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Control of innate and adaptive immunity by the inflammasome

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Abstract

The importance of innate immunity lies not only in directly confronting pathogenic and non-pathogenic insults but also in instructing the development of an efficient adaptive immune response. The Nlrp3 inflammasome provides a platform for the activation of caspase-1 with the subsequent processing and secretion of IL-1 family members. Given the importance of IL-1 in a variety of inflammatory diseases, understanding the role of Nlrp3 inflammasome in the initiation of innate and adaptive immune responses cannot be overstated. This review examines recent advances in inflammasome biology with an emphasis on its roles in sterile inflammation and triggering of adaptive immune responses.

Keywords

inflammasome; innate immunity; adaptive immunity

1. Introduction

The ability of the innate immune system to respond to a variety of stimuli is central to its function in pathogen clearance and tissue repair. Upon activation, cells of the innate immune system upregulate inflammatory mediators and induce increased expression of costimulatory and adhesion molecules which can in turn drive the activation of the adaptive arm of the immune system. Traditionally, the role of the innate immune system was thought to be to recognize harmful conditions such as the presence of microbes through its ability to distinguish pathogens as “non-self”. However, this paradigm cannot account for the activation that follows sterile insults wherein the inflammatory response is triggered in the absence of invading pathogens. It is now known the ability of cells of the innate immune system to execute their inflammatory and tissue repair programs is dependent upon germ-

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line encoded pattern recognition receptors (PRR) that identify molecular structures associated with cellular stress and death, known as damage associated molecular patterns (DAMPs), as well as conserved pathogen derived structures, or pathogen associated molecular patterns (PAMPs) [1, 2].

DAMPs can be cytosolic or nuclear components, such as the chromatin-associated protein high mobility box group 1, HMGB1, or the chaperone heat shock protein 60 (Hsp60), which under homeostatic conditions do not come into contact with PRRs. Like these intracellular DAMPs, some components of the extracellular matrix like hyaluronan and heparan sulfate can also serve as DAMPs [3]. A number of DAMPs, such as amyloid peptides and uric acid, have been implicated in the generation of a range of inflammatory diseases, including Type 2 diabetes, Alzheimer's disease and gout [4-6]. The inflammatory response can cause bystander host tissue damage and has a high metabolic cost, thus multiple regulatory mechanisms control the extent, duration and the type of response. To prevent undesirable responses, interactions of these intracellular and extracellular molecules with PRRs must occur under conditions in which an inflammatory response is required, allowing cells of the innate immune system to activate in an appropriate and timely manner. In this review we will focus on the ability of sterile stimuli to activate the cells of the innate immune system; specifically the activation of the Nlrp3 inflammasome complex and the consequences of this activation for the generation of an adaptive immune system will be examined.

2. The NLR family

PRRs include a number of cell surface receptors such as the Toll-like receptors (TLR) and the c-type lectin receptors (CLRs), whose functions are reviewed in detail elsewhere [7, 8]. These membrane-anchored receptors coordinate responses with another family of PRR, the nucleotide-binding domain leucine-rich repeat containing (NLR) family of cytosolic receptors. To date 22 members of the NLR family have been identified in humans. NLRs are characterized by a central nucleotide-binding oligomerization (NACHT) domain that is flanked by a C-terminal LRR domain, and an N-terminal effector domain [9]. The NLRs are subgrouped based upon the particular N-terminal effector domain they contain; those that contain an N-terminal pyrin domain are members of the Nlrp subgroup and those with a caspase activation and recruitment domain (CARD) are part of the Nlrc subgroup.

Three members of the NLR family (Nlrp1, Nlrp3, Nlrc4) and one PYHIN family member (AIM2) have been shown to form high-molecular weight, multiprotein complexes called inflammasomes. Inflammasomes typically contain an NLR, the adaptor protein apoptosis-associated speck-like protein containing a CARD domain (ASC) and the cysteine protease caspase-1. The precise order of events leading to inflammasome activation remains elusive for most inflammasomes. By bringing two or more monomers into proximity it is believed these complexes serve to initiate the autocatalytic processing of caspase-1, which then process the zymogens pro-IL-1 β and pro-IL-18 to their biologically active forms [9]. In addition to the release of cytokines, inflammasome activation also leads to a proinflammatory form of programmed cell death termed pyroptosis [10]. IL-1 β and IL-18 are potent proinflammatory mediators, which are also important in driving antigen specific adaptive immune responses [11]. However, unchecked IL-1 β secretion can lead to fibrosis as well as autoinflammatory disorders. Activation of inflammasomes requires two signals. Signal one, typically TLR activation, causes the production of pro-IL-1 β and pro-IL-18 as well as upregulates the transcription and translation of molecules such as Nlrp3 [12]. Signal two causes the assembly and activation of the inflammasome and can be provided by a variety of stimuli [9]. The Nlrp1 inflammasome responds to anthrax lethal toxin [13]. Nlrc4 is activated by bacterial type III and type IV secretion systems or cytosolic flagellin through the utilization of the NLR family members NAIP2 or NAIP5, respectively [13, 14]. The

inflammasome that forms around AIM2 responds to cytosolic dsDNA [13]. The Nlrp3 inflammasome is to date the best characterized of the inflammasomes, and in terms of activating stimuli seems to be the most promiscuous (Fig. 1).

3. Nlrp3 inflammasome

Nlrp3 was originally identified by a gain of function mutation in a gene associated with the autoinflammatory disorders familial cold autoinflammatory syndrome (FCAS), chronic infantile neurologic cutaneous and articular syndrome (CINCA, also known as neonatal-onset multisystem inflammatory disease of NOMID) and Muckle-Wells syndrome, together now called the cryopyrin-associated periodic syndromes or CAPS [15]. The Nlrp3 inflammasome is activated by a large number of diverse stimuli that have divergent molecular characteristics making it unlikely that Nlrp3 recognizes each directly. Rather, it is more probable the actions of the various agonists converge to generate, modify or expose a common cytosolic product that serves as the physical and activating ligand of Nlrp3. One broad category of Nlrp3 activators is crystalline substances, phagocytosis of which has been shown to activate the Nlrp3 inflammasome. The crystals can be environmental irritants such as asbestos, silica or alum [15-19], or can be host derived DAMPs, like uric acid or cholesterol crystals [5, 20]. Uric acid has been shown to be responsible for Nlrp3 inflammasome activation in the context of gout [5] and bleomycin induced lung injury and fibrosis [21], while cholesterol crystals and oxidized LDL were shown by Duewell *et al.* [20] to activate the Nlrp3 inflammasome in atherosclerosis. A study by Hornung *et al.* [19] showed that phagocytosis of crystals leads to lysosomal disruption, supporting a hypothesis that lysosomal damage triggers Nlrp3 inflammasome activation. They found that disruption of the lysosome, even in the absence of particulate DAMPs within the phagosome, was able to activate the Nlrp3 inflammasome [19]. Additionally, upon phagocytosis both β -amyloid and islet amyloid polypeptide (IAPP), associated with Alzheimer's disease and type 2 diabetes respectively, induce lysosomal damage and IL-1 β secretion that is dependent upon the Nlrp3 inflammasome [4, 22]. Damage or rupture of the lysosome leads to the release of lysosomal components into the cytosol. It has been proposed that cathepsin B released from these damaged lysosomes could be the direct activator of the Nlrp3 inflammasome, as inhibition of lysosomal acidification and the cathepsin B inhibitor CA-074-Me were both able to prevent inflammasome activation by silica and alum [19]. However, the role of cathepsin B in inflammasome activation has been called into question because a deficiency in cathepsin B did not prevent the activation of caspase-1 [23], suggesting that a target of CA-074-Me, other than cathepsin B, may be responsible for Nlrp3 inflammasome inhibition. Lysosomal disruption also fails to explain how soluble DAMPs such as ATP activate the Nlrp3 inflammasome [19, 24].

Numerous studies have confirmed that both potassium efflux and reactive oxygen species (ROS) are necessary for Nlrp3 inflammasome activation [17, 25-27]. The requirement for potassium efflux, at least *in vitro*, has been shown indirectly through the use of high concentrations of extracellular potassium that removes the concentration gradient of potassium between the intra- and extra-cellular spaces as well as directly through pharmacological inhibition of potassium channels and is associated with inhibition of Nlrp3 inflammasome activation [17-19, 23, 27-29]. While it is known that potassium efflux is necessary for Nlrp3 inflammasome activation, its precise role in Nlrp3 inflammasome activation remains unclear.

A second requirement for Nlrp3 inflammasome activation is the production of ROS. The importance of ROS production in Nlrp3 inflammasome activation has been shown through the use of ROS scavengers such as diphenyleneiodonium chloride (DPI) [17, 25, 27]. Recently, Park *et al.* solved the crystal structure of the Nlrp3 pyrin domain [30]. They

identified two conserved cysteine residues at positions 8 and 108, which potentially allows for the formation of a disulfide bridge. The formation of such a bridge might endow Nlrp3 with a level of redox sensitivity, and therefore may shed light on the apparent requirement for ROS in Nlrp3 inflammasome activation [30]. The source of ROS has also been an area of interest. It had been postulated that the ROS was derived from phagosomal NADPH oxidase as Dostert *et al.* showed that shRNA knock down of p22^{phox}, an essential part of a number of phagosomal NADPH oxidases, decreased IL-1 β secretion [17]. However, subsequent studies have shown that genetic deletion, or mutation, of various components of the NADPH oxidase does not affect Nlrp3 inflammasome activation, suggesting the source of ROS is not phagosomal NADPH oxidase [19, 31, 32].

Mitochondria are a primary source of cellular ROS and are known to increase ROS production in response to stress conditions including membrane destabilization [33]. Recently two publications have suggested that mitochondria are the source of ROS involved in Nlrp3 inflammasome activation [34, 35]. Zhou *et al.* [35] showed that induction of mitochondrial ROS production by specific inhibition of Complex I and Complex III, through the use of rotenone and Antimycin A respectively, not only induced robust ROS production but also IL-1 β secretion that was Nlrp3 dependent. Impairment of the production of mitochondrial ROS through shRNA knockdown of voltage dependent anion channel VDAC1 and VDAC2 decreased both ROS production and IL-1 β secretion upon the addition of Nlrp3 agonists [35], providing further evidence that mitochondria play a role in Nlrp3 inflammasome activation. This connection is supported by the finding that although both Nlrp3 and ASC are associated with the ER under resting conditions, upon activation both relocate to the mitochondria [35]. It was also shown that mitophagy, a specialized form of autophagy that targets dysfunctional mitochondria, can influence Nlrp3 inflammasome activation [34, 35]. RNA mediated knockdown of the autophagy proteins beclin-1 and ATG5 [35], as well as genetic disruption of LC3, ATG16L1 and beclin-1 [34, 36] caused increased IL-1 β secretion upon challenge with Nlrp3 agonists. It has also been shown that disruption of mitophagy through deletion of LC3 not only increased basal production of mitochondrial ROS, but mitochondria also showed increased damage upon treatment with the Nlrp3 agonist ATP [34]. Such damage to mitochondria can release their components into the cytosol, and it has been proposed that such a component could be the ligand for Nlrp3. It has been noted that thioredoxin interacting protein (TXNIP) dissociates from thioredoxin and can associate with Nlrp3 under conditions of oxidative stress [37], and also localize to the mitochondria upon addition of Nlrp3 agonists [35]. However, another report indicates that mitochondrial DNA is released into the cytosol following mitochondrial damage, and this release is necessary for Nlrp3 inflammasome activation [34]. Together these reports make it clear that mitochondria are critically involved in Nlrp3 inflammasome activation at least in part through serving as the main cellular source for the requisite ROS, and potentially as the source of the activating signal for Nlrp3. While ROS may be directly required, it is equally likely ROS may be an earlier common step in the path to Nlrp3 activation, triggering events in the mitochondria leading to Nlrp3 activation. Further studies are required to determine how ROS is involved in Nlrp3 activation and to identify the endogenous ligand for Nlrp3.

4. Inflammasomes and adaptive immunity

In very general terms, the immune system is commonly divided into two branches that are responsible for both immediate and long-term immunity to pathogen and non-pathogen derived substances, called the innate and adaptive immune systems respectively. Thus far the immediate or innate immune responses mediated by inflammasomes and in particular Nlrp3 have been discussed. There is no doubt that the innate and adaptive immune responses are intimately connected, which invites an ostensibly simple question: Does activation of the

inflammasome complex provide instruction to the adaptive immune system in a manner similar to the other PRRs such as the TLRs?

The production of proinflammatory cytokines is a critical step for an effective innate response, and is also a pivotal mechanism by which the innate immune system influences the subsequent development of adaptive immune responses. IL-1 β (IL-1F2), IL-18 (IL-1F4) and IL-33 (IL-1F11), all members of IL-1 family, are examples of key cytokines that not only activate monocytes, macrophages and neutrophils but have also been shown to specifically drive the development of CD4⁺ T cell adaptive responses in both mice and human. CD4⁺ T cell differentiation in the presence of IL-1 β , IL-18 or IL-33 results in Th17, Th1 or Th2 effector cells, respectively [38-41]. Secreted forms of IL-1 β and IL-18 are synthesized as inactive precursors and mature following cleavage by the enzyme caspase-1 [9] as discussed above. Similarly to IL-1 β and IL-18, the 30 kDa precursor of IL-33 can be cleaved by caspase-1 *in vitro* resulting in an 18 kDa IL-33 [42]. Further evidence states that, unlike IL-1 β and IL-18, there are other enzymes that might contribute to processing of IL-33. Cleaved IL-33 secretion was found in caspase-1 deficient, caspase-8-inhibitor or calpain-inhibitor treated cells, suggesting a partial role for each of these enzymes in the maturation of IL-33 in macrophages or mast cells [41]. However, recent findings demonstrate that IL-33 is biologically active in its full-length form and actually processed and inactivated by caspase-3, but not by caspase-1 [43, 44]. Thus the role of inflammasome pathways in IL-33 signaling remain unclear.

The role of IL-1 β and IL-18 in regulating Th1 and Th17 responses has been examined through the use of a variety of different disease models. Autoimmune disease is a pathologic response by the adaptive immune system but its development is dependent in part upon the production of IL-1 and IL-18 by the innate system. As NLRs drive the production of these critical cytokines their role in the development of these diseases has been investigated. In experimental autoimmune encephalitis (EAE), a mouse model for multiple sclerosis that is driven by Th1 and Th17 responses, Gris *et al.* reported Nlrp3 deficient mice had a delay in the progression of disease as well as a significant decrease in IL-18 production [45]. IL-18 knockout mice displayed a similar disease course to Nlrp3 knockout mice. Furthermore, Nlrp3- and IL-18-deficient mice had reduced IFN- γ and IL-17, together suggesting Nlrp3 activation and IL-18 production are necessary for the normal development of Th1 and Th17 responses [45]. In contrast, a separate study by Kanneganti's group proposed that the progression of EAE is dependent on ASC and caspase-1, but not Nlrp3 [46]. The same study demonstrated that although there were fewer T cells in the ASC deficient mice, MOG (myelin oligodendrocyte glycoprotein)-specific T cell proliferation and cytokine production were intact in ASC deficient mice [46]. There are significant discrepancies amongst these recent reports on the roles of ASC and inflammasomes in regulating adaptive immunity; the potential independent role of ASC in driving adaptive responses is discussed separately below.

Although the role of caspase-1 and inflammasomes in IL-33 processing is not as well-established as for IL-1 β and IL-18, the role of IL-33 in the generation of Th2 responses has been examined [42, 47]. As mentioned above, the IL-1 family member IL-33 has been suggested to be modulated by caspase-1 and thus may be regulated, either positively or negatively, through the action of one or more of the NLR inflammasomes. Although IL-33 has homology to IL-18 at the amino acid level its function on the adaptive immune response is distinct in that it predominantly drives Th2 responses. IL-33 was reported as a ligand for the orphan IL-1 family receptor T1/ST2 and this receptor has been used as a selective marker for both murine and human Th2 lymphocytes [48]. One of the earlier studies by Schmitz *et al.* demonstrated immunomodulatory functions of IL-33 in the gene expression of IL-4, IL-5 and IL-13 *in vivo* which are known to induce Th2 response [42]. Subsequent

studies have confirmed IL-33 injection results in elevation of markers of Th2 associated immune responses including eosinophilia, elevated serum IgE levels and inflammatory responses in the lung and gut [42, 49]. The Nlrp3 inflammasome activator alum has been used for years as an adjuvant in a Th2 dependent allergic airway disease model in mice; it is attractive to consider that IL-33 released following Nlrp3 activation could potentially explain the link between alum and this Th2 response.

5. Sterile inflammation and adaptive immunity

T cell mediated immune responses to haptenated allergens have been shown to be impaired in ASC- and Nlrp3-deficient mice in response to trinitrophenylchloride in a contact hypersensitivity model [50, 51]. These haptens serve as danger signals that affect Th1, Th17, and regulatory T cell development via inflammasome-mediated caspase-1 and IL-1 β activation [51]. In addition, ATP, alum and uric acid are non-pathogenic stimulants of Nlrp3 that are also well known T cell adjuvants. Alum is a mix of aluminum and, variably, magnesium salts that is a powerful and effective adjuvant that has been used for decades to stimulate robust adaptive immune response to vaccines in humans as well as to drive a Th2-mediated adaptive response in mouse models of allergic disease. Numerous groups have tested the effects of different forms of alum on the activation of the Nlrp3 inflammasome and shown a significant role for this inflammasome complex in mediating alum's effect on innate immunity [52-54]. However, the role of the Nlrp3 inflammasome in driving the adaptive response triggered by alum has been less clear. While some reports have shown a requirement for Nlrp3 in the T and B cell responses initiated by alum [18, 28, 53, 54] there have also been reports that failed to observe a Nlrp3 dependency in the activation of adaptive immunity and in particular for antibody production in response to alum [18, 55, 56]. In addition to alum, uric acid (UA) is a DAMP that is secreted from dying or stressed cells and can efficiently stimulate neutrophilic inflammation through Nlrp3 inflammasome activation and the release of IL-1 β [57]. The fact that alum can induce a Th2 response coupled with this new finding that UA triggers the same inflammatory pathway as alum together raised the question of whether UA also can induce and amplify Th2 cell responses. Kool *et al.* have recently shown that intraperitoneal injection of UA induced Th2 responses in mice but that this Th2 cell adjuvant effect did not require the Nlrp3-ASC complex or IL1R signaling [58]. In this study, spleen tyrosine kinase (Syk) and Phosphatidylinositol 3-kinase (PI3K) δ signaling were shown to be involved in the UA induced Th2 response.

The role of Nlrp3 in the development of the CD4⁺ T cell response was also studied by Meng *et al.* who generated a knock-in mouse expressing a missense mutation in *Nlrp3* [59]. Importantly, this mutation was associated with Muckle-Wells syndrome and mice expressing this constitutively active mutant Nlrp3 exhibited a dominant Th17 cytokine response [59]. This suggests the adaptive immune response is driven by Nlrp3 inflammasome activation. However, another study utilizing independently generated mutant Nlrp3 knock-in mouse also to model Muckle-Wells syndrome had an autoinflammatory phenotype that was independent of CD4⁺ T cells and the adaptive immune system [60].

6. Pathogen induced inflammasome activation and adaptive immunity

Nlrp3 can also respond to pathogens by initiating adaptive immune responses. A number of studies have demonstrated that the Nlrp3 inflammasome plays an important role in the control of the fungal pathogen *Candida albicans* [23, 61, 62]. A recent study by van de Veerdonk *et al.*, found that caspase-1- and ASC- deficient mice had impaired Th1 and Th17 responses and were more susceptible to disseminated candidiasis, suggesting an important protective role for the inflammasome during fungal infections through the activation of Th1 and Th17 responses [63]. However, it should be noted that this study [63] did not

specifically examine Nlrp3-deficient mice and given the Nlrp4 inflammasome has also been implicated in *C. albicans* pathogenesis it will be important to determine the individual contribution of these two pathways in driving effector CD4⁺ responses [64]. The effects of *C. albicans* and *S. cerevisiae* on inflammasome activation were also examined by Kumar *et al.* who showed that stimulation of dendritic cells and macrophages with zymosan and curdlan (beta-1,3-glucan) resulted in the production of IL-1 β through the Nlrp3 inflammasome [65]. Interestingly, this activation was necessary for the effects of curdlan on B cells but was not required for Th1 and Th17 cell differentiation. Additionally, B cells were stimulated upon direct exposure to curdlan through a mechanism that was dependent upon Nlrp3 but did not require MyD88 in the B cells. The IL-1 receptor cannot signal in the absence of MyD88, thus activation of B cells was not dependent upon the activation of the IL-1 receptor, proposing a distinct role for Nlrp3 in B cells for normal antibody responses. Hence, the Nlrp3 inflammasome is an important contributor to the regulation of β -glucan-induced innate and humoral adaptive immunity [65]. In another study it was observed that ASC- and Nlrp3-deficient mice had impaired antigen specific Th1, Th2 and Th17 responses to *Schistosoma mansoni* infection. The authors also importantly noted that schistosomal egg antigens (SEA) could activate the Nlrp3 complex via the c-type lectin receptor Dectin-2. [66].

7. Inflammasome-independent, ASC-dependent adaptive immunity

A collection of recent studies have suggested a definitive role for ASC in the adaptive immune response. ASC functions as an important adaptor molecule by linking NLRs to procaspase-1. This association is required for the eventual activation of caspase-1 and its cleavage of IL-1 β and IL-18. Recently, ASC has been proposed to possess inflammasome independent roles. A report by Ippagunta *et al.* shows a discrete role for ASC in the regulation of adaptive immune cells [67]. In this study, ASC-deficient mice were found to have impaired antigen presentation and migration by dendritic cells and lymphocytes. Using a genome wide analysis, the function of ASC in shaping adaptive immunity was suggested to be Nlrp3 and caspase-1 independent, and to be mediated via Dock2-mediated Rac activation and actin polymerization [67]. Earlier studies by the same group looked at a collagen-induced arthritis (CIA) model and showed ASC-deficient mice were protected from arthritis, whereas disease stage and severity in mice lacking Nlrp3 or caspase-1 was unchanged from wild-type mice. In this study, antigen induced proliferation of ASC-deficient T cells was restored when incubated with wild-type dendritic cells, but not with ASC-deficient DCs, suggesting in this system ASC is required only in DCs for antigen-induced T cell activation [68]. These findings were partially consistent with a previous study focusing on the involvement of inflammasome components in T cell activation and proliferation during the course of CIA wherein CD4⁺ T cells from mice lacking ASC had impaired function [69]. In contrast to the findings by Kanneganti and colleagues, this study suggested a CD4⁺ T cell intrinsic defect in ASC deficient cells as the response of ASC-deficient CD4⁺ T cells activated *in vitro* with anti-CD3 was inferior to that of wild type CD4⁺ T cells [69].

8. Conclusion

The activation of the innate immune system is intimately tied to the activation of the adaptive side as well. A number of studies show that the Nlrp3 inflammasome plays a pivotal role in influencing this interaction of innate and adaptive immunity. Understanding the regulation of this communication between innate and adaptive immunity is essential for the understanding of the pathways that underlie a broad range of human disease. This knowledge can potentially be exploited in the design and development of new vaccines and drugs for the treatment and even prevention of these disorders. While the studies

determining these roles remain in their early stages the groundwork for this work is underway and shows promise for many significant advancements to come.

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Figure 1. Proposed model for the activation of Nlrp3 inflammasome through non-pathogenic insults

A divergent group of stimuli leads to a cascade of events that culminate in the activation of the Nlrp3 inflammasome, which results in processing and secretion of IL-1β and IL-18 by active caspase-1. These events include: a potassium efflux, the generation of mitochondrial ROS and the assembly of the Nlrp3 inflammasome complex.