

Innate instruction of adaptive immunity revisited: the inflammasome

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Introduction

The immune system of mammals can be divided into two arms that act together to provide both immediate and long-term immunity to pathogens.

The former has been termed the innate immune system and is composed of phagocytes, complement, antimicrobial peptides, etc and the latter is called the adaptive immune system and consists of T- and B-lymphocytes. This latter branch of immunity provides long-term protection with exquisite antigen specificity; however, adaptive immunity is unbiased *a priori*, meaning T- and B-cell receptors can collectively recognize any antigen—whether non-self (pathogen-associated) or self. Thus lymphocyte activation must be tightly regulated and it was not until major advances in our understanding of the more primitive

The innate immune system regulates initial responses to pathogen invasion through a set of conserved pattern recognition receptors (PRR). The best-characterized PRRs are the Toll-like receptors, which regulate not only the initial pathogen defense response, but also adaptive immune responses. Thus, insight into the function of PRRs has major implications for our understanding of the physiology of vaccination and the pathophysiology of human disease. Recent advances in our understanding of a new class of pattern recognition receptors—NOD-like receptors (NLR)—have similarly provided insight into both innate and adaptive immunity. In particular, the NLR Nlrp3 (also known as Nalp3 or Cias1) forms an intracellular multimolecular complex with active caspase-1, called an inflammasome, creating a platform for regulating secretion of interleukin-1 (IL-1) family members. Given the important role of IL-1 in inflammatory diseases, from gout to rheumatoid arthritis, the importance of understanding the regulation of such a cytokine cannot be underestimated. In this review, we address new evidence supporting a role for adaptive immune activation by recently identified NLR agonists, with a particular focus on Nlrp3. Basic questions in our understanding of Nlrp3 inflammasome activation are also presented.

innate branch of immunity that immunologists recognized the fundamental principle of innate–adaptive crosstalk in immune regulation (Iwasaki & Medzhitov, 2004; Medzhitov & Janeway, 1999).

The players of the innate–adaptive crosstalk

A central tenet of T-cell priming is dendritic cell (DC) maturation. DCs function as sentinels at sites of invasion and are uniquely programmed to respond to perturbations of homeostasis with a maturation profile that limits bystander activation and targets naïve T-cells circulating through the draining lymph nodes. This activation state tightly regulates antigen phagocytosis and presentation ('signal 1') as well as the expression of necessary co-stimulatory molecules such as B7 family members ('signal 2'), thus licensing antigen-specific naïve T-cells for priming rather than tolerance. Critical to this

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'two signal model' of T-cell activation is the cue to the antigen-presenting cell (APC) to engage in this program of maturation. Over the past decade, we have come to understand that the innate immune system provides this fundamental cue and thereby regulates the generation of adaptive immunity. Therefore, understanding how the innate immune system is activated is a pre-requisite to manipulate (both positively and negatively) long-lived adaptive immune responses.

A large number of germline-encoded pattern recognition receptors (PRRs) exist, which recognize repetitive motifs of potential pathogens such as lipopolysaccharide (LPS) of Gram-negative bacteria. These receptors are central to the rapid innate immune response and include endocytic PRRs (*e.g.*, macrophage mannose receptor) and secreted PRRs (*e.g.*, mannose

binding lectin). However, an additional group of PRRs that have direct effects on adaptive immunity are the signalling PRRs, which can be divided into three subgroups: the Toll-like receptors (TLR), RIG-I-like receptors (RLRs) and NOD-like receptors (NLRs). There are now a total of 10 TLRs in humans (TLR 1–10) and 12 TLRs in mice (TLR 1–9 and 11–13). TLRs recognize conserved microbial molecules expressed across a wide range of pathogens (pathogen-associated molecular patterns; PAMPs) thereby making them potent sensors of infection and endowing them with the ability to distinguish self from non-self molecules. Many of these TLR agonists have been used as adjuvants in animal models for decades, prior to any understanding of their mechanism of action. The RLRs are a recently discovered group of intracellular sensors of viral RNA (Pichlmair & Reis e Sousa, 2007). The NLR (NOD-like receptor or nucleotide-binding domain, leucine-rich repeat (LRR) containing) family is an evolutionarily conserved group of proteins with structural homology to the plant disease resistance (R) proteins. Our understanding of this latter group of PRRs has rapidly evolved and will be focus of this review.

Glossary

Adaptive immune system (or acquired immunity)

A branch of the immune system composed of lymphocytes with antigen-specific receptors that, upon activation, use multiple effector mechanisms to respond to antigen challenge. This branch of the immune system is classically distinguished from the innate immune system by its ability to collectively respond to *any* antigen, the exquisite specificity of the receptor for its target, and the generation of long-lived memory.

Adjuvant

Derived from the latin word to help (*adjuvare*). In this case, it refers to a material used to help initiate an immune response to an antigen.

Antigen

Any molecule that can be specifically recognized by the randomly-generated T- and B-cell receptors. Historically, 'antigen' referred to molecules that generated, and could be recognized by, antibodies.

Bystander activation

Priming of T-cells that have a T-cell receptor not specific for the target antigen due to its proximity to a site of inflammation. This term can apply to other cell types as well.

Complete Freund's adjuvant (CFA)

A water-in-oil emulsion with dead mycobacteria. CFA was originally described by J. Freund in 1942 to induce a potent immune response. This adjuvant is not used in humans due to toxicity.

Innate immune system

A branch of the immune system composed of cells, such as dendritic cells, macrophages, neutrophils and NK cells, with germline-encoded receptors specific for markers of host perturbation (*e.g.*, infection, damage or loss of expression of self molecules). Triggering of these receptors results in a rapid but short-lived inflammatory response. In addition to the cellular component, complement proteins and barriers such as the skin are constituents of the innate branch of immunity.

Pattern recognition receptor (PRR)

Patterns of the innate immune system that are not randomly generated (*i.e.*, germline) that recognize makers of pathogens or cell stress/damage.

T-Cell priming (or sensitization)

Activation of naïve T-lymphocytes to cognate antigen under immunogenic conditions.

Th1

A CD4⁺ helper T-cell that produces the cytokines IFN- γ and IL-2.

Th2

A CD4⁺ helper T-cell that produces the cytokines IL4, IL-5 and IL-13.

NLRs and the inflammasome

TLRs are membrane-bound proteins with the majority expressed on the cell surface; in contrast, the NLRs are cytosolic counterparts of TLRs and sense both microbial products that gain access to the cell interior (*e.g.*, intracellular flagellin) and host molecules released during states of cell stress or damage (*e.g.*, extracellular ATP) (Martinon, 2008; Miao et al, 2006; Sutterwala & Flavell, 2009). These markers of cell damage have been called, by some, 'danger signals' or danger-associated molecular patterns (DAMPs), which distinguish this latter class of innate agonists from PAMPs by their derivation from 'self' molecules. Regardless of the nomenclature, it is clear that the innate immune system is capable of sensing both pathogen-derived motifs directly and the markers of cell damage inflicted on the host by pathogens. Therefore, the NLRs can act as a second line of defense to detect potential pathogens capable of evading cell surface PRRs and might induce distinct, but overlapping, effector responses.

Unlike the TLRs, NLRs are intricately linked not only to the induction of pro-inflammatory responses but also to the apoptosis with pro-inflammatory aspects (Ting et al, 2008b). Apoptosis is a program of cell death that has traditionally been thought of as 'silent' to the immune system. In contrast, NLR-induced forms of apoptosis-pyroptosis (caspase-1 and ASC dimerization-dependent) and pyronecrosis (ASC-dependent but caspase-1-independent)–are inflammatory and share features with necrosis (Fernandes-Alnemri et al, 2007; Fink & Cookson, 2006; Willingham et al, 2007). Determination of cell fate upon activation of NLRs, such as the secretion of pro-inflammatory cytokines or cell death, is thought to be influenced by the strength and duration of the NLR signal; however, the mechanistic details of these pathways remain to be elucidated.

NLRs contain an N-terminal effector domain, a central nucleotide-binding domain and a C-terminal leucine-rich repeat (LRR) domain. The first NLRs described were nucleotide-binding

oligomerization domain (Nod1) and Nod2, which recognize different mucopeptides of peptidoglycan from Gram-positive and Gram-negative bacteria (Carneiro et al, 2007). There are now more than 20 described NLRs, which fall into four main subclasses based on their variable N-terminal domain, accordingly: (1) acidic transactivation domain/NLRA family; (2) baculoviral inhibitory repeat (BIR)-like domain/NLRB family; (3) caspase-recruitment domain (CARD)/NLRC family; and (4) pyrin domain/NLRP family. One NLR member, Nlrp1, is an independent member without N-terminal homology to any of the described families. Unfortunately, as multiple groups independently described the NLRs, many of the well-characterized NLRs have multiple names. Recently, a consortium of investigators met to standardize the NLR nomenclature (Ting et al, 2008a); the new terminology for the NLR members and families will be used in this review, but for reference, a table with the more commonly used names has been included (Table I). For a majority of these NLRs, their triggers and physiologic function remain unknown.

The term 'inflammasome' was coined in 2002 to describe a multi-protein complex formed in the cell cytosol upon stimulation and is composed of an NLR, an adaptor protein ASC (Apoptosis-associated speck-like protein containing a CARD) and the active form of caspase-1 (Martinon et al, 2002). Through the proteolytic action of caspase-1, this molecular platform cleaves a large cohort of proteins without traditional signal peptides resulting in their secretion. Thus far, only Nlrp1, Nlrp3 and Nlrc4 inflammasomes have been identified. Specific to the immune function of the inflammasome is its ability to transform pro-IL-1 β , pro-IL-33 and pro-IL-18 into their mature, active forms resulting in their secretion and the potent inflammatory response associated with

these cytokines. The specificity of the immune response is determined by the NLR member incorporated into the inflammasome; however, prior to NLR agonist signalling, most studies *in vitro* have found the need for an initial, or 'first' signal, to induce pro-cytokine formation and to allow inflammasome activation. In a majority of cases, the first signal is provided by LPS, a TLR agonist, suggesting PRR cooperation is necessary to prevent inappropriate NLR activation by ubiquitously present self-molecules. It is not clear whether the same rule applies *in vivo*, as will be discussed later in this section.

The Nlrp3 inflammasome has been most thoroughly characterized and is activated by several chemically and structurally diverse triggers (Kanneganti et al, 2006; Mariathasan et al, 2006; Martinon et al, 2006; Sutterwala et al, 2006), including markers of cell damage (*e.g.*, ATP), multiple microbial toxins that disrupt the cell membrane (*e.g.*, *Listeria's* LLO and *Aeromonas's* aerolysin) and phagocytosed insoluble crystals (for review see Dostert et al, 2008a; Martinon, 2008). A feature common to all of these agonists is induction of potassium efflux from the cell (Petrilli et al, 2007), an indicator of cell membrane disruption as potassium flows down its concentration gradient. Consistent with the model that NLRs provide a second line of defense, sensing membrane disruption is a sensitive method of detecting pathogens that have evaded cell surface or endocytic PRRs and have gained access to the cytosol; yet how potassium flux activates Nlrp3 remains unknown. Extracellular ATP is the best-studied stimulus of the Nlrp3 inflammasome and is thought to act as an indicator of local cell death; it is the only agonist with a known cell surface receptor (P2X7R) and a clear mechanism of inducing potassium efflux (opening of nonselective pannexin-1 channels) (Pelegri &

Table I. Murine NOD-like receptor members with selected synonyms and agonists

Family	Members	Commonly used alternatives	Selected agonists
NLRA	Clta	C2ta	IFN- γ
NLRB	Naip1	Birc1a; Naip-rs1	
	Naip2	Birc1b; Naip-rs6	
	Naip3	Birc1c; Naip-rs5	
	Naip4	Birc1d; Naip-rs2	
	Naip5	Birc1e; Naip-rs3	Legionella, Flagellin
	Naip6	Birc1f; Naip-rs4	
	Naip7	Birc1g; Naip-rs4B	
NLRC	Nod1	Card4	Meso-DAP (PGN from Gm-, Some Gm+, Mycobacterium)
	Nod2	Card15	MDP (PGN from Gm+, Gm-, Mycobacterium)
	Nlrc3	CLR16.2	
	Nlrc4	Ipaf; Card12; Clan	Pseudomonas, Salmonella, Legionella, Shigella
	Nlrc5		
NLRP	Nlrp1a-c	Nalp1a-c	Anthrax Lethal Toxin ^a
	Nlrp2	Nalp2; Pypaf2; Nbs1; Pan1	
	Nlrp3	Nalp3; Cias1; Pypaf1; Mmig1; Cryopyrin	Pore-forming toxins, ATP, Crystals, Chemical Sensitizers
	Nlrp4a-g	Nalp4a-g	
	Nlrp5	Nalp5; Mater; Op1	
	Nlrp6	Nalp6	
	Nlrp9a-c	Nalp9a-c	
	Nlrp10	Nalp10; Pynod	
	Nlrp12	Nalp12	
	Nlrp14	Nalp14; GC-LRR; Nalp-iota	

^aNlrp1b

Surprenant, 2006). For the remaining Nlrp3 agonists, specific receptors have not been identified.

What any NLR within an inflammasome directly senses remains unknown; some have proposed that NLRs bind directly to PAMPs resulting in activation (Franchi et al, 2006) and, indeed, NLRs contain an LRR much like the proposed ligand-binding recognition site within TLRs. However, definitive evidence of this interaction is still missing. To complicate matters, the large numbers of chemically distinct crystals that have recently been shown to activate the Nlrp3 inflammasome do not contain known PAMPs or DAMPs. Rather, it is thought that they induce release of markers of cellular stress from the stimulated cell upon their endocytosis, thereby signalling damage through inflammasome activation. Recent work has attempted to define the pathway between endocytosis of crystals such as silica and aluminium hydroxide and Nlrp3 activation. Some groups have observed a requirement for the production of reactive oxygen species through NADPH oxidase while others have identified a destabilization pathway of lysosomes, whose content is released into the cytosol (Cassel et al, 2008; Dostert et al, 2008b; Halle et al, 2008; Hornung et al, 2008). In particular, inhibition of cathepsin B, a lysosomal enzyme that requires an acidic environment for activation, blocks many of the Nlrp3-mediated inflammasome functions through an as yet unclear mechanism (Fujisawa et al, 2007; Hornung et al, 2008; Willingham et al, 2007). These intermediaries are attractive candidates in the pathway towards NLR activation as they signal disruption of cell integrity. However, what directly activates Nlrp3 still remains a mystery.

Another outstanding question in Nlrp3 function is whether the two-signal model of inflammasome activation described *in vitro* applies to *in vivo* activation. Of note, some *in vitro* models, such as stimulation of the PMA-treated human monocyte cell line THP-1 or cycloheximide-treated macrophages, appear to require only the second (NLR) signal for IL-1 β production (and in one case, independently of caspase-1) (Dostert et al, 2008b; Maelfait et al, 2008; Pan et al, 2007). Yet these treatments prior to NLR activation likely provide a surrogate signal in place of the standard LPS stimulus in untreated phagocytes *in vitro*. The relationship of these alternate first signals *in vitro* to *in vivo* inflammasome activation is not clear. Regardless, many of the known *in vitro* Nlrp3 agonists can be administered alone, *in vivo*, and induce an inflammatory profile consistent with inflammasome activation. Whether a surrogate signal initiates the *in vivo* response and transcription of IL-1 family cytokines remains unknown.

The inflammasome in chronic inflammation

The potent role of NLRP3 in chronic inflammatory responses was initially identified in a group of disorders called systemic autoinflammatory syndromes, characterized by periodic fevers and localized inflammation, and were linked to activating mutations in *NLRP3* (Church et al, 2008). More recently, triggers of the Nlrp3 inflammasome have emerged, providing a mechanistic understanding for chronic inflammatory conditions with previously unknown etiologies. The first was the realization that uric acid crystals induce Nlrp3-mediated IL-1 β activation

and likely result in the inflamed joints of patients with gout (and similarly calcium pyrophosphate dihydrate crystals in pseudogout) (Martinon et al, 2006). Silica and asbestos crystals were subsequently demonstrated to induce Nlrp3-dependent caspase-1 activation; in the absence of any one of the Nlrp3 inflammasome components, the macrophage-driven pulmonary inflammation of silicosis and asbestosis was attenuated in animal models of these diseases (Cassel et al, 2008; Dostert et al, 2008b). Nlrp3 activation has also been linked to the activation of microglia by fibrillar amyloid- β in senile plaques of Alzheimer's disease (Halle et al, 2008). In these conditions, it appears that Nlrp3 stimulation results in a chronic state of (innate) inflammation, IL-1 β production and, in some cases, fibrosis.

The inflammasome in adaptive immune responses

Returning to the central role of the innate-adaptive immune system crosstalk, a logical extension of the studies of inflammasome-mediated innate immune responses described above is to ask whether Nlrp3 can provide instruction to the adaptive immune system as do the TLRs and if so, is the resultant adaptive immune response similar in nature? Precedent exists for NLR instruction of adaptive immunity. Nod2 has been shown to mediate the adjuvant effect of muramyl dipeptide (MDP) when administered intra-peritoneally with a protein antigen (Kobayashi et al, 2005). Further, lymphocyte responses to antigen priming with the common adjuvant, CFA, were severely diminished in Nod1 KO mice (Fritz et al, 2007). Two groups have also demonstrated that contact hypersensitivity (T-cell mediated immune response to allergens) with the skin contact allergens trinitrophenylchloride (TNP-Cl) or dinitrofluorobenzene (DNFB) were severely attenuated in inflammasome-deficient mice due to a defect in lymphocyte sensitization (Sutterwala et al, 2006; Watanabe et al, 2008). The first identified crystal agonist of the Nlrp3 inflammasome, uric acid, was shown to induce cytotoxic T-cell priming when used as an adjuvant (Shi et al, 2003). All of these studies suggest that Nlrp3 activation is capable of providing the requisite signals necessary to instruct long-lived adaptive immune responses and therefore, might be a useful target in vaccination.

The most commonly used adjuvant in human vaccines is aluminium hydroxide ('alum'), a suspension of insoluble crystals that adsorb antigens and has been used over many decades to induce effective and safe immune responses. Given the need for innate immune system instruction of adaptive immune cells and given the potent adjuvant activity of alum, it seemed likely that alum exerted its effect by triggering a PRR. However, a number of earlier studies found no role for TLRs in alum-induced immunity (Gavin et al, 2006; Piggott et al, 2005; Schnare et al, 2001). Therefore, whether alum followed the canon of innate immune system-instructed adaptive immunity remained in question. Indeed the historical explanation of its adjuvant effect relied on a 'depot' theory in which particulate antigen adsorbed to alum was slowly released to APCs. However, how this was immunostimulatory was not clear. Five recent reports have now described activation of the Nlrp3 inflammasome by various forms of

aluminium adjuvants (Eisenbarth et al, 2008; Franchi & Nunez, 2008; Hornung et al, 2008; Kool et al, 2008; Li et al, 2008). In all studies, alum adjuvants activated APCs *in vitro* leading to caspase-1 activation and IL-1 β secretion in an Nlrp3-dependent manner. Alum triggers the same signalling pathway as all known insoluble Nlrp3 agonists to activate the Nlrp3 inflammasome (e.g., potassium efflux and particle endocytosis with possible lysosomal disruption). Further, we and two other groups confirmed that in the absence of this NLR activation, the adaptive immune response was impaired upon antigen priming. Interestingly, one group did not find a difference in antigen priming in Nlrp3-deficient mice possibly due to differences in the sensitization models (Franchi & Nunez, 2008). It was further shown by Li et al that other commonly studied particulate adjuvants such as Quil A and chitosan could also induce Nlrp3-dependent IL-1 β secretion (Li et al, 2008). Kool et al then provided evidence that DC maturation was decreased in the absence of inflammasome-induced IL-1 β secretion upon immunization with alum (Kool et al, 2008). This latter finding returns us to the initial unifying hypothesis of innate immune system control of T-lymphocyte priming through cues delivered to the DC. Summing up, these studies broaden our understanding of the potential repertoire of innate immune system receptors that regulate activation *versus* tolerance of the adaptive immune system.

While these findings define a role for the inflammasome in adjuvant control of immunity, they raise a number of key questions. First, how does the Nlrp3 inflammasome control adaptive immunity? IL-1 has long been known to promote the initiation of immune responses and has direct effects on lymphocyte activation (Curtsinger et al, 1999; Dinarello, 2002; Greenbaum et al, 1988; Kurt-Jones et al, 1987). It is tempting to speculate that activation of IL-1 family members by the inflammasome directly regulates initiation of antigen-specific inflammation (Fig 1). However, there are reasons to question whether IL-1 is the intermediary between NLR activation and lymphocyte responses. One major issue that has not yet been resolved is the IL-1 receptor's requirement for the adaptor protein MyD88. This molecule is an adaptor for most TLRs and is also known to transmit signals from the IL-1, IL-18 and IL-33 receptors, yet MyD88 has repeatedly been shown to be dispensable for alum's initiation of immunity (Adachi et al, 1998; Gavin et al, 2006; Piggott et al, 2005; Schnare et al, 2001). Two alternatives exist to explain this paradox: (1) IL-1 family cytokines are dispensable in Nlrp3-initiated adaptive immune responses; or (2) IL-1 family cytokines are required, but can signal through MyD88-independent signalling pathways. Evidence supporting both possibilities exists. Two studies recently described a large cohort of secreted molecules putatively cleaved by caspase-1 (Keller et al, 2008; Lamkanfi et al, 2008). It is plausible that caspase-1-dependent secretion of one or more of these proteins transmits the activation signal from the NLR to the adaptive immune system. Alternatively, other studies identified a MyD88-independent signalling pathway involving PI3K and AKT downstream of the IL-1 receptor (Cahill & Rogers, 2008; Davis et al, 2006). Identification of the signals induced by the inflammasome to the adaptive immune cells will likely shed light on many crucial pathways in the control of immunity.

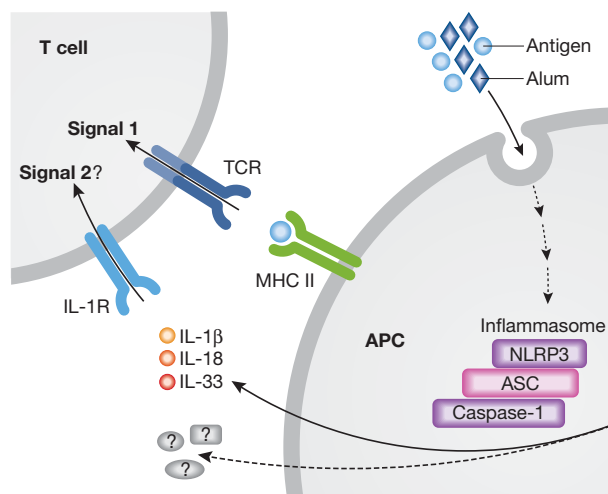


Figure 1. Proposed model of adaptive immune system activation by inflammasome stimulation with the adjuvant aluminium hydroxide. Alum and antigen are phagocytosed by an APC and, through an unknown mechanism, activate the Nlrp3 inflammasome, resulting in secretion of the pro-inflammatory cytokines IL-1 β , IL-18 and IL-33 and potentially other immune modulatory molecules. In conjunction with antigen recognition on the APC by the T-cell receptor (TCR), IL-1 receptor (IL-1R) stimulation (or another inflammasome-dependent signal) provides 'signal 2' for CD4⁺ T-cell priming, the first step in generation of adaptive immunity.

A second question raised, in particular by the studies on alum-induced Nlrp3 stimulation, is whether NLR activation induces a qualitatively different adaptive immune response when compared with other innate immune receptors. Triggering of TLRs is typically thought to induce a strong Th1-biased helper T-cell

Pending issues

- What is the ligand or ligands that directly activate Nlrp3?
- For other NLR family members without known triggers, what do they recognize?
- What is the first signal for inflammasome priming *in vivo*?
- What signal determines caspase-1-regulated cell death *versus* cytokine secretion?
- Which cells respond to Nlrp3 inflammasome triggers *in vivo*?
- What products of Nlrp3 inflammasome activation regulate T-cell priming and polarization?
- What role does the MyD88-independent signalling pathway have in the response to IL-1, IL-18 or IL-33?
- How do other inflammasomes affect the adaptive immune response?
- How can Nlrp3 activation be harnessed in future adjuvant development?
- Do other adjuvants activate the Nlrp3 inflammasome?

response in mice, especially given the potent induction of pro-Th1 differentiation cytokines such as IL-12. In contrast, alum immunization with antigen results in a strong Th2 skewed immune responses in mice, including the induction of IgE and IgG1 antibody isotypes. Thus, alum immunization is used in the study of asthma and allergy in animals, despite the questionable relevance of this adjuvant to the mechanism of allergen sensitization in humans (Eisenbarth, 2008). Therefore, one could suggest that NLR, in contrast to TLR, activation promotes Th2 immunity to deal with (large and 'insoluble') parasites. However, there is ample evidence to suggest that not all Nlrp3 triggers induce Th2 responses including uric acid-induced CD8⁺ T cell responses (Cassel et al, 2008; Shi et al, 2003). And conversely, not all Th2 responses require Nlrp3 activation; we have preliminary evidence that other Th2-skewed models of inflammation in mice are intact in Nlrp3-deficient animals. Further, the immune response to alum in humans is not clearly Th2-biased. In fact, alum has actually been used to *desensitize* allergic patients to their offending allergen (Francis & Durham, 2004). It is not clear why alum can be used both to sensitize and desensitize the human immune system, and it could be that another inflammasome-independent pathway is preferentially harnessed during allergen desensitization, but nevertheless, the Nlrp3 inflammasome should probably not be considered a preferentially Th2-inducing pathway.

While alum-triggered NLR activation might not be Th2-biased, it certainly does induce an inflammatory profile distinct from that of TLR activation. Many TLR agonists can incite an overwhelming and toxic inflammatory response. Nlrp3 activation in humans has not thus far been shown to induce the same response. For example, uric acid activation of Nlrp3 in gout induces a potent but local reaction and clearly the response to aluminium adjuvants during immunization is mild and well tolerated. In contrast, systemic exposure to LPS or other TLR ligands can induce massive cytokine production in conditions such as septic shock. Therefore, there is a qualitative difference in the innate immune response generated by Nlrp3 stimulation, perhaps to reflect the distinct nature of the triggers (*i.e.*, infection *vs.* host damage). These immediate differences in the immune response might translate into an adaptive response with an altered character, although currently this is only speculation. Despite this difference, it is important to recognize that the previously established rules of innate-adaptive cross-talk can be applied to this emerging class of PRRs, the NLRs, which have an apparently unique sentinel role in the immune system.

CONCLUSIONS

The recent pace of discovery in the NLR field has been astounding; however, several key questions regarding the inflammasome have been highlighted in this review (see Pending Issues box). There is a great potential for applying these discoveries to our comprehension of both basic immune physiology and disease pathology. An exciting application derived from these studies is in the development of new vaccine adjuvants, where TLR-based adjuvants have thus far proven to

be too toxic and a molecular understanding of how to target and modulate adaptive immunity has been challenging. Although alum will likely prove to have multiple immunostimulatory properties; NLR-based activation appears to be an effective alternate strategy in the innate-adaptive paradigm to generate immunity—one that can be built upon to tailor a new generation of adjuvants.

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