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Sensing damage by the NLRP3 inflammasome

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Summary

The NLRP3 inflammasome is activated in response to a variety of signals that are indicative of damage to the host including tissue damage, metabolic stress, and infection. Upon activation, the NLRP3 inflammasome serves as a platform for activation of the cysteine protease caspase-1, which leads to the processing and secretion of the proinflammatory cytokines interleukin-1 β (IL-1 β) and IL-18. Dysregulated NLRP3 inflammasome activation is associated with both heritable and acquired inflammatory diseases. Here we review new insights into the mechanism of NLRP3 inflammasome activation and its role in disease pathogenesis.

Keywords

NLRP3; inflammasome; caspase-1; sterile inflammation

The NLR family

The immune system is capable of detecting a wide range of insults against the host including tissue damage, metabolic stress, and infections. The innate immune system possesses multiple families of germline encoded pattern recognition receptors (PRRs) through which it can detect such insults (1). These receptors recognize conserved moieties associated with either cellular damage [danger-associated molecular patterns (DAMPs)] or invading organisms [pathogen-associated molecular patterns (PAMPs)]. Activation of these receptors induces immune responses that are critical for host defense and tissue repair programs. However, when there is overwhelming tissue damage or infection, the ensuing inflammatory response can be detrimental to the host. Similarly, chronic stimulation of these pathways is also injurious and responsible for the pathology seen in a number of autoinflammatory and autoimmune disorders, such as arthritis and diabetes.

The nucleotide-binding domain leucine-rich repeat (LRR)-containing receptors (NLRs) are 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 nucleotide-binding and oligomerization domain called the NACHT domain that is located between an N-terminal effector domain and a C-terminal LRR domain (2, 3). The human NLR family consists of 22 members that can be subgrouped based on their N-terminal domain which

include caspase-recruitment domain (CARD), pyrin domain (PYD), and baculovirus IAP repeat domain (BIR).

Upon activation, the NLR family members NLRP1, NLRP3, and NLRC4 are capable of forming multiprotein complexes called inflammasomes. In addition, the cytosolic HIN200 family member AIM2 (absent in melanoma 2) is also capable of forming an inflammasome complex. The inflammasome serves as a platform for activation of the cysteine protease caspase-1. Active caspase-1 cleaves the pro-forms of the cytokines IL-1 β and IL-18 to their active and secreted forms. Caspase-1 may also possess additional functions including regulation of glycolysis pathways (4) and unconventional protein secretion (5); however, *in vivo* studies to clearly demonstrate a role for caspase-1 in these processes are still lacking.

The murine *Nlrp1b* gene has been shown to regulate macrophage cell death in response to anthrax lethal toxin (6). In addition, sequence variants in the human *NLRP1* gene have been linked to vitiligo-associated autoimmune and autoinflammatory diseases (7). The NLRC4 inflammasome is activated by Gram-negative bacteria that possess either a type III or type IV secretion system (8–10). NLRC4 in conjunction with another cytosolic NLR, Naip5, can also recognize the presence of cytosolic flagellin (11–13). The AIM2 inflammasome also plays a crucial role in host defense by recognizing the release of dsDNA into the cytosol by a number of bacterial and viral pathogens (14–17).

The NLRP3 inflammasome has been associated with a wide range of diseases including infectious, autoinflammatory, and autoimmune disorders. Bacterial, fungal, viral, and protozoan parasitic pathogens have all been demonstrated to activate the NLRP3 inflammasome (reviewed in 18, 19). However, it is unlikely that NLRP3 is detecting the presence of cytosolic PAMPs but is more likely responding to the cellular stress induced by the infectious agents. In this review we will examine the mechanism by which the NLRP3 inflammasome is activated and its role in sterile inflammatory diseases (Fig. 1).

The NLRP3 inflammasome

Insights into the function of NLRP3 first came through studies that identified mutations within *NLRP3* (also known as NALP3 or cryopyrin) as being responsible for the autoinflammatory Muckle-Wells syndrome. Mutations in *NLRP3* are also responsible for the autoinflammatory syndromes familial cold autoinflammatory syndrome and neonatal-onset multisystem inflammatory disease, which together with Muckle-Wells syndrome are known collectively as cryopyrin-associated periodic syndrome (CAPS) (20–22). These *NLRP3* mutations, over 40 of which have been identified to date, result in a constitutively active form of NLRP3 that leads to increased inflammasome activation with a resultant increase in secretion of IL-1 β (23). Inhibition of this pathway with the recombinant human IL-1 receptor antagonist (IL-1Ra) anakinra has proven to be highly successful in abrogating disease severity in CAPS (24, 25).

Activation of NLRP3 results in the assembly of the NLRP3 inflammasome composed of NLRP3, the adapter molecule ASC and caspase-1 (26, 27). This association and resultant activation of the inflammasome lead to the activation of caspase-1, leading to processing of pro-IL-1 β and pro-IL-18 to their mature and secreted forms. The activators of NLRP3 are quite varied and include environmental irritants, endogenous danger signals, pathogens, and distinct PAMPs. Given this diversity in agonists, it is likely that there is a point of convergence resulting in the release of an endogenous molecule into the cytosolic space that is directly sensed by NLRP3. Latz and colleagues (28) demonstrated that lysosomal disruption might be one such common pathway. Phagocytosis of particulate activators of the NLRP3 inflammasome such as silica, asbestos, alum, and uric acid crystals leads to lysosomal rupture with the release of proteolytic lysosomal contents into the cytosol. In

support of this model, inhibition of lysosomal acidification or use of the cathepsin B inhibitor CA-074-Me results in inhibition of inflammasome activation (28). However, macrophages from cathepsin B-deficient mice do not have a marked defect in inflammasome activation, suggesting that CA-074-Me may be affecting other pathways (29). In addition, lysosomal rupture is not required for NLRP3 inflammasome activation in response to non-particulate activators such as ATP and nigericin, suggesting that lysosomal rupture results in yet another intracellular event prior to NLRP3 inflammasome activation (28).

Detection of mitochondrial dysfunction by the NLRP3 inflammasome

Two common events that are required for all described activators of the NLRP3 inflammasome are a potassium efflux and the generation of reactive oxygen species (ROS). Inhibition or scavenging of ROS blocks NLRP3 inflammasome activation in response to a wide variety of agonists (30–33). It was initially postulated that the source of ROS was from the phagosome-associated NADPH oxidase (33). However a number of subsequent studies utilizing macrophages deficient in components of the NADPH oxidase system demonstrated that the NADPH oxidase was dispensable for NLRP3 inflammasome activation (28, 34), suggesting that the source of the ROS may be of mitochondrial origin.

Two recent studies have provided evidence that mitochondria are the principal source of ROS required for inflammasome activation (35, 36) (Fig. 2). Inhibition of mitochondrial Complex I or Complex III with rotenone or antimycin A, respectively, results in loss of mitochondrial membrane potential and the generation of ROS. Both rotenone and antimycin A were capable of activating the NLRP3 inflammasome. Additionally, treatment of macrophages with Mito-TEMPO, a scavenger of mitochondrial ROS, resulted in inhibition of NLRP3 inflammasome activation (36). Some caution must be taken when interpreting these studies, as the inhibitors used may potentially have off-target effects.

To avoid cellular damage mediated by ROS-generating mitochondria, these mitochondria are eliminated through a specialized form of autophagy known as mitophagy. Pharmacologic or genetic inhibition of autophagy/mitophagy results in the accumulation of ROS-producing mitochondria and subsequently increased NLRP3 inflammasome activation (35, 36). The role of mitochondria in NLRP3 inflammasome activation is further supported by the finding that cyclosporine A, a potent inhibitor of mitochondrial permeability transition (MPT), inhibits the release of IL-1 β in response to the NLRP3 agonist ATP (36). Similarly, short hairpin RNA (shRNA) knockdown of the MPT- mediating protein voltage-dependent anion channel (VDAC) or overexpression of the VDAC closing protein Bcl-2 results in a marked inhibition of NLRP3 inflammasome activity (35). Interestingly, upon activation of the inflammasome, both NLRP3 and ASC relocate to the mitochondria and mitochondria-associated ER membranes (35). This positions the NLRP3 inflammasome in an ideal place to receive signaling cues from the mitochondria.

A second event required for NLRP3 inflammasome activation is a decrease in cytoplasmic potassium concentrations. Inhibition of the potassium efflux *in vitro* by increasing extracellular potassium concentrations results in the abrogation of NLRP3 inflammasome activation (32, 33, 37). The exact role of the potassium efflux is unclear; however, it is tempting to speculate that a low potassium environment is required for subsequent signaling events mediated by the mitochondria.

These studies suggest that the NLRP3 inflammasome ultimately senses mitochondrial dysfunction and initiates inflammatory responses following specific forms of cellular stress. It remains unclear what the ligand for NLRP3 is, but one possibility is this ligand is released from the ROS-generating damaged mitochondria. There is additional appeal to this model,

as it parallels the mechanism by which the structurally related apoptosome is activated, wherein elevated ROS production facilitates the release of cytochrome c from the inner mitochondrial membrane into the cytosol where it activates Apaf-1 (38). Increased ROS levels have been shown to cause the interaction of NLRP3 with thioredoxin-interacting protein (TXNIP) (39), although it remains unclear if TXNIP is involved in NLRP3 inflammasome activation (40). In a separate study, Nakahira and colleagues (36) show that mitochondrial DNA (mtDNA) is released into the cytosol following stimulation with NLRP3 agonists such as LPS and ATP and that this mtDNA release is required for subsequent IL-1 β secretion. Intriguingly, in this model NLRP3 also appears to play a role upstream of MPT and mtDNA release. Further studies are required to determine which if any of these models represent the true mechanism by which the NLRP3 inflammasome is activated.

Ischemia-reperfusion injury

Ischemia-reperfusion injury (IRI) refers to tissue or organ damage, which occurs when blood flow is restored after a period of ischemia. IRI is a common and significant clinical problem in a wide range of organs that is encountered in many clinical conditions such as trauma/shock, stroke, transplantation vascular and cardiac surgery, and treatment of vascular occlusions. During IRI, a multifaceted complex cascade of events is induced that ultimately leads to necrotic and apoptotic cell death and organ dysfunction. With loss of blood flow, parenchymal cells are injured as a result of ATP depletion, microvessels begin to become dysfunctional and a subsequent inflammatory response is induced that increases local damage. The restoration of blood flow causes further damage to the ischemic tissue through neutrophil infiltration, ROS accumulation, dysregulation of cellular ion homeostasis, and inflammatory responses to necrotic cell death. Beyond local damage, IRI can also induce deleterious remote and systemic effects resulting in the development of systemic inflammatory response and multiple organ dysfunction syndrome.

Despite the substantial incidence of and the profound mortality and morbidity associated with IRI, no specific therapy exists for the prevention or treatment of IRI. Therefore, knowledge of the primary mechanisms involved in the development and regulation of IRI is essential for the development of therapeutic strategies. It is established that inflammation plays a pivotal role in the pathogenesis of IRI and a growing body of evidence demonstrates in particular the importance of innate immunity and pattern recognition receptors (41–43). In the following section, we provide an overview of recent data implicating the NLRP3 inflammasome in IRI, supporting the possible strategy to inhibit NLRP3 inflammasome activation in order to improve clinical outcomes following IRI (Fig. 3).

Renal IRI

Renal IRI is a leading cause of acute kidney injury in both transplanted and native kidneys. This disorder is occurring with increasing incidence and lacks effective therapeutic interventions, resulting in substantial cost to society (44). Ischemic acute renal injury causes localized massive infiltration of inflammatory cells accompanied by fulminant tubular necrosis and apoptosis resulting in significant renal dysfunction. 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 (45–49). In our initial description of the involvement of NLRP3 in ischemic disease (45), we found that acute necrotic cell death due to renal IRI triggers an inflammatory response through the NLRP3 inflammasome and results in collateral tissue damage. *In vitro* data suggests that NLRP3 activation was triggered through ATP produced by mitochondria released from damaged cells (45). Whether this mechanism is also taking place during renal IRI *in vivo*

warrants further investigation. We also found NLRP3 deficiency protected mice from lethal renal ischemic injury with 86% of NLRP3-deficient mice surviving lethal renal IRI challenge in comparison to less than 10% of wildtype (WT) mice. In addition, deficiency in NLRP3 or ASC was associated with reduced neutrophilic infiltration and IL-1 β in the kidney in comparison to WT mice and resulted in marked conservation of renal function. In concordance with our results, Shigeoka *et al.* (50) also found profound functional protection of NLRP3-deficient mice against renal IRI. By transplanting NLRP3-deficient bone marrow into irradiated WT hosts, the investigators further showed the absence of NLRP3 solely in hematopoietic cells was not sufficient to protect against loss of renal function following renal IRI (50). The extent to which parenchyma-associated NLRP3 might contribute to renal IRI remains undetermined. Both studies also showed that the absence of NLRP3 protected kidneys against renal IRI to a greater extent than the absence of ASC, suggesting NLRP3 may play an additional role in renal IRI independently of ASC and caspase-1 (45, 50).

Myocardial IRI

Myocardial IRI is predominantly associated with myocardial infarction but also occurs during surgical interventions such as cardiac transplantation and coronary artery bypass grafting. Myocardial IRI induces cardiomyocyte damage and a subsequent profound inflammatory response. Recently, ASC has been implicated in the pathogenesis of myocardial IRI. Kawaguchi *et al.* (51) demonstrated different levels of ASC expression in infiltrating inflammatory cells, cardiomyocytes, and (fibroblast-like) interstitial cells in cardiac tissue from patients who have died after an acute myocardial infarction and in ischemic myocardium from mice in which the left anterior descending artery was occluded. Of particular interest was the observation that ASC or caspase-1 deficiency diminished myocardial inflammation, subsequent infarct development, myocardial fibrosis and dysfunction in mice during cardiac IRI. Using bone marrow chimeric mice, they showed the effect of ASC deficiency in protecting the heart from IRI was mediated by both bone marrow-derived cells and myocardial resident cells (51). *In vitro* studies further revealed that cardiac fibroblasts, not cardiomyocytes, induce inflammasome activation during hypoxia/reoxygenation. Thus, similarly to findings for renal IRI models, it appears that inflammasome function in both hematopoietic and stromal cells may drive inflammation following cardiac ischemic events.

Previous studies have also reported that caspase-1 and IL-1 β play a pivotal role in augmenting myocardial IRI (52, 53). Overexpression of IL-1Ra by gene transfection in an *in vivo* rat model ameliorated myocardial IRI injury by reducing the inflammatory response and apoptosis of cardiomyocytes (54). Experimental studies in mice and rats also showed that anakinra, a recombinant IL-1R antagonist, is able to improve cardiac remodelling after acute myocardial infarction by inhibiting cardiomyocyte apoptosis (55, 56). Interestingly, in a small pilot study in human patients with acute myocardial infarction, anakinra was found not only to be safe but also demonstrated benefit in left ventricular remodelling (57).

Hepatic IRI

Recent work has suggested that NLRP3 is also critical in hepatic IRI, seen following liver transplantation and certain hepatic surgical procedures. Zhu *et al.* (58) found increased levels of NLRP3 protein and ROS in hepatic nonparenchymal cells and enhanced plasma levels of IL-1 β after segmental (70%) warm liver IRI. Interestingly, gene silencing of NLRP3 with shRNA significantly decreased the severity of inflammation and subsequent hepatocellular injury after IRI. This protection was associated with a reduction in expression of cleaved caspase-1, IL-1 β , IL-18, TNF- α , IL-6, and HMGB1 (58). Previous work has also implicated IL-1 β and IL-18 in the induction of inflammation (59, 60) and injury (60) after hepatic IRI, and targeting of these cytokines was protective in liver IRI (60–62). These

results suggested that, next to renal and myocardial IRI, NLRP3 activation is also critical in mediating liver IR damage.

Atherosclerosis

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. Duewell *et al.* (63) showed that the crystalline form of cholesterol, frequently detected in atherosclerotic lesions, activates the NLRP3 inflammasome and induces caspase-1 dependent IL-1 β maturation in murine macrophages *in vitro*. Rajamäki *et al.* (64) obtained similar results when human macrophages were exposed to cholesterol crystals. In line with this observation, injection of cholesterol crystals into the peritoneal cavity of mice induced NLRP3-dependent IL-1 β production and peritoneal polymorphonuclear (PMN) influx (63). Further analysis revealed that NLRP3 and ASC in hematopoietic cells contributed to atherosclerosis lesions using atherosclerotic-prone low-density lipoprotein receptor-deficient mice fed a high-cholesterol diet. A role for NLRP3 inflammasome activation in parenchymal cells during atherogenesis was not explored in this study. It is important to note that a recent study by Menu *et al.* (65) did not find a role for the NLRP3 inflammasome in atherosclerosis in an *in vivo* ApoE mouse model, and further studies will be required to determine the reason for the discrepancy between these two mouse models.

Despite the wealth of information regarding the role of inflammation in atherosclerosis, little had been known about the molecules that elicit inflammation in atherosclerotic lesions. These studies provide the first evidence that cholesterol crystals via the Nlrp3 inflammasome are important inflammatory stimuli in atherosclerosis development and reveal potentially new therapeutic strategies to manage atherosclerosis and cardiovascular disease.

Obesity and diabetes

It has become clear over the past few years that IL-1 β and IL-18 play a crucial role in the pathogenesis of diabetes (66–69). Although it is known that increased levels of IL-1 β are associated with an increased risk for developing diabetes, the mechanism behind this increase was unclear until recently. Zhou *et al.* (39) were the first to report a role for NLRP3 in the glucose-mediated release of IL-1 β from islet cells in the pancreas and identified the ROS-TXNIP axis as a possible signalling pathway involved in NLRP3 inflammasome activation. They found that murine islet preparations, consisting of β cells and resident leukocytes, expressed NLRP3, ASC, and caspase-1 and released IL-1 β at a low level. Upon glucose treatment, islets start to produce IL-1 β at much higher levels, a response that was severely reduced in the absence of NLRP3 and TXNIP. Similar to these findings, Koenen *et al.* (70) reported that glucose-induced activation of TXNIP induced IL-1 β transcription in adipose tissue as well. The study of Zhou *et al.* (39) furthermore showed that mice deficient in NLRP3 were more glucose tolerant and had improved insulin sensitivity. An endogenous trigger that could activate the NLRP3 inflammasome and drive increased IL-1 β in type 2 diabetes came to light through the study of Masters *et al.* (40). They showed that soluble oligomers of human islet amyloid polypeptide (IAPP), a protein that is deposited in the pancreas in type 2 diabetes, can be phagocytosed by murine bone marrow-derived dendritic cells *in vitro* and activate the inflammasome to produce active IL-1 β in a caspase-1 and NLRP3-dependent fashion. In contrast to the above study (39), a role for TXNIP was not found in this study (40). Priming for IAPP-induced NLRP3 activation seemed to require sufficient glucose metabolism and was facilitated by minimally oxidized low density lipoprotein. To translate these *in vitro* findings to an *in vivo* model, mice transgenic for

human IAPP were placed on a high-fat diet for a year. Analysis of the pancreas of these mice revealed that IL-1 β was increased and localized together with amyloid and macrophages in the islets. It was not reported whether the amyloid deposits in these mice also led to progressive loss of β -cell function and diabetes, and this warrants further investigation. In addition, it is not yet clear whether amyloid deposits are a cause or effect of type 2 diabetes.

Two other recent papers elaborate on these findings and provide compelling evidence for a central role for the NLRP3 inflammasome and caspase-1 in the induction of obesity and insulin resistance (71, 72). Stienstra *et al.* (71) identified a new role for NLRP3 inflammasome-mediated caspase-1 in adipose tissue. They show that NLRP3 inflammasome-mediated caspase-1 activation can influence the differentiation of adipocytes and in this way contribute to insulin sensitivity associated with obesity. Inhibition of caspase-1 by pharmacological inhibitors or specific siRNA, or the absence of NLRP3 improved insulin sensitivity and differentiation of adipocytes. Further analysis revealed that the activity of caspase-1, IL-1 β and IL-18 was increased in white adipose tissue of obese mice. Stienstra *et al.* (71) also showed that caspase-1 deficiency profoundly improved insulin sensitivity in mice and also had a beneficial effect on adipose tissue *in vivo* as reflected by the presence of smaller adipocytes, a lower percentage of total fat mass, and signs of increased mitochondrial energy dissipation in white adipose tissue. To study whether inhibition of caspase-1 might have therapeutic possibilities in the treatment of diabetes type 2 during obesity, the investigators treated genetically obese mice with the caspase-1 inhibitor pralnacasan (71). These mice had a profound improvement in insulin sensitivity along with a diminished increase in body weight despite equivalent caloric intake.

Vandanmagsar *et al.* (72) identified an important role for the NLRP3 inflammasome in sensing obesity-associated DAMPs and regulating inflammation and insulin signalling in obesity. In this study, NLRP3 mRNA expression in visceral fat of mice fed a high fat diet correlated to body weight and IL-1 β levels. In line with this, calorie-restricted mice and obese individuals with type 2 diabetes who have lost weight had reduced IL-1 β and NLRP3 mRNA expression in adipose tissue. Reduced IL-1 β but not NLRP3 mRNA was also seen in a separate study of subcutaneous fat and liver of severely obese patients that underwent laparoscopic adjustable gastric banding (73). Similar to the findings by Stienstra *et al.* (71), Vandanmagsar *et al.* (72) reported that mice deficient in caspase-1 or NLRP3 had enhanced insulin sensitivity, reduced hepatic steatosis, and a reduced size of visceral adipocytes when fed a high fat diet. The authors next looked for the endogenous trigger stimulating NLRP3 inflammasome activation during obesity and found that both macrophages and adipose tissue explants sense ceramide and induce activation of caspase-1 in a NLRP3-dependent manner. The authors propose that the NLRP3 inflammasome plays a crucial role in obesity-related inflammation by inducing macrophage and subsequent T-cell activation in adipose tissue. Together, these studies may provide new strategies for the treatment or even prevention of diabetes and obesity in the clinic. In fact, blocking IL-1 β with anakinra has already been shown to improve glycemic control and reduce systemic inflammation in patients with type 2 diabetes (68).

Alzheimer's disease

In Alzheimer's disease, progressive dementia is associated with cerebral accumulation of amyloid β plaques as well as neuronal cell death. 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. Microglia, the predominant phagocyte in the central nervous system (CNS), have been shown to be activated in increased numbers in Alzheimer's disease patients and in particular in association with amyloid plaques (74, 75).

These plaques have been shown to serve as a pro-inflammatory stimulus by activating microglia and inducing cytokine expression following their phagocytosis (76). In addition, numerous studies have suggested a critical role for IL-1 β in the pathogenesis of Alzheimer's disease. Injection or overexpression of IL-1 β in rodent brains results in the activation of astrocytes and microglia as well as the recruitment of numerous other inflammatory cells into the CNS (77). The mechanism by which these amyloid fibrils trigger IL-1 β release was unclear until a study by Halle *et al.* (78) showed the link between the amyloid fibrils and IL-1 β release is activation of the NLRP3 inflammasome. In their study, Halle *et al.* (78) show release of IL-1 β by microglia is dependent upon their phagocytosis of amyloid fibrils and the NLRP3 dependent activation of caspase-1. Amyloid and structurally similar fibrillar molecules can also be found in deposits at various sites of inflammation. It is unclear if amyloid is simply a marker of chronic inflammation in other disease processes or if it can act as a danger signal that further drives subsequent inflammation in the amyloid effected tissue.

Gout and pseudogout

Gouty arthritis occurs with acute attacks associated with the abrupt onset of pain, swelling, and redness of the affected joint or extremity. It has long been known that these attacks are associated with the presence of monosodium urate (MSU) crystals within the joint (79). While gout is associated with hyperuricemia, it is the loss of solubility and assumption of the crystalline form of MSU that triggers the inflammation, not simply the degree of elevation of urate. *In vitro*, MSU triggers the death and release of lysosomal contents by neutrophils (80), while interaction of MSU with macrophages resulted in the release of proinflammatory cytokines including IL-1 β (81). A study by Jürg Tschopp's group (82) found this release of IL-1 β by macrophages was dependent upon the components of the NLRP3 inflammasome. This inflammatory response was not specific, however, to the chemistry of the MSU crystal, as calcium pyrophosphate dihydrate (CPPD) crystals, the inciting agent of pseudogout, also elicited IL-1 β release in an NLRP3-dependent fashion. Targeting IL-1 using the IL-1R antagonist anakinra has also proven effective in treating the symptoms associated with acute gouty arthritis in patients, further reinforcing the role of this inflammatory pathway in gout (83).

Environmental hazards and alum adjuvants

The above finding by Tschopp and colleagues (82), that two chemically divergent crystals were capable of activating NLRP3 immune responses, led to of the examination of other crystalline molecules, including the environmental pollutants asbestos and silica and the adjuvant alum. Similar to MSU and CPPD, following phagocytosis, silica and asbestos trigger IL-1 β release from macrophages in a NLRP3 inflammasome-dependent manner (28, 32, 33). In addition, the NLRP3 inflammasome has been linked to the development of pulmonary fibrosis: following inhalant exposure to silica, NLRP3-deficient animals had less pulmonary inflammation and collagen deposition than that found in WT mice (32). Inhalational exposure of asbestos in NLRP3-deficient mice also resulted in a diminished inflammatory response compared to WT animals (33). Thus, it may be that NLRP3 plays a role in the fibrotic diseases silicosis and asbestosis.

Aluminum salts (alum) have long been used in vaccines as an adjuvant to trigger a sustained immunologic response, although the mechanism of that response had been unknown. A critical study by Fabio Re's group (84) tied the immunostimulatory function of alum to the release of IL-1 β . Subsequently, this group and others (28, 37, 85–87) reported that this release of IL-1 β was due to activation of the NLRP3 inflammasome. Intriguingly, two of these studies (37, 86) found a critical requirement for the NLRP3 inflammasome in the

adjuvant effect of alum in certain IgG responses, and one study (87) showed a defect in IgE responses in NLRP3-deficient animals. Other studies however have reported the induction of antibody response is intact in mice deficient in components of the NLRP3 inflammasome (85, 88), leaving the exact role for NLRP3 in this adaptive immune response unclear.

Targeting the NLRP3 inflammasome

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-1 β blockade. Three agents targeting IL-1 β 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-1 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 (89, 90). Anakinra was however highly efficacious as a therapy in gout, as it significantly lowered acute and chronic gout symptoms in a small group of patients (n=10) (83). Consistently, rilonacept (IL-1 Trap), a glycoprotein that neutralizes IL-1 α and IL-1 β yielded clinical improvements in patients with chronic active gouty arthritis (91). In a small, randomized clinical trial in 70 patients with type 2 diabetes, anakinra was shown to improve glycemia and β -cell secretory function and to reduce markers of systemic inflammation (68). A follow-up study showed that part of this beneficial effect was sustained for 39 weeks following treatment withdrawal (92). Clinical trials of IL-1 β blockade also show efficacy for symptomatic treatment of the genetic disorders of the inflammasome-IL-1 system that cause autoinflammatory diseases such as cryopyrin-associated periodic syndromes, familial Mediterranean fever, and PAPA syndrome, as reviewed by others (93–95). Reducing IL-1 activity might also be a therapeutic option for treating human cancer and metastatic disease, as reviewed by Dinarello (96). However, one must bear in mind when treating cancer patients with IL-1 β blockers that inflammation has both tumor-suppressive and tumor-promoting functions (97).

An alternative to blocking the NLRP3 inflammasome pathway through interference with IL-1 β 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. Although no significant adverse events associated with liver toxicity were observed in patients participating in these studies, the use of pralnacasan led to liver abnormalities in animal toxicological studies, and the rheumatoid arthritis clinical trial was therefore discontinued (98). The results of the clinical trial for the treatment of psoriasis with VX-765 have as yet not been reported (98).

Other options for targeting the NLRP3 inflammasome pathway could be the use of NLRP3 or ASC antagonists. Such agents might be good alternatives for IL-1 β blockers, as they may be closer to the initiation phase of the pathological disorder. Specific NLRP3 inflammasome inhibitors are not yet available but are currently being developed for the treatment of rheumatoid arthritis, gout, and asthma.

One concern that should be taken into account when using agents that modulate the NLRP3 inflammasome pathway in the clinic is that this modulation can interfere with immune surveillance and tissue repair mechanisms. Thus, targeting upstream mechanisms of NLRP3 activation (e.g. DAMPs) may be an alternative therapeutic approach for the treatment of sterile inflammatory diseases, as it leaves host defence responses intact. Maintaining the balance between host defense, tissue repair, and prevention of deleterious NLRP3

inflammasome-mediated inflammation in sterile inflammatory disease continues to be a challenge in future medical care and research.

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Fig. 1.
Pathophysiological role of the NLRP3 inflammasome in sterile inflammatory diseases.

Fig. 2. Mitochondrial dysfunction triggers NLRP3 inflammasome activation

Cellular stress or damage induced by DAMPs and PAMPs in the setting of a low intracellular potassium concentrations results in mitochondrial dysfunction and the generation of ROS. An unknown intermediate, likely of mitochondrial origin, triggers the activation of the NLRP3 inflammasome. Clearance of ROS-producing mitochondria by mitophagy prevents further NLRP3 inflammasome activation.

Fig. 3. Role of the Nlrp3 inflammasome in the cycle of organ damage and dysfunction induced by ischemia reperfusion

Ischemia reperfusion injury results in tissue necrosis with the subsequent release of DAMPs into the extracellular space. These DAMPs induce the priming and activation of the NLRP3 inflammasome in both leukocytes and parenchymal cells resulting in inflammation and possible organ damage.