally AIM2, a PRR that senses cytosolic double-stranded DNA (dsDNA) (dsDNA) (Fig. 2), is also capable of forming inflammasomes. Since ligand requirements for AIM2 include dsDNA from viruses, bacteria, or the host itself, it may also contribute to autoimmune responses (49).

Regulation of Inflammasome Activity

Inflammasome activity requires precise regulation to avoid an excessive production of cytokines and its deleterious effects. Regulation takes place at transcriptional and posttranscriptional levels. For instance, NLRP3 is expressed at limited levels in macrophages and is highly inducible in response to proinflammatory stimuli such as LPS, cytokines, or ROS (5). Moreover, differential splicing of ASC can generate protein variants with an inhibitory function, instead of the classical adaptor molecule (8). Another level of regulation is the sub-
cellular location of the inflammasome components; one example is the aforementioned redistribution of ASC from the nucleus to the cytoplasm in activated inflammatory cells (9). Additional regulation of the inflammasome activity can be achieved by secreted factors. In fact, type I interferons can suppress inflammasome activation and its subsequent production of IL-1β and IL-18 into the extracellular milieu. Currently, the nature of the second signal is debated. The 3 proposed models of activation are shown: 1) extracellular ATP, which activates the purinergic P2X7 receptor and causes subsequent recruitment of pannexin-1 hemichannel to the plasma membrane and K+ efflux; 2) lysosomal rupture after engulfment of crystalline or particulate agonists; and 3) reactive oxygen species (ROS), which upregulate NLRP3 expression and activate the inflammasome. PAMP, pathogen-associated molecular pattern; DAMP, danger-associated molecular pattern; TLR, Toll-like receptor; dsDNA, double-stranded DNA.

Role of the Inflammasome in Lung Diseases

Lung infections. Community-acquired pneumonia (CAP) is the most common cause of severe sepsis and the leading cause of death from infection in the United States (60). Since the mortality rate from CAP has not changed significantly in the past four decades (44), a thorough comprehension of its pathogenesis is mandatory to find a suitable treatment and effective vaccines.

Many bacterial pathogens that can cause CAP have been shown to activate the NLRP3 inflammasome; the most common mechanism involves the secretion of pore-forming toxins. The virulence of *Streptococcus pneumoniae*, the leading cause of life-threatening infections such as CAP, meningitis, and sepsis, depends on the polysaccharide capsule and pore-forming toxins, such as cytolysin pneumolysin (PLY) (61). The disruption of the plasma membrane caused by PLY induces K+ efflux, one of the proposed mechanisms of activation of the NLRP3 inflammasome. Listeriolysin O, a pore-forming toxin produced by *Listeria monocytogenes*, and the α-hemolysin...
and caspase-1. NLRPs require the adaptor protein ASC, inflammasomes are also composed of ASC except for NLRP1, which can bind directly to procaspase-1 and does not terminal PYD, a conserved NACHT domain, and a COOH-terminal LRR domain. With the exception of NLRP1, which can bind directly to procaspase-1 and does not require the adaptor protein ASC, inflammasomes are also composed of ASC and caspase-1.

Toxin produced by *Staphylococcus aureus* activate the NLRP3 inflammasome in human and mouse monocyctic cells in a similar manner (13, 37).

Infection by certain viruses also results in inflammasome activation. dsDNA viruses can activate the AIM2 inflammasome, while DNA- and RNA viruses can trigger assembly of the NLRP3 inflammasome. Influenza A virus (IAV), a major cause of lung infections and mortality, is known to activate the NLRP3 inflammasome (4, 58, 62), but the mechanism is unclear. Recent reports indicate that the IAV ion channel M2, which is involved in fusion during viral entry and in synthesis of new virions, can trigger inflammasome assembly and activation (26, 48). Infection of mice with IAV induces secretion of ATP into the bronchoalveolar lavage fluid (1), and ATP released from influenza-infected dying cells may also trigger the inflammasome-dependent response (1).

Active tuberculosis is primarily a disease of the lung, but it can progress to a generalized inflammatory disease. *Mycobacterium tuberculosis* (MTB) is a peculiar pathogen, because it resides within a phagosomal-like compartment of host macrophages during infection, where it can suppress inflammasome activation (39). However, MTB has the ability to activate the NLRP3 inflammasome, depending on the expression of a functional protein secretion system (ESX-1). (17) The pathways that lead to inflammasome activation in this pathogen are not completely understood, but the export of ESAT-6 (early secreted antigenic target, 6 kDa, a family of small proteins secreted by MTB) via ESX-1 appears to be of critical importance (43).

**Airway diseases.** Although the incidence and severity of asthma and chronic obstructive pulmonary disease (COPD) are increasing worldwide (32), there is no treatment to slow the progression of the latter, and a significant group of asthmatic patients remain resistant to available therapies. Hence, understanding the pathophysiology of these diseases to find effective therapies is of prime importance. Chronic inflammation of the airway is the common feature of both diseases, and a growing body of evidence suggests a role for the NLRP3 inflammasome in the pathogenesis of this inflammation.

Extracellular ATP is strongly and persistently upregulated in the airways of patients with COPD (31), and this is associated with a decline in lung function (12) and an increase in airway infiltration by inflammatory cells. As we previously mentioned, extracellular ATP activates the NLRP3 inflammasome by engaging the purinergic P2X7 receptor. This receptor is also

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NLR, nucleotide oligomerization domain (NOD)-like receptor; NLRP, NLR subfamily containing a pyrin domain; NLRC, NLR subfamily containing a caspase recruitment domain; MSU, monosodium urate; ROS, reactive oxygen species; dsDNA, double-stranded DNA.
upregulated in alveolar macrophages and blood neutrophils from patients with COPD (31), as well as in blood and airway neutrophils and alveolar macrophages in the mouse model of lung inflammation induced by cigarette smoke (CS) (33).

The role of the inflammasome components NLRP3 and ASC in CS models has not been fully investigated. However, there is evidence of inflammasome activation, since levels of caspase-1 are increased in lung tissue after CS challenge in mice. Caspase-1 levels are also higher in lung tissue from COPD patients and smokers than from nonsmoking donors (19). Selective inhibition of caspase-1 significantly decreased inflammation after CS challenge in animal models (11). These data support a role for the inflammasome in the airway inflammation in COPD and asthma, although further studies are required.

The role of the NLRP3 inflammasome in the development of allergic airway disease is controversial. As with COPD, ATP levels are also elevated in the airways of asthmatic patients and in animal models in response to allergen challenge (6). Moreover, IL-1β levels are higher in serum of asthmatic than nonasthmatic patients (59), and induced sputum contains higher levels of IL-1β in symptomatic than in nonsymptomatic asthmatic patients, suggesting a putative role of the NLRP3 inflammasome in chronic disease and acute exacerbations. However, recently, Allen et al. (3) failed to see a difference in clinical outcome of four allergic asthma models in NLRP3−/− mice compared with controls.

Pulmonary fibrosis. The term “pulmonary fibrosis” includes a broad range of lung disorders characterized by progressive and irreversible destruction of normal lung architecture by excessive accumulation of collagen and other extracellular matrix (ECM) components in basement membranes and interstitial tissues. ECM expansion impairs effective gas exchange and leads to death due to extreme respiratory failure (30). However, the pathophysiological mechanisms underlying pulmonary fibrosis are not completely understood.

The importance of inflammation in initiation and progression of pulmonary fibrosis has been established (63). Silica, asbestos, and bleomycin, among other irritants, can injure lung epithelial cells and activate the NLRP3 inflammasome in macrophages, leading to IL-1β secretion (18, 21, 25). IL-1β secretion is also increased in macrophages treated with pravastatin, as demonstrated by Xu and colleagues (64), suggesting a role for statins in pulmonary fibrosis. IL-1β promotes production of TGF-β, the most potent and ubiquitous profibrotic cytokine (30), which triggers activation, proliferation, and transdifferentiation of epithelial cells and resident fibroblasts into collagen-producing myofibroblasts. IL-1β also promotes the secretion of neutrophil-attracting CXC chemokines, which favors the influx of neutrophils and exacerbate the damage to epithelial cells, and PDGF, which further induces fibrosis. Finally, TGF-β and IL-1β can increase the expression of plasminogen activator inhibitor 1, which inhibits ECM degradation, promotes recruitment of more inflammatory cells (67), and suppresses the release of antifibrogenic growth factors (30). The initial proinflammatory scenario of inflammasome activation can rapidly progress to a profibrotic one, leading to the chronic and devastating disease.

Acute respiratory distress syndrome. Acute lung injury and its most severe form, acute respiratory distress syndrome (ARDS), are frequent complications of patients admitted to the intensive care unit and are associated with a mortality of ~25% (54). ARDS can be triggered by different disorders, such as pneumonia, sepsis, ischemia, and trauma; however, there is neither an effective tool to predict patients’ susceptibility to ARDS nor an effective therapy. Pathophysiologically, the disease is characterized by dysregulated inflammation, with excessive permeability of epithelial and endothelial barriers leading to lung edema formation and severe hypoxemia (40). Inflammasome-regulated cytokines appear to play a major role in the development of ARDS, as demonstrated by Dolinay and colleagues (16), since increases in the levels of circulating IL-18 in critically ill patients correlate with disease severity and mortality, even after adjustment for important confounders, such as Acute Physiology and Chronic Health Evaluation (APACHE) II score.

Conclusion

The lung is exposed to a variety of insults that can be detected by different PRRs, such as microbial molecules (LPS and flagellin), inhaled particles (asbestos and silica), and cell injury-associated endogenous molecules (ATP and potassium). The activation of different PRRs and the resultant signaling pathways play a critical role in host protection and in the pathology of lung diseases. New reports (21) implicate the inflammasome in the sensing of danger/stress signal, leading to increased levels of active IL-1β. Furthermore, the role of IL-1β as a critical inflammatory mediator of acute inflammation and tissue remodeling has been well established (22). Over the last 10 years, the discovery and characterization of the inflammasome have led to a comprehensive insight into the innate and adaptive immune responses to different lung insults. However, our knowledge of activation and regulation of the inflammasome is incomplete. Further understanding of the inflammasome complex would be advantageous in the development of new treatment modalities in acute lung injury and chronic lung disease.

GRANTS

This work was supported by a Merit Review grant from the Department of Veterans Affairs (K. M. Ridge).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

G.D.S. drafted the manuscript; M.A.K. edited and revised the manuscript; K.M.R. approved the final version of the manuscript.

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