The role of the NLRP3 inflammasome in pulmonary diseases

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***Abstract*:** Respiratory diseases and lung injuries are one of the leading causes of death in the world. One critical component of these diseases is exaggerated inflammatory response. The recently discovered inflammasome is believed to play a key role in inflammation. The inflammasome is an oligomer of intracellular proteins that, once activated by an insult or damage signal, produces mature cytokines from the interleukin-1 family that mediate an inflammatory response. Previous research has provided evidence that suggests the role of the inflammasome in the pathogenesis of many chronic respiratory diseases and acute lung injuries, such as transfusion-related acute lung injury, ventilator-induced lung injury, asthma, chronic obstructive pulmonary disease and pulmonary fibrosis. This article summarizes recent research on the inflammasome and reviews proposed molecular models of the role of the inflammasome in several prominent lung diseases and injuries.

Keywords: acute lung injury, chronic respiratory disease, inflammasome, inflammation, interleukin 1, lung, NLRP3

Introduction

Chronic respiratory diseases and acute lung injuries are common and widespread problems affecting millions of people worldwide, especially in developing countries. The Centers for Disease Control and Prevention estimated in 2012 that 18 million Americans had asthma, one of the most prevalent of the chronic respiratory diseases [Blackwell *et al.* 2014]. For some of these diseases, medication and therapy are available. However, these treatments are not particularly effective for long-term remedy.

Lung diseases can have harmful effects on the airways, alveoli, interstitium or capillary network. Although these respiratory diseases may be distinct, they appear to share chronic inflammation as the main factor of their pathogenesis. There is new hope for respiratory diseases characterized by chronic inflammation. In 2002, Martinon and colleagues discovered the inflammasome, a protein complex known to promote inflammation [Martinon *et al.* 2002]. The role of the inflammasome in innate immunity and inflammation may hold the key to understanding the pathogenesis of many chronic respiratory diseases and acute lung injuries, along with new methods of treatment. With the discovery of the inflammasome and recent research linking it to many respiratory diseases, it is now believed that the inflammasome and its inflammatory products are the connection between many chronic respiratory diseases and acute lung injuries.

Inflammation in innate immunity

Inflammation is one of the immediate responses of the innate immune system. Its purpose is to prevent the spread of infection by providing a barrier and allowing repair of damaged tissue after the pathogens have been eliminated. Inflammation is initiated by the introduction of pathogens or mechanical injury to cells or tissue. These events are recognized by pattern recognition receptors (PRRs), which can either be attached to the membrane of the cell on toll-like receptors (TLRs) or found within the cytoplasm on nucleotide-binding oligomerization domain-like receptors (NLRs) [dos Santos, 2012]. In the case of an infection, pathogen-associated molecular patterns (PAMPs) are recognized by the PRRs. Damage-associated molecular patterns (DAMPs) are recognized by

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the PRRs when mechanical damage is present. After the recognition of a PAMP or DAMP, the innate immune system responds with an infectious or sterile inflammatory response [Land, 2013]. These responses utilize innate immune cells such as macrophages, neutrophils and proinflammatory chemical factors known as cytokines to promote inflammation. The intentions of inflammation are benevolent, and a controlled response is a necessary defense mechanism that is crucial for the host's recovery. However, an uncontrolled or exaggerated response often occurs, resulting in severe inflammation that can lead to acute or chronic diseases.

Inflammasome

The recently discovered inflammasome has a substantial impact on tissue inflammation. The inflammasome is a complex of proteins that oligomerize and activate the caspase-1 cascade which produces the active proinflammatory cytokines interleukin (IL)-1β and IL-18 when triggered by various environmental, pathogenic or endogenous danger signals [dos Santos, 2012; Brickey *et al.* 2011]. The inflammasome contains the adaptor protein ASC [apoptosis-associated speck-like protein containing a caspase recruitment domain (CARD)], pro-caspase 1 and the receptor which activated its formation. The inflammasome has four known structural subsets which include nucleotide-binding oligomerization domain receptors (NLR) family, pyrin domain containing 1 (NLRP1), NLRP3, NLR family CARD domain-containing protein 4 (NLRC4) and absent in melanoma 2 (AIM2) [van de Veerdonk *et al.* 2011].

In the caspase-1 cascade, the inflammasome positions several 45 kDa (p45) pro-caspase 1 molecules. They are then autocatalytically cleaved into 10 kDa (p10) and 35 kDa (p35) subunits. The p35 subunit is further processed into 20 kDa (p20) and its CARD [dos Santos *et al.* 2012]. The active caspase 1 is formed when two p20 molecules heterodimerize with two p10 molecules, resulting in two active sites [Wilson *et al.* 1994]. The active caspase 1 then carries out the proteolytic cleavage of pro-IL-1β and pro-IL-18 into their active forms. These cytokines are then excreted into the extracellular medium, where they promote inflammation. While all inflammasomes carry out the same function, they are activated by different agonists due to their unique structures.

NLRP3

NLRP3 is the most studied inflammasome and will be the focus in this review. NLRP3 contains leucine-rich repeats, nucleotide-binding domains (NBDs), and an N-terminal pyrin domain, allowing for the recruitment of ASC to activate pro-caspase 1. Oligomerization of NLRP3 requires adenosine triphosphate (ATP) or deoxyATP to bind to the NBD of the inflammasome [Duncan *et al.* 2007] and can be inhibited by high K^+ concentrations [Petrilli *et al.* 2007]. Only one NLRP3 inflammasome inhabits a cell, and it is relatively large compared with the other inflammasomes, reaching up to 2 μm in diameter [Stutz *et al.* 2009].

It is postulated that a two-step mechanism is required for the full activation of the NLRP3 inflammasome [Bauernfeind *et al.* 2011; Gross *et al.* 2011]. The first is a priming step that is initiated by various DAMPs and PAMPs, which results in upregulation of pro-IL-1β, pro-IL-18 and the components of the inflammasome. The second step is the activation step which is the assembly of the components into the inflammasome structure and production of proinflammatory interleukins. The mechanism of activation is not known, but NLRP3 is shown to have an abundance of activators. NLRP3 activity can be triggered by a low K^+ concentration environment [Petrilli *et al.* 2007]. A wide range of bacteria and viruses have been shown to activate NLRP3, including influenza A virus (IAV), Sendai virus, *Neisseria gonorrhoeae* and *Mycobacterium marinum* [Duncan *et al.* 2009; Koo *et al.* 2008; Thomas *et al.* 2009]. Studies have also shown that the *Candida albicans* fungus induced IL-1β production from NLRP3 [Hise *et al.* 2009]. Microbial substances such as muramyl dipeptide, lipopolysaccharide and bacterial RNA were found to activate NLRP3 in the presence of ATP [Kanneganti *et al.* 2006]. Bacterial toxins like nigericin (from *Streptomyces hygroscopicu*) and maitotoxin (from *Gambierdiscus toxicus*) can also activate NLRP3 [Mariathasan *et al.* 2004]. Crystalized endogenous molecules are the most notable activators of NLRP3. Cholesterol crystals and monosodium urate crystals were shown to activate NLRP3 and are associated with arthritis and gout symptoms [Martinon *et al.* 2006]. Urban particulate matter, which is the metal, aromatic hydrocarbon, dust and emission particles commonly found in the air, has been shown to activate the NLRP3 inflammasome specifically in airway epithelial cells [Hirota *et al.* 2012]. Inorganic materials such as titanium

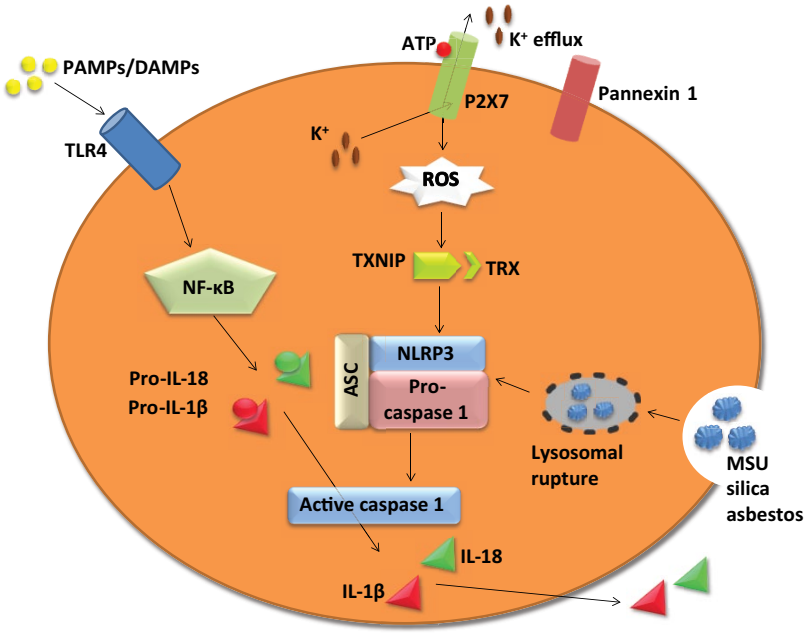

Figure 1. Activation of the NLRP3 inflammasome. The 'priming' step consists of the recognition of PAMPs or DAMPs by a TLR, which results in the production of the precursor components of the inflammasome such as Pro-IL-18 and -1β, and NLRP3. Three 'activating' steps have been hypothesized that lead to the oligomerization of NLRP3, ASC, and pro-caspase 1, and the activation of the caspase-1 cascade: (1) K^+ efflux, initiated by extracellular ATP interacting with $P2X_7$ receptors; (2) lysosome degradation, leading to the release of particulate activators into the cytosol; (3) ROS-mediated breakdown of thioredoxin and TXNIP, and subsequent interaction of TXNIP with NLRP3. ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; ATP, adenosine triphosphate; DAMP, damage-associated molecular pattern; IL, interleukin; NF-κB, nuclear factor κB; NLRP3, nucleotide-binding oligomerization domain-like receptor pyrin domain containing 3; PAMP, pathogen-associated molecular pattern; ROS, reactive oxygen species; TLR, toll-like receptor; TRX, thioredoxin; TXNIP, thioredoxin interacting protein; TRX, thioredoxin; MSU, monosodium urate.

dioxide, silica and asbestos trigger NLRP3 response as well [Yazdi *et al.* 2010].

There are several hypotheses for the mechanism that activates NLRP3. One model proposes that a low K^+ concentration is needed for NLRP3 activation. Some of the stimuli for NLRP3 allow for this by causing K^+ efflux. For example, some toxins can form pores in the membrane of cells allowing for K^+ efflux [Perregaux and Gabel, 1994]. Also, the activation of $P2X_7$ receptors on pannexin 1 by extracellular ATP causes K^+ efflux [Colomar *et al.* 2003]. Another model proposes that the NLRP3 inflammasome senses the degradation of lysosomes carrying crystallized and inorganic particles (which are known activators themselves) as a DAMP, which leads to NLRP3 activation [Hornung *et al.* 2008]. It has also been suggested that reactive oxygen species (ROS) cause the breakdown of thioredoxin and its interacting protein (TXNIP) [dos Santos *et al.* 2012]. TXNIP therein binds to an NLRP3 receptor and induces the recruitment of ASC and pro-caspase 1 into the inflammasome structure [Zhou *et al.* 2010] (Figure 1).

The inflammasome and the pathology of lung disease and injury

Inflammation is a major symptom of many lung injuries and diseases. When inflammation is present, the inflammasome is a possible suspect in their pathology. Much research has been undertaken to discover the possible role the inflammasome plays in the cause and development of various lung disorders.

Transfusion-related acute lung injury

Blood transfusions are often necessary for patients who experience severe traumatic injuries, but the majority of the population assumes that this procedure can be performed without later consequences. Many complications could possibly occur, and one of the leading causes of morbidity and mortality as a result of a blood transfusion is

transfusion-related acute lung injury (TRALI) [Land, 2013]. TRALI is the bilateral pulmonary edema as a result of the increased permeability of the microvasculature of the lungs that occurs within 6 h of a whole blood or blood component transfusion [Land, 2013]. The pathogenesis of TRALI remains unclear, but a 'two-hit' model has been suggested that resembles the two-step hypothesis of the activation of the NLRP3 inflammasome [Looney *et al.* 2009]. In the two-hit theory, the 'first hit' is an underlying injury to the endothelium of the lungs leading to the attachment of neutrophils to the capillary network. This initial proinflammatory response could arise from a previous infection, invasive surgery or mechanical ventilation. The 'second hit' is received with the blood transfusion. The recognition of foreign biological markers may serve to activate primed neutrophils from the 'first hit' leading to the symptoms of TRALI.

Not only are the 'two-hit' model of TRALI and the two-step activation of NLRP3 similar, they may be linked to each other [Land, 2013]. The priming factors of TRALI are known to produce PAMPs and DAMPs that can prime the NLRP3 inflammasome. The 'second-hit' of the blood transfusion can then serve to activate NLRP3 inflammasome. Changes that occur in stored blood eventually promote the hemolysis of aged erythrocytes, which involves the release of DAMPs that could prime the NLRP3 inflammasome. It has been found that the release of ATP after substantial hemolysis could activate the $P2X_7$ receptors on T cells [Scheuplein *et al.* 2009]. The association of the activation of $P2X_7$ receptors by ATP to K^+ efflux suggests another possibility of NLRP3 inflammasome activation due to blood transfusion.

Although it is not yet proven, it is likely that the inflammasome plays a role in the pathogenesis of TRALI. As mentioned earlier, it is suggested that TRALI develops from an underlying injury or infection. Mechanical ventilation, invasive surgery or traumatic injury to the lung could serve as DAMPs or allow the entrance of PAMPs that can be detected by PRRs. High mobility group box 1 is a DAMP common to surgery and traumatic injury and is shown to be related to blood loss. The recognition of these DAMPs and PAMPs primes the inflammasome by producing pro-IL-1β and pro-IL-18, and upregulating the expression of NLRP3 inflammasome components. A subsequent blood transfusion may activate the NLRP3 inflammasome. The extracellular ATP found in transfused blood (due to hemolysis during storage) can interact with $P2X_7$ receptors on pulmonary neutrophils, macrophages and endothelial cells, causing K^+ efflux and introduction of ROS. These signals cause the oligomerization of the NLRP3 inflammasome and proteolytic cleavage of pro-IL-1β and pro-IL-18 into their mature forms. Excretion of IL-1β and IL-18 into the pulmonary milieu would then cause the inflammation shown in TRALI.

Ventilator-induced lung injury

Another type of acute lung injury in which the inflammasome is suspected to play a role is ventilator-induced lung injury (VILI). VILI is caused by injurious overinflation of the lungs during mechanical ventilation [dos Santos and Slutsky, 2006]. VILI may arise in patients who require mechanical ventilation of the lungs due to a previous lung injury or because the patient has acute respiratory distress syndrome. In mechanical ventilation, air flows more easily into areas of the lung with less resistance than areas that are collapsed or filled with fluid, leading to overstretching of tissue and eventual damage. The damage to the tissue initiates an immune response characterized by inflammation. The inflammasome is believed to be the link between mechanical damage of tissue and hyperinflammation in VILI.

In one study, gene expression analysis was undertaken on *ex vivo* and *in vivo* mice lungs subjected to mechanical ventilation and induced VILI [Dolinay *et al.* 2012]. Retrospective analysis of this study found a significant change in gene expression of components related to the inflammasome. In the *ex vivo* model, changes in the expression of IL-1α, caspase-activator domain 10 and IL-1 receptor 1 and 2 were observed. In the *in vivo* model, significant changes in the gene expression of caspase-activator domain 10 and 15, IL-18 receptor 1, IL-1 receptor 1 and 2, and IL-1β were also noted. The changes in the expression of these genes may suggest inflammasome activity in VILI. In addition, the number of alveolar macrophages increased and cleaved IL-18 was colocalized with the cells. Since the inflammasome is known to cleave IL-18 in macrophages, this further indicates the role of the inflammasome in the inflammation seen in VILI.

To support this, the same study exposed mice containing suppressed IL-18 and caspase 1 genes

to mechanical ventilation and induced VILI [Dolinay *et al*. 2012]. Neutrophil cell counts in the bronchoalveolar lavage fluid of IL-18 and caspase 1 deficient mice were lower than the control mice. IL-18 deficient mice also showed to be without the alveolar–capillary barrier dysfunction compared with control mice. If the lower neutrophil count in IL-18 and caspase 1 deficient mice is related to decreased inflammation, then these results may show that the inflammasome function was reduced as a result of the genetic deletions.

Although the role of the inflammasome in the VILI inflammation pathway is not exactly known, it is possible that VILI may cause or introduce PAMPs and DAMPs directly or indirectly. It could be the case that the injury to the lung as a result of overinflation during mechanical ventilation may be sensed as a DAMP. It is also plausible that overstretching of lung tissue during mechanical ventilation makes PRRs vulnerable to microbes, toxins and other PAMPs that were unintentionally introduced. Nevertheless, either method may result in activation of the nuclear factor κB (NF-κB) and activator protein 1 (AP-1) pathway, resulting in the production of pro-IL-1β and pro-IL-18 in lung tissue. The membrane damage and cell death caused by VILI has been shown to increase the levels of extracellular ATP, which could then interact with $P2X_7$ receptors to induce K^+ efflux [dos Santos, 2012]. The damage to mitochondria caused by VILI can also generate ROS [dos Santos, 2012]. Oligomerization of NLRP3, ASC and pro-caspase 1 in the lung tissue can be activated by K^+ efflux and ROS caused by VILI, leading to the caspase 1 cascade, resulting in the production and excretion of IL-18 and IL-1β, and subsequently ending in the lung inflammation that is present in VILI.

Pulmonary fibrosis

Pulmonary fibrosis is a deadly disorder that results in collection of extracellular matrix (ECM) in interstitial tissue and basement membranes [dos Santos *et al*. 2012]. Pulmonary fibrosis can lead to respiratory failure due to damage to the lung structure and reduced gas exchange [dos Santos *et al*. 2012]. Inflammation is known to have an important role in fibrogenesis or development of fibrosis. It is thought that a dysregulated wound repair response by the pulmonary epithelial cells or damage to the pulmonary epithelial cells causes fibrosis [Wynn, 2011]. This results in over release of inflammatory mediators such as IL-1β that cause excessive fibroblast proliferation and ECM accumulation.

There is evidence that the inflammasome is involved in the development of fibrosis, since asbestos and silica, the inorganic particulates associated with the development of pulmonary fibrosis, are also known to permeate lysosomal membranes, a phenomenon that can activate the NLRP3 inflammasome and subsequent IL-1β production. IL-1β is known to advance the production of a major profibrotic cytokine called transforming growth factor β (TGFβ). Increased levels of TGFβ can activate epithelial cell and fibroblast proliferation and conversion into myofibroblasts [Liu, 2008]. This results in increased collagen production, a critical step in fibrogenesis. CXC chemokines are another product of IL-1β that attract neutrophils to the epithelium, where they can cause further damage and initiate fibrosis [Mortaz *et al*. 2011]. Thus, it is evident that the pathway which leads to inflammation and development of fibrosis is associated with IL-1β, which is contingent on inflammasome assembly that is initiated by the same agents believed to cause pulmonary fibrosis.

Most interestingly, antifibrotic growth factors and other molecules that break down ECM are inhibited by a product of IL-1β and TGFβ known as plasminogen activator inhibitor 1 (PAI-1) [Zmijewski *et al*. 2011]. Bronchoalveolar lavage fluid (BALF) from patients with idiopathic pulmonary fibrosis (IPF) showed decreased fibrinolytic activity associated with an increase expression of PAI-1. The deletion/insertion polymorphism (4G/5G) in the PAI-1 gene promoter has been associated with increased PAI-1. *In vitro* experimentation showed that PAI-1 RNA levels were increased sixfold when the 4G allele was exposed to IL-1β compared with the 5G allele. These findings indicate that people that are 4G homozygous are more susceptible to developing pulmonary fibrosis, especially in the presence of IL-1β, which is known to be the primary cytokine product of the inflammasome.

There has been growing concern over the role of epithelial–mesenchymal transition (EMT) in pulmonary fibrosis. Many studies present conflicting evidence for EMT in fibrogenesis, which may cast doubt on the role of the inflammasome in pulmonary fibrosis. One study showed an incomplete expression of EMT markers in human IPF lung

tissue through immunohistochemistry (IHC) staining [Morbini *et al.* 2011]. These findings were supported by another study that used dual IHC staining for epithelial and mesenchymal markers on mouse and human IPF tissue [Yamada *et al.* 2008]. A study examining normal and fibrotic mouse and human lungs showed that there was no evidence at the cellular level for EMT [Rock *et al.* 2011]. Despite evidence mounting against EMT in pulmonary fibrosis, there are still studies that point to the critical role of EMT in fibroblast accumulation in IPF [Yang *et al.* 2013; Zhu *et al.* 2013].

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is a pulmonary disorder that burdens millions of people worldwide and costs the global economy billions of dollars in treatment. COPD is characterized by the symptoms of emphysema and chronic bronchitis [Rabe *et al.* 2007]. Chronic cigarette smoke exposure is the leading cause of COPD. With inflammation being a critical part of COPD, it is speculated that the inflammasome may take part in its pathogenesis.

One study showed that exposure of cigarette smoke and airways with COPD had increased levels of ROS [Domej *et al.* 2006]. As mentioned earlier, it is believed that ROS may be able to activate the NLRP3 inflammasome leading to the inflammation seen in COPD, but no further studies solidify this statement. Another study on mice showed that TLR4, a PRR, is critical to the inflammatory response in COPD [Muller *et al.* 2011]. Mice with genetic alterations for TLR4 dysfunction did not show the inflammation that was found in the wild type mice. However, stimulus of TLR4 is not thought to activate the inflammasome response, so this evidence regarding the role of the inflammasome in COPD is debatable.

Nevertheless, further studies show that the $P2X_7$ receptor, one that is believed to play a role in the activation of the inflammasome, also plays a role in inflammation found in mouse lungs exposed to cigarette smoke [Muller *et al.* 2011]. In conjunction with this evidence, other evidence shows increased levels of ATP in COPD and smoke-exposure models *in vivo* [Mohsenin and Blackburn, 2006]. In addition, an increased level of ATP has been found in the lungs of patients with COPD, and is linked to inflammation [Cicko *et al.* 2010]. Correlation of $P2X_7$ receptor activity and increased ATP found in COPD may point to the role of the inflammasome in the inflammation in COPD.

Caspase 1, a key part of the inflammasome and its proinflammatory function, has been studied in regard to COPD. Caspase 1 activity has been found to increase in lung tissue exposed to cigarette smoke, as well as in human lung tissue in patients with COPD [Muller *et al.* 2011]. Caspase inhibitors have been shown to block lung inflammation in mouse model [Churg *et al.* 2009]. It is also proposed that cytokines produced by inflammasome activation are linked to the inflammation in COPD. Increased levels of IL-18 and IL-1β, which are products of inflammasome activities, have been detected in the lungs of mice exposed to cigarette smoke and in human patients with COPD [Churg *et al.* 2009].

Collectively, a good case can be made for the role of the inflammasome in COPD. The priming signal for the activation of the NLRP3 inflammasome in COPD could be the TLR's recognition of inhaled DAMPs, caused by cigarette smoke exposure in the bronchial tubes. This signal could activate NF-κB. NF-κB and AP-1 could then promote the transcription of pro-IL-1β and pro-IL-18 in the bronchial tissue. Now that NLRP3 has been primed, it can be activated. The cigarette smoke exposure in the bronchial tubes may induce increased levels of extracellular ATP in the bronchial tissue. The extracellular ATP can activate $P2X_7$ receptors, which results in K^+ efflux and the introduction of ROS. The presence of ROS is detected by thioredoxin and TXNIP and could cause disjunction of the complex. TXNIP can then bind to NLRP3 and cause the oligomerization of NLRP3, ASC and pro-caspase 1, resulting in the full NLRP3 inflammasome in the cells of bronchial tissue. The NLRP3 inflammasome can then activate the caspase 1 cascade, which serves to cleave pro-caspase 1 into active caspase 1, which subsequently cleaves pro-IL-18 and pro-IL-1β into their mature forms. IL-18 and IL-1β can then be excreted from the cells of the bronchial tissue, where they carry out their proinflammatory functions in the bronchial tubes which is evident in COPD.

Asthma

Asthma is a very common pulmonary disease, especially in children. Patients with asthma

experience chronic inflammation of the airways, and when allergens are inhaled, the muscles of the airways tighten, causing difficulty with breathing and mucus build up [Godfrey, 1985]. The NLRP3 inflammasome is suspected to play a part in the inflammation in patients with asthma.

ROS and inhaled pathogens are linked to asthmatic inflammation but their connection with the inflammasome is yet to be elucidated [Nadeem *et al*. 2008]. However, there is evidence that extracellular ATP may be responsible for inflammasome activation in asthma. Dust mites, a common asthmatic allergen, were observed to prompt the release of ATP from macrophages, epithelial and dendritic cells [Suzuki *et al*.]. More interestingly, increases in ATP were measured in patients with asthma as a result of an allergen [Idzko *et al*. 2007]. Coupled with this, $P2X_7$ receptors have been shown to be involved in several asthma models, and their expression is increased in these models and in human samples [Muller *et al*. 2011].

Elevated caspase 1 activity has also been demonstrated in ovalbumin and aluminum oxide mouse models [Muller *et al*. 2011]. Along with this, a decrease in airway inflammation has been detected in mouse asthma models after caspase inhibitors were introduced. IL-1β activity is also present in asthma models and may be related to the increased caspase activity. Patients with asthma were shown to have increased levels of IL-1β in their sputum compared with patients without asthma [Thomas and Chhabra, 2003]. Symptomatic patients with asthma were also shown to have higher IL-1β levels than asymptomatic patients with asthma [Konno *et al*. 1996]. The introduction of recombinant adenovirus expression human IL-1 receptor antagonist to a mouse model prior to allergen challenge showed a dramatic decrease in airway hypersensitivity response, attachment of neutrophils and eosinophils, as well as reduced peribronchial inflammation [Wang *et al*. 2006].

The culmination of evidence regarding ROS, ATP, $P2X_7$, caspase 1 and IL-1β in human and mouse asthmatic models provide a strong argument for the role of the inflammasome in asthma. DAMPs or PAMPs associated with asthma could serve to complete the priming step by activating TLRs, followed by the transcription of pro-IL-18 and pro-IL-1β through the NF-κB and AP-1 pathway. Then, the inhalation of allergens such as dust mites and aluminum oxide crystals can induce higher levels of extracellular ATP in bronchial tissue, which could lead to the activation of $P2X_7$ receptors, resulting in K^+ efflux and the addition of ROS. ROS could then lead to NLRP3 inflammasome assembly, which could initiate the caspase 1 cascade and result in the excretion of mature IL-18 and IL-1β and achieve the airway inflammation commonly seen in asthma. Additionally, other allergens associated with asthma such as silica and asbestos can activate NLRP3 inflammasome assembly. As mentioned previously, it is hypothesized that the introduction of such allergens through lysosomal rupturing in the cytosol is sensed by NLRP3 and activates the NLRP3 inflammasome by an unknown mechanism.

Other diseases

As previously stated, IAV is a known activator of the NLRP3 inflammasome and is also a foundation for lung infection. The mode of IAV-induced NLRP3 activity is not fully understood yet. Some studies submit that inflammasome production and activation is elicited by the M2 ion channel of IAV [Ichinohe *et al*. 2010], which is responsible for the entry of virus into the cell and production of virions. Increased ATP has been observed in the BALF of mice infected with IAV, which points to IAV-induced secretion of ATP [Aeffner *et al*. 2011]. ATP has also been seen to be released from dying cells infected with IAV [Aeffner *et al*. 2011]. These high levels of released ATP may interact with $P2X_7$ receptors of macrophages and cause inflammasome activation.

Tuberculosis is a bacterial infection spread through the air and primarily affects the lungs, although it can spread to other organs. Active TB results in symptoms caused by inflammation, and may result in abscesses in the lungs and possibly bronchopleural fistulas. The bacteria that causes TB, *Mycobacterium tuberculosis* (MTB), are found in host macrophages in phagosome-like vesicles, and suppress inflammasome activity [Master *et al*. 2008]. However, it can also activate the inflammasome. The mechanism by which MTB causes inflammasome activity is not yet fully known, but it is believed to involve the export of early secreted antigenic target, 6 kDa (ESAT-6), a group of proteins secreted by MTB, through a functional protein secretion system ESX-1 [Mishra *et al*. 2010].

Community-acquired pneumonia (CAP) is a common lung infection particular to people who

have not been in a hospital or other healthcare institution. The infection can result from a virus, bacterium or fungus. CAP is characterized by the inflammation that results from the damage of the pathogen and the immune system's detection of the infection. The bacteria that cause CAP can also activate the NLRP3 inflammasome. *Streptococcus pneumoniae* produces pore-forming toxins like cytolysin pneumolysin which can disturb the plasma membrane and cause K^+ efflux, a potential activator of the NLRP3 inflammasome [Witzenrath *et al*. 2011].

Conclusion

The inflammasome has the potential to be the key that unlocks the pathogenesis of pulmonary diseases characterized by inflammation and expedite the development of more effective therapeutic treatments. Although the mechanism of inflammasome assembly and activation is not well established, there is overwhelming evidence in support of its role in multiple pulmonary disorders. The lung is subjected to a wide range of injuries and insults which are detected by PRRs. The recognition of these DAMPs and PAMPs is crucial to innate host defense and the pathology of lung diseases. The inflammasome is now believed to be the link between innate immune response and lung disease pathology. The recognition of molecular patterns has been shown to produce pro-IL-18, pro-IL-1β and induce upregulation of inflammasome components. The inflammasome is activated by several mechanisms that are related to lung injury and disease. Activation results in caspase 1 cleaving pro-form ILs into their active forms, which are critical to acute inflammation in the lungs. Although our knowledge of the inflammasome is growing, further study needs to be undertaken to fully understand how the inflammasome assembles and activates, as well as its roles in inflammation and associated diseases.

Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

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