The Inflammasomes: Guardians of the Body

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Abstract

The innate immune system relies on its capacity to rapidly detect invading pathogenic microbes as foreign and to eliminate them. The discovery of Toll-like receptors (TLRs) provided a class of membrane receptors that sense extracellular microbes and trigger antipathogen signaling cascades. More recently, intracellular microbial sensors have been identified, including NOD-like receptors (NLRs). Some of the NLRs also sense nonmicrobial danger signals and form large cytoplasmic complexes called inflammasomes that link the sensing of microbial products and metabolic stress to the proteolytic activation of the proinflammatory cytokines IL-1β and IL-18. The NALP3 inflammasome has been associated with several autoinflammatory conditions including gout. Likewise, the NALP3 inflammasome is a crucial element in the adjuvant effect of aluminum and can direct a humoral adaptive immune response. In this review, we discuss the role of NLRs, and in particular the inflammasomes, in the recognition of microbial and danger components and the role they play in health and disease.

INTRODUCTION AND OVERVIEW

Pattern-recognition receptor (PRR): membrane receptor expressed by cells of the immune system to identify molecules associated with microbial pathogens or cellular stress

Pathogen-associated molecular pattern (PAMP): highly conserved microbial structure that is essential for microbial survival and is detected by host innate immune receptors

Danger signal: signal released by injured or damaged tissues that trigger an innate immune response

Toll-like receptor (TLR): membrane receptor involved in innate immune sensing

Vertebrates have evolved two complementary systems to detect and clear pathogens: the innate and the adaptive immune systems. The innate immune system is the first one to be activated by pathogens (1) and is usually sufficient to clear the infection. However, when the innate immune system is overwhelmed, it triggers and directs the adaptive arm, thus activating specific B and T cells for pathogen clearance. Receptors expressed by B and T cells are generated through somatic gene rearrangement and hypermutation. This process enables the generation of a virtually infinite repertoire of antigen receptors, allowing the adaptive immunity to specifically recognize any type of microorganism.

In contrast, innate immunity is characterized by its ability to recognize a wide range of pathogens such as viruses, bacteria, and fungi, but through a limited number of germline-encoded receptors called pattern-recognition receptors (PRRs) (2, 3). PRRs are expressed by many cell types including macrophages, monocytes, dendritic cells (DCs), neutrophils, and epithelial cells, and they allow the early detection of pathogens directly at the site of infection. PRRs recognize conserved microbial signatures (4) termed pathogen-associated molecular patterns, or PAMPs (see below). Once activated, the innate immune system initiates the inflammatory response by secreting cytokines and chemokines, inducing the expression of adhesion and costimulatory molecules in order to recruit immune cells to the site of infection and to trigger the adaptive immune response.

Pathogens can rapidly evolve and, in principle, could avoid detection by the innate immune system by simply altering the targeted PAMPs. By doing so, the pathogen would not only escape the recognition by the innate immune system but also avoid the adaptive immune response. However, the immune system has evolved to recognize PAMPs that are essential for the viability of microbes and are thus less prone to modifications. PAMPs can be of diverse origins; sugars, flagellin, and the cell wall components peptidoglycan (PGN) and lipopolysaccharide (LPS) are all recognized by the innate immune system.

The model proposed by Charles Janeway based on PAMP recognition is, however, too simplistic. Indeed, if PAMPs activate the immune pathway, how does the immune system distinguish pathogenic microorganisms from commensal and other non-pathogenic bacteria? To answer this question, Matzinger (5, 6) suggested that the activation of the innate immune system is not only based on the recognition of PAMPs but also relies on the presence of danger signals or danger-associated molecular patterns (DAMPs) released by injured cells. These two models seem completely opposed, but several reports now show activation of innate immunity by host molecules. Indeed among others, mammalian dsDNA (7) and uric acid crystals (8) activate an inflammatory response.

The release of DAMPs is a common event, as tissue damage and cell lysis are often associated with infections and lead to the release of host molecules. Recognition of these DAMPs by the immune system not only allows the sensing of an ongoing infection and subsequent recruitment of more immune cells, but also can initiate the repair of the damaged tissue (9). It seems then that the innate immune pathway not only scans the cellular environment for signs of invading pathogens, but also recognizes the damage caused by them.

PAMPs are recognized by PRRs that are either cytoplasmic, membrane-bound, or secreted; the most intensely studied are the Toll-like receptors (TLRs). These receptors are expressed by many cell types including mononuclear, endothelial, and epithelial cells. Once activated by PAMPs, the TLRs induce different signaling cascades depending on the adaptor protein, ultimately leading to the activation of the transcription factors NF-κB, AP-1, and interferon-regulatory factor (IRF)-3. TLR activation results in the production of antimicrobial peptides, inflammatory cytokines and chemokines, tumor necrosis factor (TNF)-α, and costimulatory and adhesion

molecules, as well as in the upregulation of major histocompatibility complexes (MHCs). As one given pathogen does not trigger only one specific TLR, but rather a set of TLRs leading to the expression of different proteins depending on the nature of the activated TLRs, the immune system is instructed on the type of the invading microorganism and mounts the most appropriate response to fight it. Excellent reviews on the biology of TLRs have been published recently (10–12). TLRs are therefore not the focus of this review.

Recently, two other families of PRRs were described: the NLRs (NOD-like receptors) and the RLHs (RIG-like helicases). Unlike TLRs, these families consist of soluble proteins that survey the cytoplasm for signs that advertise the presence of intracellular invaders. Two RNA helicases, namely RIG-I and MDA5 (13–15), were identified as cytoplasmic, viral RNA sensors. Upon viral stimulation of the two RLHs, NF-κB and IRF3/7 are activated and, in turn, induce the transcription of type I interferon (IFN). Based on the CARD (caspase recruitment domain) homology with RIG-I or MDA5, the CARD adaptor (Cardif, also known as IPS-1, MAVS, or VISA) that induces IFN-β was identified (16–18). Both the CARD domain and the mitochondrial localization of Cardif are required to induce NF-κB and IRF3/7 activation (16, 18).

Studies on TLR signaling pathways and the analysis of key TLR-deficient mice revealed that TLRs could not be the only innate immune receptors responsible for cytokine production. Indeed, computational analysis of the genome identified the NLR proteins. NLR proteins are intracellular LRR (leucine-rich repeat)-containing proteins that resemble plant disease–resistance genes. The characterization of these NLRs has advanced greatly in recent years, underscoring their essential roles in innate immunity. In particular, cytoplasmic complexes called inflammasomes, in which the scaffolding and sensing proteins are members of the NLR family, have been found to be central platforms of innate immunity.

In this review, we examine the remarkably important and emerging functions of inflammasomes as guardians of the body. We begin by describing the general molecular nature of the inflammasome complexes and the known pathways that activate them. We then highlight the current understanding of the function of this pathway—its role in orchestrating host defenses and in the pathogenesis of inflammatory diseases.

THE NLR FAMILY

In recent years, the central role of the NLR family has become increasingly appreciated (19, 20). NLRs form central molecular platforms that organize signaling complexes such as inflammasomes and NOD signalosomes. Most NLRs are expressed in the cytosol. Structurally, NLRs are multidomain proteins with a tripartite architecture containing a C-terminal region characterized by a series of LRRs, a central nucleotide domain termed the NACHT domain (also referred to as NOD domain), and an N-terminal effector domain (**Figure 1**).

The LRR domain has been implicated in ligand sensing and autoregulation of NLRs, yet the precise mechanism of how NLR LRRs sense their ligands is largely unknown. The LRR is a widespread structural motif of 20–30 amino acids with a characteristic pattern rich in the hydrophobic amino acid leucine. LRR domains are formed by tandem repeats of a structural unit consisting of a β strand and an α helix, and are organized in such a way that all the β strands and the helices are parallel to the same axis, resulting in a nonglobular, horseshoe-shaped molecule with the curved β parallel sheet lining the inner circumference and the α helices lining the outer circumference (21). These modules are associated with a wide range of functions including a role as pathogen sensors in various innate immune receptors such as TLRs and NLRs. TLRs contain LRRs that recognize or sense the presence of a wide range of PAMPs including LPS, lipoproteins, flagellin, and RNA from bacteria or viruses. They are believed to sense directly

NOD-like receptor (NLR): cytosolic protein involved in innate immune sensing

RIG-like helicase (RLH): cytosolic helicase involved in innate immune sensing of nucleic acids

Inflammasome: molecular complex involved in the activation of inflammatory caspases resulting in the processing of immature proIL-1β and proIL-18 into their mature forms

NOD signalosome: complex that is assembled upon oligomerization of NOD1 or NOD2 that activates RIP2 and triggers NF-κB activation

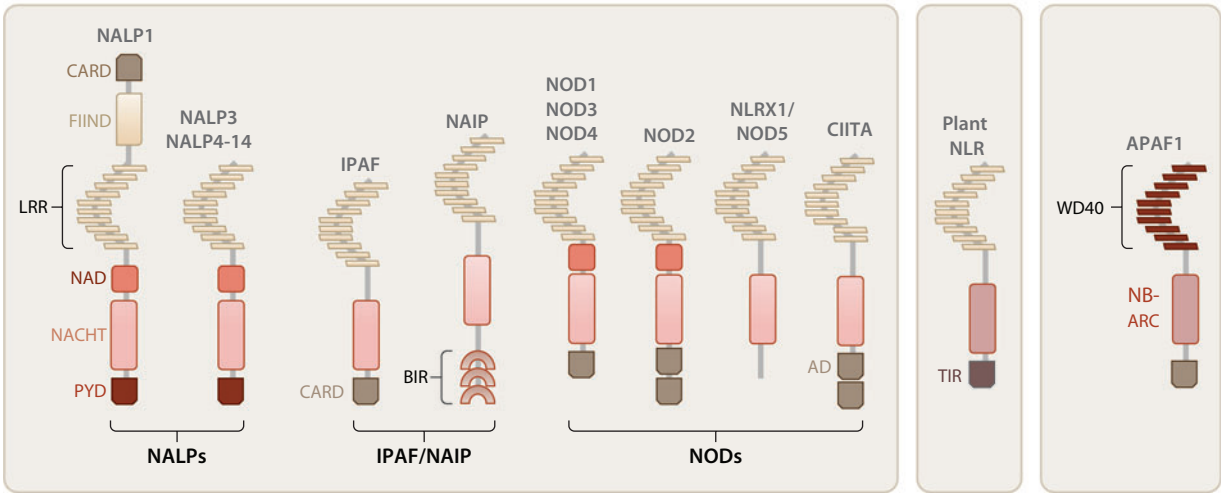

Figure 1

Domain organization of representative NOD-like receptors (NLRs). NLRs are characterized by three distinct domains: the ligand-sensing leucine-rich repeats (LRRs); the NACHT domain, which is responsible for the capacity of NLRs to oligomerize; and the effector domain, which can be a pyrin domain (PYD), CARD, or BIR domain. Most NLRs also contain a NACHT-associated domain (NAD) C-terminal of the NACHT domain. NLRs are divided into two large subfamilies: the 14 members of the PYD-containing NALP clan and the five members of the NODs and CIITA. IPAF and the BIR-containing NAIP form the remaining NLR members. For comparison, the structural organization of a plant NLR-like gene and APAF1 are shown. (Abbreviations: CARD, caspase recruitment domain; PYD, pyrin domain; FIIND, function to find; NACHT, domain conserved in NAIP, CIITA, HET-E and TP1; NAD, NACHT-associated domain; BIR, baculovirus IAP repeat; AD, activation domain; NALP, NACHT, LRR, and PYD-containing protein; CIITA, MHC class II transcription activator; IPAF, ICE-protease-activating factor.)

Apoptosome: oligomeric structure that is assembled when APAF1 interacts with cytochrome *c* released from mitochondria; triggers apoptosis by activating caspase-9

STAND family of NTPases: Subfamily of AAA+ NTPases that includes NLRs

AAA+ NTPases: superfamily of ATPases associated with a variety of cellular activities and characterized by their extended P-loop ATPase domain capable of forming donut-shaped oligomers

or indirectly their activating PAMPs. Double-stranded RNA and lipopeptides have recently been shown to bind TLR3 and TLR1/TLR2 complexes, respectively, whereas LPS-induced TLR4 activation is presumed to be indirect and to involve binding of LPS to MD2 (22, 23). In contrast, no experimental data have convincingly demonstrated a direct interaction between the LRRs of NLRs and their respective activators, suggesting that sensing of pathogens and other signals by NLRs may be indirect.

The NACHT domain, which is central to all NLRs, has similarity to the NB-ARC motif of the apoptotic mediator APAF1. APAF1 performs its cellular function through the formation of a caspase-9 activating, heptameric platform termed an apoptosome. The NB-ARC domain is responsible for dATP/ATP-dependent oligomerization of APAF1 upon cytochrome *c* binding, a process that initiates apoptosis. Both the NACHT and NB-ARC domains belong to the recently defined STAND family of NTPases (24). Oligomerization has been reported for several STAND family proteins, as well as in other related AAA+ NTPases. Similarly, it is believed that the crucial step in NLR activation lies in the oligomerization of the NACHT domain, thereby forming active, high molecular weight complexes that characterize inflammasomes and NOD signalosomes (25, 26).

NLR subfamilies differ in their N-terminal effector domains, which mediate signal transduction to downstream targets, leading to activation of inflammatory caspases by inflammasomes or NF-κB by NOD signalosomes. The vast majority of NLRs harbor a death-fold domain at the N terminus, which is either a CARD or a pyrin domain (PYD) (**Figure 1**, **Table 1**). The death-fold domain superfamily was originally described in proapoptotic signaling pathways and, in addition to CARD and

Table 1 NOD-like receptors (NLRs)

NLR subfamily[a]	Commonly used nomenclature		Other names and aliases	Structure
	human	mouse		
NALPs	NALP1		DEFCAP, NAC, CARD7, NLRP1	PYD-NACHT-NAD-LRR-FIIND-CARD
		NALP1a		NACHT-NAD-LRR-FIIND-CARD
		NALP1a		NACHT-NAD-LRR-FIIND-CARD
		NALP1a		NACHT-NAD-LRR-FIIND-CARD
	NALP2		Pypaf2, NBS1, PAN1, NLRP2	PYD-NACHT-NAD-LRR
		NALP2		PYD-NACHT-NAD-LRR
	NALP3 (Cryopyrin)		Pypaf1, CIAS1, NLRP3	PYD-NACHT-NAD-LRR
		NALP3	Cias1, Pypaf1, Mmig1	PYD-NACHT-NAD-LRR
	NALP4		Pypaf4, PAN2, RNH2, NLRP4	PYD-NACHT-NAD-LRR
		NALP4a	Nalp-η, NALP9D	PYD-NACHT-NAD-LRR
		NALP4b	Nalp-γ, NALP9E	PYD-NACHT-NAD-LRR
		NALP4c	Nalp-α, Rnh2	PYD-NACHT-NAD-LRR
		NALP4d	Nalp-β	PYD-NACHT-NAD-LRR
		NALP4e	Nalp-ε	PYD-NACHT-NAD-LRR
		NALP4f	Nalp-κ, NALP9F	PYD-NACHT-NAD-LRR
		NALP4g		PYD-NACHT-NAD-LRR
	NALP5		Pypaf8, Mater, PAN11, NLRP5	PYD-NACHT-NAD-LRR
		NALP5	mater, Op1	NACHT-NAD-LRR
	NALP6		Pypaf5, PAN3, NLRP6	PYD-NACHT-NAD-LRR
		NALP6		PYD-NACHT-NAD-LRR
	NALP7		Pypaf3, NOD12, NLRP7	PYD-NACHT-NAD-LRR
	NALP8		PAN4, NOD16, NLRP8	PYD-NACHT-NAD-LRR
	NALP9		NOD6, NLRP9	PYD-NACHT-NAD-LRR
		NALP9a	Nalp-θ	PYD-NACHT-NAD-LRR
		NALP9b	Nalp-δ	PYD-NACHT-NAD-LRR
		NALP9c	Nalp-ζ	PYD-NACHT-NAD-LRR
	NALP10		PAN5, NOD8, Pynod, NLRP10	PYD-NACHT-NAD
		NALP10	Pynod	PYD-NACHT-NAD
	NALP11		Pypaf6, NOD17, NLRP11	PYD-NACHT-NAD-LRR
	NALP12		Pypaf7, Monarch1, RNO2, PAN6, NLRP12	PYD-NACHT-NAD-LRR
		NALP12		PYD-NACHT-NAD-LRR
	NALP13		NOD14, NLRP13	PYD-NACHT-NAD-LRR
	NALP14		NOD5, NLRP14	PYD-NACHT-NAD-LRR
		NALP14	Nalp-ι, GC-LRR,	PYD-NACHT-NAD-LRR

(*Continued*)

Table 1 (*Continued*)

NLR subfamily[a]	Commonly used nomenclature		Other names and aliases	Structure
	human	mouse		
IPAF/NAIP	Ipaf	Ipaf	CARD12, CLAN, NLRC4	CARD-NACHT-LRR
		Ipaf	CARD12, CLAN	CARD-NACHT-LRR
	NAIP		BIRC1	BIR3x-NACHT-LRR
		NAIPa	Birc1a, NAIP1	BIR3x-NACHT-LRR
		NAIPb	Birc1b, Naip-rs6, NAIP2	BIR3x-NACHT-LRR
		NAIPc	Birc1c, Naip-rs5, NAIP3	BIR3x-NACHT-LRR
		NAIPd	Birc1d, Naip-rs2, NAIP4	BIR3x-NACHT-LRR
		NAIPe	Birc1e, Naip-rs3, NAIP5	BIR3x-NACHT-LRR
		NAIPf	Birc1f, Naip-rs4, NAIP6	BIR3x-NACHT-LRR
		NAIPg	Birc1g, NAIP7	BIR3x-NACHT-LRR
NODs	NOD1		CARD4, CLR7.1	CARD-NACHT-NAD-LRR
		NOD1	CARD4	CARD-NACHT-NAD-LRR
	NOD2		CARD15, CD, BLAU, IBD1, PSORAS1, CLR16.3	CARD2x-NACHT-NAD-LRR
		NOD2	CARD15	CARD2x-NACHT-NAD-LRR
	NOD3		CLR16.2, NLRC3	CARD-NACHT-NAD-LRR
		NOD3		CARD-NACHT-NAD-LRR
	NOD4		NOD27, CLR19.3, NLRC5	CARD-NACHT-LRR
		NOD4		CARD-NACHT-LRR
	NOD5 (NLRX1)		NOD9, CLR11.3, NLRX1	X-NACHT-LRR
		NOD5(NLRX1)	BC034204	X-NACHT-LRR
	CIITA		MHC2TA, C2TA	(CARD)-AD-NACHT-NAD-LRR
		CIITA	C2TA	(CARD)-AD-NACHT-NAD-LRR

[a]NLRs are divided into two large subfamilies: the 14 members of the PYD-containing NALP clan and the five members of the NODs and CIITA. IPAF and the BIR-containing NAIP form the remaining NLR members.

PYD, includes the death domain (DD) and the death effector domain (DED) (27). The death-fold domain is characterized by six α helices that are tightly packed in a Greek key fold and form trimers or dimers with other members of the same subfamily. In most known cases a DD interacts with a DD, a CARD with a CARD, a DED with a DED, and a PYD with a PYD. These four domains are frequently found in pathways that lead to the activation of caspases or that activate the transcription factor NF-κB. The observation that every death-fold-containing family member is able to interact with another partner harboring the same domain was instrumental in the identification of major signaling pathways involved in apoptosis and immunity. The death fold acts as a molecular velcro that bridges receptors to adaptors and effector proteins. Similarly, the N-terminal PYD or CARD present in NLRs recruits PYD- or CARD-containing molecules to signaling platforms.

NLR Subfamilies

Structurally and functionally, NLRs are divided into subfamilies. In this review, we use the nomenclature that is most commonly used and highlight the various NLR subfamilies. An overview of the family members and their historical or alternative nomenclature is listed in **Table 1**. The NLR family emerged almost ten years ago with the cloning of NOD1 and NOD2 and the subsequent identification of

the PYD domain (28–31). Based on phylogenetic distribution, we distinguish three different NLR subfamilies, all characterized by a specific molecular structure (29, 32) (**Figure 1**). NALPs represent the largest NLR subfamily and have 14 genes identified in humans (29) (**Table 1**). Some of them, such as NALP1, NALP2, and NALP3, were shown to be the central scaffold of caspase-1-activating complexes known as inflammasomes. NALP proteins harbor a NACHT and a LRR and are characterized by an N-terminal PYD domain. Interestingly, the LRR region within NALPs is organized in the genome in a very conserved and precise manner. NALP LRR regions are formed by tandem repeats of exons of exactly 171 nucleotides and are defined completely by a preserved intron-exon structure. Each exon encodes one central LRR and two halves of the neighboring LRRs (33). The phasing and position of the introns are consistent with rapid and efficient exon amplification during evolution. This particular modular organization possibly allows extensive alternative splicing of the LRR region without disturbing the three-dimensional fold of the region and, as a consequence, maximizing variability in the ligand-sensing unit.

Evolutionarily, IPAF and NAIP group together and are well separated from other NLRs. IPAF contains an N-terminal CARD, whereas NAIP has three BIR domains, which are often found in proteins involved in apoptosis such as the IAP family of caspase inhibitors. Both IPAF and NAIP are involved in the formation of inflammasomes, either alone or in combination with one another.

The third class of NLRs includes the remaining CARD-containing NLRs such as NOD1, NOD2, NOD4, and CIITA. This clade also contains a slightly separated group with NOD3 and NOD5/NLRX1 (19, 32). NOD5/NLRX1 does not have a defined N-terminal domain, whereas at least one splice variant of CIITA has been reported to harbor a CARD. NOD1 and NOD2 activate the transcription factor NF-κB, a major regulator of inflammatory responses. CIITA regulates the transcriptional regulation of genes encoding MHC II (34). NOD5/NLRX1 is recruited to the outer membrane of mitochondria (35). The function of NOD5/NLRX1 is still controversial. One study suggested that NOD5/NLRX1 interacts and negatively regulates the antiviral pathway involving the CARD-containing adapter MAVS/CARDIF/IPS-1/VISA (36), whereas another study proposed that NLRX1 promotes the production of reactive oxygen species (ROS) (37). NOD3 and NOD4 have no identified functions yet.

NLR Expression Patterns and Gene Regulation

Expression patterns of most NLRs in various cell populations and tissues have not yet been studied in detail. Nevertheless, the importance of NLRs in defense strategies of the body is supported by the fact that several NLRs are expressed in cells and tissues that have a role in immunity such as phagocytes. Some NLRs are also critical in epithelial cells, which form the first barrier of defense against bacteria in human tissues and express NOD1, NOD2, NALP3, and NAIP (20, 38, 39). NALP1 is widely expressed, whereas NALP3 is found mainly in immune cells, epithelial cells, and osteoblasts (40). NAIP and IPAF are expressed in the brain and in macrophages and macrophage-rich tissues such as spleen, lung, and liver (41, 42). The expression of some NLRs seems to be highly restricted; for example, NALP5, NALP8, NALP4, NALP7, NALP10, and NALP11 are mainly expressed in germ cells and preimplantation embryos (43). Most NLRs may be induced by other branches of the innate immunity as part of a regulatory network. TLR stimulation, for example, increases the expression of NLRs, such as NOD1, NOD2, and NALP3, possibly reflecting enhancement of NLR responses after TLR stimulation (44).

NLRs: Lessons from Evolution

Ever since the discovery of NLRs in mammals, the similarity of these genes with a family of

plant genes involved in immune defenses has cross-fertilized NLR research (28). The plant genes, known as R-genes (R for resistance), are crucial for the immune defense of plants against bacteria, fungi, viruses, and other pathogens. The largest known class of R-genes structurally resembles mammalian NLRs. They have a C-terminal LRR, a central oligomerization module related to the NB-ARC subtype of STAND domains, and an N-terminal effector domain that is generally either a coiled-coil domain or a TIR domain (45). The TIR domain is a well-known recruitment domain involved in the TLR and IL-1R family of immune mediators. There are more than 150 NLR-like R-genes in *Arabidopsis* (46). Many of these genes have been implicated in sensing pathogens. The vast repertoire of NLRs in plants is believed to be the result of a complex host-pathogen race that promoted the evolution of specific NLR genes that genetically interact with specific avirulence genes from distinct pathogens (47). Although plant NLR-like proteins are functionally and structurally similar to mammalian NLRs, there is no evidence for a common evolutionary origin. It is more likely that these innate immune sensors are an example of convergent evolution. The NLR structure (based on a C-terminal LRR sensing unit, a STAND oligomerization module, and an N-terminal recruitment domain) probably originated independently through evolution, which emphasizes key molecular constraints required to design successful innate immune systems (48, 49). Supporting the convergent evolution idea, no NLR-like proteins have been found in insects. In the animal kingdom, the first evolutionarily conserved NLRs are observed in the echinoderm sea urchin.

The observation by Mechnikov of phagocytosis in echinoderms is considered to be a critically defining moment that led to the original concept of immune defenses and, more specifically, innate immunity (50). More than one century after these experiments, the sequencing of the sea urchin genome provided us with another appreciation of the complexity and general conservation of innate immune system mechanisms (51, 52). The sea urchin genome contains 222 TLRs and 203 NLRs. Interestingly, most of the NLRs are expressed in the gut, suggesting that gut-related immunity is a likely driving force behind the expansion of this family in echinoderms (51). These findings highlight NLRs as well as TLRs as evolutionarily important immune genes that preceded the acquisition of the adaptive immune system in vertebrates.

In nonmammalian vertebrates such as zebrafish, three distinct families of NLRs have been identified (53, 54). The first subfamily is related to the NOD subclass of mammalian NLRs and contains orthologs of NOD1, NOD2, NOD3, and NOD4. The second subfamily resembles the NALPs and contains at least six genes. Finally, the last subfamily has the highest similarity with the NACHT domain of NOD3 and has expanded in teleost fish into several hundreds of predicted genes. Most of these genes encode a PYD domain at the N terminus (similar to the one that characterizes NALPs). Interestingly, some of these NLRs have a C-terminal extension following the LRR that contains a PRY-SPRY domain. The PRY-SPRY domain is also found in human Pyrin, a PYD-containing regulator of the inflammasome (see below). The precise role of the PRY-SPRY in Pyrin or in nonmammalian, vertebrate NLRs is unknown; however, this finding further highlights the role of Pyrin and possibly other PRY-SPRY-containing proteins in the regulation of NLRs (55, 56).

Extensive diversification of the NLRs occurred also within the mammalian lineage; this is particularly true for NALPs that mainly evolved through gene duplication events. Some NALPs such as NALP2 and NALP7 in humans are clearly paralogs, whereas others, such as NALP4 and NALP9, expanded only in the mouse (**Table 1**). A similar evolutionary trend was followed by NAIP in mice, where the locus expanded to seven NAIP genes (33).

All these observations indicate that the NLR repertoire within a species, and across vertebrates, is large. Some of these NLRs have undergone lineage-specific amplification, such as NOD3-related NLRs in zebrafish or NALPs in

mammals. Genes involved in interactions with pathogens are likely to diversify by undergoing lineage-specific expansion (57), reflecting the adaptive dynamics of a species to new environments with emerging pathogens.

NOD Signalosomes

NOD1 was one of the first NLRs to be described (58, 59). Both NOD1 and NOD2, once activated, recruit and engage the kinase RIP2 through CARD-CARD interactions. Oligomerization of RIP2 in the NOD signalosome results in the activation of the transcription factor NF-κB (60). Recently, the CARD-containing protein CARD9 has also been found to interact with NOD2 and RIP2 and to be involved in the activation of JNK and p38 by NOD2 (61). Both NOD1 and NOD2 detect muropeptides released from bacterial PGNs. NOD1 and NOD2 sense distinct PGN structures. Whereas NOD2 detects muramyl dipeptides (MDP), the largest motif common to Gram-negative and Gram-positive bacteria, NOD1 detects meso-diaminopimelic acid (meso-DAP), which is mainly found in Gram-negative bacteria (62, 63). Both PGNs are degradation products of bacterial cell wall components released by intracellular or phagocytosed bacteria. Pathogens that do not reach the intracellular compartment of the host cell may use specialized secretion systems to inject the PGN fragment into the host cytosol. *Helicobacter pylori*, for example, elicits NOD1 activation by delivering PGN fragments into the host cell through a mechanism that requires a functional type IV secretion system (64).

NOD1 and NOD2 are crucial innate immune receptors in epithelial cells, where they are important to control infection via the gastro-intestinal route, for example, by *H. pylori* and *Listeria monocytogenes* (64, 65). Importantly, mutations in NOD2 have been associated with Crohn's disease, a form of inflammatory bowel disease. Most NOD2 mutations in these patients affect the LRR region of NOD2 and are believed to disrupt the protein's ability to sense bacteria (66). This probably confers a loss of tolerance toward commensal bacteria or allows the proliferation of pathogenic bacteria in the gut. Gain-of-function mutations in the NACHT domain of NOD2 have been shown to be responsible for Blau syndrome, a rare autoinflammatory disorder starting in childhood and characterized by skin rashes, uveitis, and joint inflammation (67). More recently, NOD2 has been suggested to play a role in the activation of some types of inflammasomes (68, 69). These findings are discussed in more detail below.

INFLAMMASOMES

The term inflammasome was coined to describe a high molecular weight complex that activates inflammatory caspases and the cytokine IL-1β (70). Inflammasome is assembled from the word inflammation—to reflect the function of this complex—and the suffix "some" from the Greek soma meaning body, which is frequently used in cell biology to define entities or molecular complexes such as proteasome, liposome, ribosome, etc. Importantly, the term inflammasome was also chosen to highlight structural and functional similarities with another well-known caspase-activating complex, the apoptosome, a molecular platform that triggers apoptosis (71).

Inflammasomes Lead to Activation of Inflammatory Caspases

Caspases are proteases produced in cells as catalytically inactive zymogens and usually undergo proteolytic processing during activation (72). The subset of caspases that cleave substrates during apoptosis are known as executioner caspases. These executioner caspases (caspase-3, -6, and -7 in mammals) are generally activated by the initiator caspases such as caspase-8, caspase-10, caspase-2, or caspase-9. Initiator caspases harbor an N-terminal death-fold domain (CARD or DED in mammals) that is required for the activation of their C-terminal catalytic region. The mechanism of activation of initiator caspases depends on the engagement and activation of platforms such as the

INFLAMMATORY CASPASES

In mammals, the inflammatory caspases include human and murine caspase-1, human and murine caspase-12, murine caspase-11, and the two caspase-1-related human caspases, caspase-4 and caspase-5 (33, 204). Inflammatory caspases in mammals have a CARD domain followed by a domain containing the catalytic residue cysteine. These caspases are termed inflammatory because the main substrates of caspase-1 identified to date are cytokines (such as IL-1β, IL-18, and possibly IL-33) that are crucial mediators of the inflammatory response. Caspase-1 was the first caspase to be discovered in mammals, but only recently have the pathways (inflammasomes) leading to its activation been discovered (70). Although both human caspase-5 and mouse caspase-11 have been associated with caspase-1 activation, no specific substrates have been described for them. Caspase-5 is recruited by the C-terminal CARD of NALP1, suggesting that it may be involved in the activity of inflammasomes harboring NALP1. Caspase-12 and caspase-4 are activated by endoplasmic reticulum (ER) stress, an adaptive response that copes with protein overload in the ER. However, the function of these inflammatory caspases upon ER stress is unclear (75). Moreover, caspase-12 appears to be an inhibitor of the inflammasome, possibly by interfering with caspase-1 activation, a process that has been associated with susceptibility to sepsis (209).

death-inducing signaling complex (DISC) for caspase-8 and -10, the PIDDosome for caspase-2, and the apoptosome for caspase-9 (73). These platforms integrate cellular signals, recruit initiator caspases via their death-fold domain, and promote dimerization of the caspases, which all lead to the formation of an active enzyme proficient enough to initiate specific signaling cascades (74). Inflammasomes activate a class of caspases known as inflammatory caspases (25, 75) (see sidebar, Inflammatory Caspases). An increasing number of studies highlight the importance and complexity of inflammatory caspase activation.

Prototypical Inflammasomes

Although the biochemistry and diversity of inflammasomes are still poorly understood, we distinguish three prototypes of inflammasomes: The NALP1 inflammasome, the NALP3 inflammasome, and the IPAF inflammasome. For several NALPs, there is evidence for their roles as scaffolding proteins of inflammasomes (76). It is assumed that the PYD of NALPs interacts and recruits the adaptor ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) via PYD-PYD interaction (**Figure 2**). ASC contains an N-terminal PYD and a C-terminal CARD and is an essential component for inflammasome formation (70, 77). The CARD domain within ASC binds and recruits caspase-1 to the inflammasome. NALP1 has a C-terminal extension that harbors a CARD, which was shown to recruit caspase-5 (70) or a second caspase-1 (26). Other NALPs do not have the NALP1 C-terminal extension; instead, CARDINAL (a protein very similar to the NALP1 C terminus) interacts with other inflammasomes such as the NALP3 or NALP2 inflammasome (78). Neither CARDINAL nor NALP1 is highly conserved in mice. The NALP1 locus in mice contains three paralogs that have no functional PYD, whereas CARDINAL is not present in the mouse genome at all. It is therefore possible that NALP1 genes fulfill CARDINAL functions in mice. IPAF has an N-terminal CARD and directly recruits caspase-1 (41) (**Figure 2**).

Basic mechanisms implicated in the activation of NLRs are also involved in inflammasome assembly. IPAF and NALP3 selectively bind ATP/dATP, and nucleotide binding is necessary for oligomerization of the NACHT domain (79, 80). Both IPAF and NALP3 bind SGT1 and HSP90 (81, 82), two proteins whose plant orthologs were previously shown to regulate and interact with plant NLRs. In mammals, as in plants, the activity of the HSP90-SGT1 complex is essential for NALP3 activation, probably for keeping the inflammasome inactive but competent for activation. HSP90 has been suggested to act upstream of NALP1 inflammasome activation by anthrax lethal toxin (83).

Although the IPAF, NALP3, and NALP1 inflammasomes form prototypical inflammasome complexes, recent genetic evidence suggests that other NLRs, such as NAIP or NOD2, may be involved in forming inflammasomes or in modulating their activity. Exactly how and at what step these proteins are connected to the formation of inflammasomes are unknown. It is also unclear whether multiple NLRs may assemble as heterocomplexes to form competent inflammasomes (84, 85). Thus, the complexity and diversity of inflammasomes may turn out to be considerable (86).

INFLAMMASOMES AS SENSORS OF DANGER

Although inflammasomes are emerging more and more as key players in inflammatory and immune responses, a growing number of studies reveal their function in the sensing of a controversial signal: danger. It is well known that a major function of the immune system is to differentiate self from nonself—to respond to self with tolerance and to mount an immune response against nonself. Innate immunity, for example, detects PAMPs from microbes, including pathogens. There is also evidence that innate immunity is able to discriminate pathogenic microbes from nonpathogenic microbes or commensals; but this raises the question of how the immune system interprets the microbial environment allowing the discrimination between nonpathogenic and pathogenic microbes (6). Matzinger and colleagues (87), to account for some unsolved questions of the self-from-nonself model, promoted an alternative hypothesis (the danger hypothesis), suggesting that it is the presentation of an antigen in the context of a danger signal that triggers an efficient immune response, not simply the foreignness of the antigen. This led to multiple studies identifying molecules and signals that are released by damaged or stressed tissues and that trigger or modulate the immune response (88, 89). Both the self-from-nonself model and the danger model may synergize to determine the quality and extent of the innate immune response (90). Although the danger hypothesis was first proposed in mammals, evidence for such a type of immune response was first documented in plants. Plant immunity relies on an effector-triggered immunity that mainly detects pathogen-driven modifications, stress, or danger signals in the infected host cell (91). Similarly, the mammalian NLR NALP3 was found to be involved in sensing danger signals.

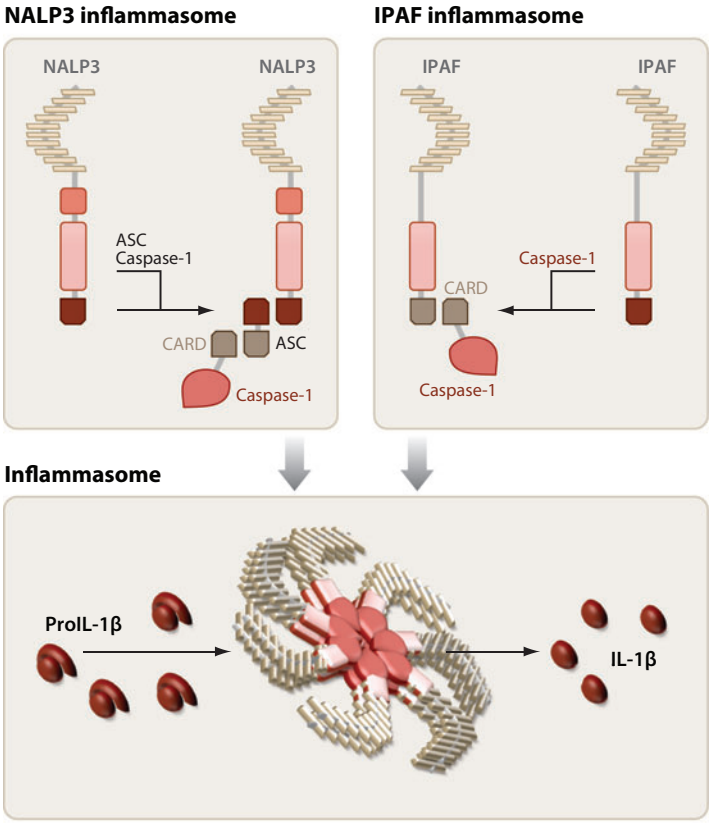

Figure 2

Structural organization of the typical NALP3 and IPAF inflammasomes. The core structure of the NALP3 inflammasome is formed by NALP3, the adaptor ASC, and caspase-1. PYD-PYD and CARD-CARD homotypic interactions are crucial for the recruitment and activation of either the adaptor ASC or the inflammatory caspases (*left panel*). IPAF recruits caspase-1 directly via CARD-CARD interactions (*right panel*). The leucine-rich repeats of NALP3 or IPAF are proposed to sense the activating signals leading to the oligomerization of the NACHT domain and initiating the formation of the donut-shaped inflammasome. Based on the structure of the apoptosome, the caspases and IL-1β processing activity most likely face toward the inside of the donut (*lower panel*).

Cell Disruption Activates the Inflammasome

Early observations that cell lysis in a hypotonic buffer can lead to the processing of proIL-1β provided an important model system that permitted the identification of caspase-1 as the protease responsible for the processing of proIL-1β (92). Using this cell-free system, investigators noticed that caspase-1 activation was restricted to a few cell types, such as the monocytic cell line THP-1. A similar assay subsequently allowed the first biochemical identification and characterization of an inflammasome complex (70). After disruption of cellular integrity, the inflammasome is spontaneously formed. Assembly can be inhibited by complementing the cell extracts with potassium levels that mimic normal levels found in the cytosol of healthy cells (over 70 mM) (70, 93, 94). The observation that subphysiological amounts of potassium are required for spontaneous inflammasome formation suggests that the inflammasome may sense drops in potassium levels. This possibility is supported by recent findings demonstrating that various danger signals and stimuli that activate the NALP3 inflammasome can trigger potassium efflux, thereby lowering the cytosolic potassium concentration of stimulated cells (93, 95) (**Figure 3**).

Sensing Extracellular ATP

Extracellular ATP serves as a danger signal that alerts the immune system by binding to the purinoreceptor P2X7, thereby activating NALP3 and caspase-1 (8, 96–98). Although ATP is emerging as an important modulator of inflammation (99), the critical role of extracellular ATP as a danger signal is unclear. The amount of extracellular ATP that is required to activate macrophages in vitro is relatively high (2 to 5 mM), and, moreover, in vivo most of the extracellular ATP may be rapidly hydrolyzed by ectonucleotidases (100). ATP is released from cells as a consequence of cell damage and/or cellular stress. In endothelial as well as in epithelial cells, ATP release is triggered by nonlytic, mechanical stimuli as diverse as compression,

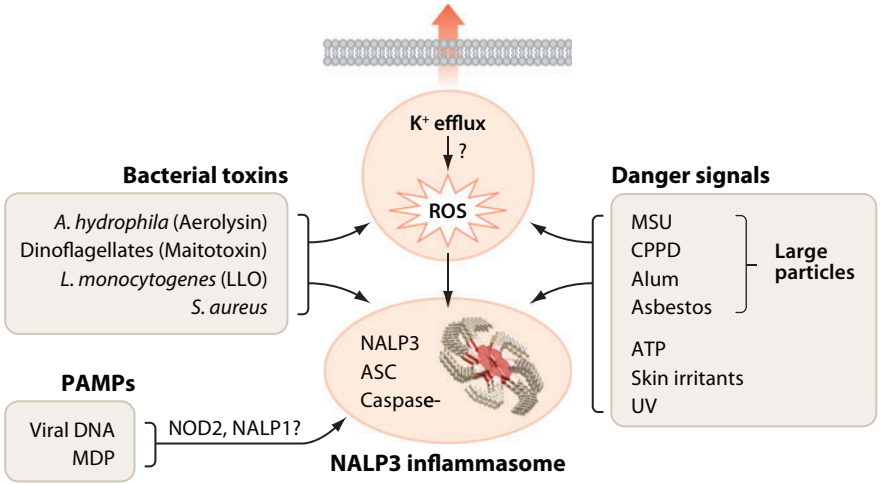

Figure 3

Multiple NALP3 inflammasome activators trigger cellular signals, such as potassium efflux and reactive oxygen species, that eventually activate an inflammasome dependent on caspase-1, ASC, and NALP3. Note that muramyl dipeptide (MDP) activation of caspase-1 may also require the NOD-like receptors NOD2 and NALP1. (Abbreviations: CPPD, calcium pyrophosphate dihydrate crystals; MSU, monosodium urate crystals; PAMP, pathogen-associated molecular pattern; UV, ultraviolet light.)

hydrostatic pressure changes, and hypotonic shock (101). ATP release can also occur through secretory organelles that store large amounts of ATP (102). Adrenal medullary chromaffin granules, which may be released upon physical or psychological stress, have concentrations of ATP around 100 mM, whereas platelet-dense granules contain concentrations of ATP that can reach 500 mM (100 times more than the cytosolic concentration). Similarly, secretion of insulin-containing granules from pancreatic β cells releases the ATP pool stored in the granules (103). ATP can also be released from microbial flora and pathogens. Salivary histatins, for example, trigger ATP efflux from *Candida albicans*, increasing extracellular ATP, a mechanism that may contribute to the antifungal properties of these proteins (104). Exposure of cells to extracellular ATP activates caspase-1 (105). Several studies have demonstrated that ATP-induced caspase-1 activation and subsequent IL-1β maturation requires activation of the purinoreceptor P2X7 in combination with another type of channel, the pannexin-1 channel (106). Interestingly, pannexin-1, besides its role as a gap junction protein, can act as a specific ATP release channel (107). This suggests an amplifying mechanism for P2X7-mediated inflammasome activation via pannexin-1, which is indeed observed, at least in vitro (108).

The generation of ASC-deficient mice demonstrated that ATP-mediated caspase-1 activation requires ASC, and it was therefore probably dependent on the activation of a NALP protein (77, 109). This hypothesis was confirmed in studies using NALP3-deficient mice (8, 96–98), demonstrating that extracellular ATP can act as a danger signal to activate the NALP3 inflammasome and promote caspase-1 activation and IL-1β maturation. Although extracellular ATP has been shown to be involved in inflammatory conditions, as in asthmatic airway inflammation (110), the physiological significance of extracellular ATP-mediated NALP3 inflammasome activation still remains to be demonstrated in vivo.

Uric Acid: A Danger Signal Involved in Gout

In addition to ATP, cells release other danger signals to activate the immune system. In a seminal paper, the Rock laboratory (111) purified, from the supernatant of dying cells, a low molecular fraction that could trigger adjuvanticity in vivo and identified uric acid as the active compound of that fraction. Uric acid is the end product of the cellular catabolism of purines and is present at near saturating amounts in body fluids and at much higher concentration in the cytosol of healthy cells. It is believed that extracellular uric acid coming in contact with the high levels of free sodium present in the extracellular environment nucleates and forms monosodium urate (MSU) crystals. MSU is considered to be the biologically active structure that is responsible for the adjuvantic effect of uric acid. Therefore, formation of this danger signal is the result of a multistep process that starts with the release of uric acid. The biological activity of MSU, including its adjuvanticity, depends on the activation of the NALP3 inflammasome and the production of IL-1, but not on TLRs (8, 112, 113). MSU stimulates the NALP3 inflammasome to produce active IL-1β (8), and macrophages from mice deficient in components of the inflammasome, such as caspase-1, NALP3, and ASC, have a highly reduced crystal-induced IL-1β activation capacity.

The in vivo relevance of uric acid signals has been addressed in a follow-up study by Rock and colleagues (114), who showed that elimination of uric acid reduced the generation of cytotoxic T cells to an antigen in transplanted syngeneic cells and the proliferation of autoreactive T cells in a transgenic diabetes model. It has also been suggested that erythrocytes infected with *Plasmodium* parasites accumulate high levels of the uric acid precursor hypoxanthine, which is released and converted into uric acid upon rupture of the erythrocytes, a process that results in inflammation (115). Uric acid release also occurs in DCs incubated in the presence of alum (aluminum hydroxide),

which is the most widely used adjuvant in human vaccines (116). Interestingly, alum was recently found to be a direct inflammasome activator (113, 117–119) (see below).

Adjuvant: substance that enhances the capacity of an antigen to stimulate the immune system

High levels of circulating uric acid (hyperuricemia) has been associated with various inflammatory diseases, including multiple sclerosis, hypertension, and cardiovascular diseases (120). Hyperuricemia and MSU formation are strongly linked to gout. The autoinflammatory disease gout is characterized by arthropathies generated by the inflammatory reaction to MSU in the joints and periarticular tissues (121). In a model of MSU crystal-induced peritonitis in mice, impaired inflammation is found in inflammasome-deficient mice or mice deficient in the IL-1 receptor (IL-1R) (8, 112), suggesting that the inflammatory response in gout is dependent on the inflammasome. The importance of IL-1 in the pathology of gout is supported by promising preliminary clinical trials in patients with acute gout. Patients responded positively to the injection of the IL-1R antagonist IL-1ra (122, 123). Similarly, patients with pseudogout, an inflammatory disease caused by the deposition of calcium pyrophosphate dihydrate crystals (CPPD), another type of pathogenic microcrystal that activates the NALP3 inflammasome (8), responded well to treatment with the IL-1ra (124).

Silica and Asbestos and Inflammation in the Lung

Alveolar macrophages reside in the respiratory surfaces, one of the major boundaries between the body and the outside world. These cells are phagocytes that play an important role in host defenses against microorganisms and remove particles such as dust. Silica and asbestos dust are particularly strong inflammation inducers in the lungs (125). Macrophages can dissolve MSU, but are not able to efficiently eliminate microparticles of silica and asbestos. Inhaling finely divided crystalline silica or asbestos dust in very small quantities over time can lead to inflammatory conditions known as silicosis and asbestosis, respectively (126). As the dust becomes lodged in the lungs, continuous irritation ensues, resulting in chronic inflammation that favors the development of cancer. This, in particular with asbestos, is associated with the development of malignant mesotheliomas.

Similar to what was found for MSU, asbestos and silica microparticles activate the NALP3 inflammasome (127–129). It is significant to note that pulmonary inflammation is greatly reduced in NALP3-deficient mice after in vivo inhalation of asbestos or silica (127, 128).

Aluminum Particles: An Inflammasome-Dependent Adjuvant

Vaccine adjuvants are exogenic preparations that boost the immune response to achieve protective immunity. Most adjuvants activate innate immune receptors such as TLRs (130). More recently, NLRs and inflammasomes were found to respond to specific adjuvants. Alum has been the most widely used adjuvant in human vaccination for more than half a century (131). Among the early observations on the adjuvantic effect of alum was that immunization with this adjuvant led to an increase in antigen-induced T cell proliferation, apparently resulting from the augmented production of IL-1 (132). Anti-IL-1 antibodies are able to inhibit an antigen-specific T cell proliferative response after immunization with alum adjuvant, but not with Freund's complete adjuvant (133). The ability of alum to activate caspase-1 and produce active IL-1β and IL-18 was demonstrated in vitro (134). Alum-induced caspase-1 activation depends on the NALP3 inflammasome (113, 117–119). Mice deficient in NALP3, ASC, or caspase-1 fail to mount a significant antibody response to antigen immunization with alum adjuvants (113, 117, 119), confirming that the NALP3 inflammasome is a key player that links the innate immune system with the adaptive immune system.

Inflammasomes and Inflammation in the Skin

The skin is the body's first line of defense against external threats and serves as an

effective barrier against ordinary environmental intrusions. As such, the skin is often damaged by various insults and proficient in mounting efficient immune responses. Ultraviolet irradiation, for example, was recently shown to activate the NALP3 inflammasome and promote IL-1β maturation in keratinocytes (135). A role of the inflammasome in the skin was also found in contact hypersensitivity, an inflammatory disease caused by irritant chemicals that penetrate the skin surface and that induce a T cell–mediated immune response (136). This response is divided into two phases. The first is a sensitization phase in which the sensitizing chemical acts both as an adjuvant and as a foreign hapten. Uptake of the chemical by skin-resident antigen-presenting cells (APCs) and their migration to draining lymph nodes ensue, promoting T cell priming. Reexposure to the chemical defines a second phase, also known as the elicitation phase. Here, challenge with the corresponding antigen triggers the activation of primed T cells. The innate immune module of the sensitization phase depends on the presence of functional caspase-1, IL-1β, and IL-18 (137–140), suggesting a potential involvement of the inflammasome. The role of the inflammasome was confirmed in ASC- and NALP3-deficient mice that showed an impaired contact hypersensitivity response to the irritants trinitrophenylchloride (TNP-Cl) (97), trinitrochlorobenzene (TNCB), and dinitrofluorobenzene (DNFB) (141). In these mice, transfer of primed T cells results in a normal contact hypersensitivity, suggesting that only the sensitization phase requires NALP3 and ASC. Interestingly, DNFB promotes the release of IL-1β in a caspase-1-dependent manner in primary keratinocytes as well as in a DC line, suggesting that the inflammasome may either detect such compounds directly or, more likely, may detect some danger signals released or produced by these irritants (142). On the contrary, dinitrothiocanobenzene (DNTB) is unable to activate the inflammasome and, as such, fails to induce a strong immune response in vivo despite the fact that DNTB is competent in inducing the effector phase if the related antigen DNFB (that activates the inflammasome) was used for sensitization (143). Together, these findings suggest that the inflammasome can bridge danger signals triggered by the irritant effect of sensitizing chemicals with the activation of IL-1β and IL-18, thus promoting efficient activation of the adaptive immune system.

Antigen-presenting cells (APCs): cells that process antigens and have special molecules (MHC) that bind the processed antigens and display them on the cell surface for T cells to recognize

ROS, the Common NALP3 Activator?

Most danger signals described to activate the NALP3 inflammasome trigger similar intracellular changes that may converge on a common mechanism of NALP3 activation (**Figure 3**). Potassium efflux, the induction of frustrated phagocytosis, and ROS production are the most striking features associated with NALP3 activators (127). Large particles and crystals such as MSU, alum, asbestos, and silica can induce the so-called frustrated phagocytosis at the surface of the cell, provoking the formation of cytoskeletal filaments (144). Inhibition of cytoskeletal filament generation with the pharmacological agents cytochalasin D or colchicine disrupts the ability of particles to trigger IL-1β activation (8, 113, 127), suggesting that the process of phagocytosis or frustrated phagocytosis is involved in NALP3 activation. Interestingly, colchicine (145) was one of the first anti-inflammatory drugs identified for the treatment of gout by the Greeks more than fifteen centuries ago (146) and is still used in modern medicine to treat inflammatory diseases. ROS production occurs quickly upon exposure of macrophages with silica or asbestos dust (127, 128, 147, 148). Similarly, MSU and alum produce ROS (113, 127). Both ATP and the toxin nigericin (that do not require phagocytosis to activate the inflammasome) activate ROS (149). A cellular redox imbalance also occurs upon cellular stimulation with the skin sensitizer DNCB, and ROS are also induced by UV (150). Along this line, knockdown of NAPDH subunits or the use of antioxidants inhibit inflammasome activation induced by alum, MSU, ATP, nigericin, asbestos, and silica (93, 113, 127, 149). It is therefore reasonable to

Receptor for advanced glycation end product (RAGE): involved in the sensing of HMGB1, a danger signal released by injured cells

propose that ROS are either directly sensed by NALP3 or indirectly sensed through cytoplasmic proteins that modulate inflammasome activity. ROS production by hydrogen peroxide activates DCs in a similar way to TLRs (151) and activates the inflammasome (127).

ROS production is a well-known, highly conserved signal involved in damage and stress sensing. ROS are also important players in innate immune responses in plants (152). *Arabidopsis* mutants that contain disruptions of NADPH oxidases fail to generate a full oxidative burst in response to infection by bacterial and fungal pathogens (48). Interestingly, plant potassium efflux has been linked to ROS production at the membrane (152). Potassium efflux has also been implicated in NADPH activation in granulocytes (153). It is therefore possible that potassium efflux by NALP3 activators may be involved in ROS generation.

INFLAMMASOMES AS SENSORS OF PATHOGENS

A key function of the innate immune system is the recognition of invading microbes. Responses to extracellular PAMPs and some extracellular danger signals are mediated by membrane receptors such as TLRs and RAGE (receptor for advanced glycation end product). However, NLRs are localized in the cytosol and thus are specialized in sampling PAMPs and danger signals that ultimately reach or affect this particular cellular compartment. Although microbes may reach the cytosol of cells during their life cycles, degradation products from phagocytosed bacteria and viruses may also be present in the cytosol and contribute to NLR and inflammasome activation (154). By definition, PAMPs represent molecules vital for microbial survival and are therefore unlikely to vary in their structures because any major change would be detrimental. Examples of PAMPs are bacterial structural components, such as LPS and PGNs, or viral nucleic acids. PAMPs are signatures that define classes of microbes and, as such, are critical in alerting the immune system. In addition to detecting PAMPs, inflammasomes also detect toxins and signals that are restricted to certain pathogens (155) (**Figures 3** and **4**). It is tempting to hypothesize that these signals may orchestrate specific innate immune responses as a result of a unique host-pathogen coevolution maximizing fitness for both the pathogen and the host. The pathogen may benefit from virulence to promote spreading replication and survival, whereas the host evolves to cope with the infection (156).

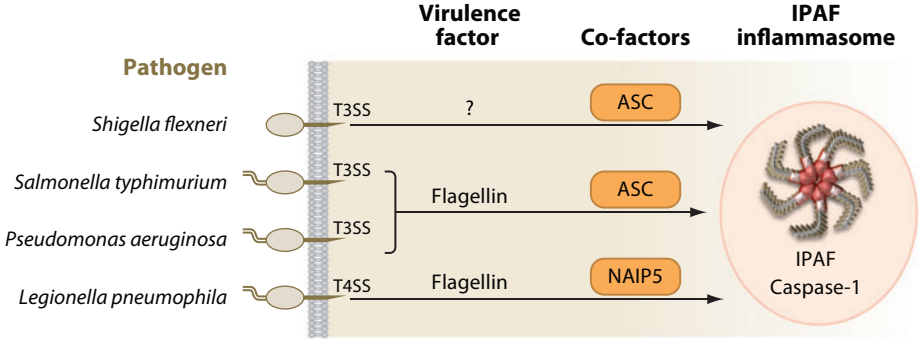

Figure 4

Gram-negative pathogens secrete factors such as flagellin and possibly other virulence factors through type III (T3SS) or type IV (T4SS) secretion systems to trigger an inflammasome dependent on caspase-1 and IPAF. Genetic studies have demonstrated that caspase-1 activation in this context may also require the adaptor ASC or the NOD-like receptor protein NAIP. Whether NAIP and ASC contribute to the formation of IPAF inflammasomes directly or indirectly is unknown.

Inflammasome Activation by PAMPs

Inflammasomes respond to immunomodulatory PAMPs, mainly bacterial PGNs and nucleic acids. PGNs are structural units of cell walls common to all bacteria (157). Degradation of PGNs leads to the release of several structural units including MDP. MDP is sensed in the cytosol by the NLR NOD2, which activates NF-κB. MDP also activates caspase-1 and IL-1β (158) via NALP3 in human monocytes, suggesting that NALP3 is an additional MDP sensor (69, 159, 160). The strength of the immune response to MDP varies greatly and depends on the animal species and genetic background. Mice are much less sensitive than humans, guinea pigs, or rats to these PGN-derived peptides; moreover, C57BL/6 mice are less sensitive than BALB/c mice (161, 162). Interestingly, both NF-κB and IL-1β activation are greatly enhanced by MDP in the presence of the protein synthesis inhibitor cycloheximide (CHX), demonstrating that a CHX-sensitive pathway may affect MDP internalization or its presentation to NLRs (69). Genetic studies in mice have shown that IL-1β activation by MDP requires both NOD2 and NALP3, suggesting that both these NLRs may cooperate either indirectly or directly as part of the same molecular complex (69). This finding is consistent with the observation that monocytes from Crohn's disease patients who have functional mutations in the *NOD2* gene fail to activate IL-1β upon MDP stimulation (163). Moreover, Muckle-Wells patients that harbor a gain-of-function mutation in the *NALP3* gene overproduce IL-1β upon stimulation with MDP (159). Similarly, a probable gain-of-function mutation in *NOD2* in the mouse leads to increased IL-1β production upon stimulation of macrophages with MDP (164). NOD2 has also been suggested to play a role, together with NALP1, in MDP-induced caspase-1 activation, further suggesting that multiple NLRs may cooperate and synergize to mount host defenses (68, 165).

TLR9 and the intracellular protein DAI have been identified as sensors for DNA resulting in the triggering of a type I IFN response (166, 167). Similar to DAI, the inflammasome is capable of sensing cytosolic DNA (168), although this is unlikely to be direct. Infection of a monocytic cell line or mouse macrophages with adenoviruses and herpesviruses leads to activation of caspase-1 and IL-1β. NALP3- and ASC-deficient mice display reduced innate inflammatory responses to infection with adenovirus. Inflammasome activation also occurs as a result of transfected cytosolic bacterial, viral, and mammalian (host) DNA; however, in this case sensing is dependent on ASC only and not on NALP3. It is also independent of TLRs and IRFs (168). Studies have also identified RNA as an activator of NALP3 (98, 169), although this study could not be confirmed by other groups.

Pore-Forming Bacterial Toxins Activate the NALP3 Inflammasome

Many bacterial pathogens produce toxins that contribute to virulence by modifying host responses. Bacterial toxins are generally either enzymes or pore-forming proteins (170). Most of the bacterial toxins that activate NALP3 are pore-forming toxins. These toxins are released by bacteria in a soluble form and subsequently polymerize into a ring-like structure forming a pore in the membrane of the host cell. Pore formation triggers ionic imbalance; in particular, potassium efflux and calcium influxes are observed. These two processes are common danger signals and are frequently associated with NALP3 inflammasome activation (155). Among pore-forming toxins, α-toxin from *Staphylococcus aureus* and aerolysin from *Aeromonas hydrophila* are potent activators of the NALP3 inflammasome (96, 171). Similarly, listeriolysin O (LLO), a toxin released by *Listeria monocytogenesis*, activates caspase-1 in an ASC- and NALP3-dependent manner (96, 172). Interestingly, ivanolysin O, a LLO-related cytolysin that exhibits quite similar function regarding the contribution to the escape of *Listeria* from the phagosome into the cytosol, is unable to restore caspase-1 activation in LLO-deficient strains, suggesting that these toxins may engage specific signals beyond their ability

Virulence factor: molecules produced by pathogens that are involved in host/pathogen interaction and aimed at increasing the rate of infection

to form pores (173). The release into the cytosol of flagellin during *Listeria* infection activates the IPAF inflammasome (but not the NALP3 inflammasome, see below), highlighting the ability of pathogens to engage specific and multiple inflammasomes (174).

Anthrax Lethal Toxin Activates NALP1

Bacillus anthracis is the causative agent of anthrax and depends for its virulence on the secretion of factors that form functional toxins. Anthrax lethal toxin (LeTx) is one of the major toxins produced by *B. anthracis* and is believed to be responsible for causing death in systemic anthrax infections.

Macrophages from inbred mice are either susceptible or resistant to cell death in response to LeTx. This trait difference has been mapped to a locus on chromosome 11 and is associated with a polymorphism in the *nalp1b* gene that impinges on caspase-1 activation (175). How LeTx activates NALP1 and the role of the inflammasome in anthrax pathology are still unknown. Murine NALP1b does not contain a PYD; hence, it is not clear whether it requires ASC or dimerization with another NALP for caspase-1 recruitment. On the other hand, NALP1b possesses a CARD and a region related to CARDINAL. It is therefore possible that this CARD-containing region is able to activate caspase-1 in an ASC-independent manner, as was suggested for human NALP1 in vitro (26). Activation of caspase-1 by LeTx requires binding, uptake, and endosome acidification to mediate translocation of lethal factor (a functional subunit of LeTx) into the host cell cytosol. Interestingly, catalytically active lethal factor activates caspase-1 by a mechanism involving proteasome activity and potassium efflux (176–178).

IPAF Inflammasome Activation by Injected Virulence Factors

IPAF and NAIP5 have been involved in the detection of virulence factors from Gram-negative bacteria. Recognition of *Salmonella typhimurium* and *Shigella flexneri* activates the IPAF inflammasome that requires the ASC adaptor (77, 179). The role of ASC in the IPAF inflammasome is still unclear, but ASC may stabilize or facilitate caspase-1 recruitment to IPAF. Alternatively, IPAF may cooperate with a yet to be defined NALP to activate caspase-1. In contrast to *S. typhimurium* and *S. flexneri*, *Legionella pneumophila* requires two murine NLRs, NAIP5 and IPAF, for inflammasome formation, but does not require ASC (180–182). The role of NAIP5 in IPAF inflammasome activation is unknown. It has been suggested that defective NAIP5 signaling renders macrophages permissive to *L. pneumophila* despite caspase-1 activation, suggesting that NAIP5 may have additional functions beyond its role in IPAF and caspase-1 activation (183). Unlike the seven NAIP genes found in the murine genome, humans only harbor one copy. Consistent with findings in the mouse, knockdown of NAIP or IPAF in human cell lines leads to enhanced susceptibility to *L. pneumophila* (38). IL-18 production and protection against *Anaplasma phagocytophilum*, a neutrophilic obligate bacteria that causes human anaplasmosis, relies on ASC and caspase-1 and partially on IPAF, but not on NALP3 (184). Similarly, *Pseudonomas aeruginosa* specifically activates the IPAF inflammasome (185–187).

Most Gram-negative pathogens that activate IPAF require the type III secretion system (T3SS) or the type IV secretion system (T4SS) to inject into the host cell IPAF-activating virulence factors, mainly flagellin. These bacterial injection machines span the two membranes from the bacteria and the host cell membrane (84). Polymers of flagellin form the flagella, a structure anchored to the bacterial cell wall that enables bacterial motility. Flagellin is a well-known activator of host innate immunity through its capacity to trigger TLR5 activation. In this context, flagellin is considered a PAMP as it is a vital, evolutionarily conserved element of mobile bacteria. In the context of IPAF activation, flagellin could be interpreted as a

virulence factor, as flagella formation is not required and flagellin release in the cytosol is apparently the result of an active process dependent on T3SS or T4SS injection systems (84). Interestingly, *S. flexneri*, a pathogen that does not have a flagellum and apparently does not express flagellin, still requires the T3SS for IPAF-induced caspase-1 activation, indicating that flagellin may not be the only T3SS virulence factor used by pathogens to activate IPAF inflammasomes (188). How flagellin or other virulence factors engage IPAF inflammasomes as well as the precise function of ASC or NAIP in detecting these signals and activating caspase-1 are fascinating questions that need to be solved in future studies.

INFLAMMASOME REGULATORS

Little is known about the mechanisms that regulate inflammasome activity. Inflammasome, caspase-1, and IL-1β activation are best performed in cells that express all the components at high concentrations and in active forms. Most cells do not express all the components required for inflammasome activation, necessitating prior stimulation or sensitization. THP-1 cells, for example, require differentiation of this monocytic cell line into a macrophage-like cell (70). Similarly, mouse macrophages are generally primed with LPS, and the response to MDP may require incubation with the protein synthesis inhibitor CHX (69). The pathogen *Francisella tularensis* first triggers type I IFN activation to enable ASC-dependent inflammasome activation (189). These experimental findings hint of synergisms, feedback loops, and checkpoints that ultimately control inflammasome activation and orchestrate the physiological inflammatory response. Of particular interest are negative feedback loops that are crucial for the resolution phase of inflammation. NF-κB for example, a well-known proinflammatory transcription factor, is also a crucial player in the down-modulation of the inflammatory response including inflammasome activation (190). Although we know little at the physiological level, investigators have identified various proteins that may interfere with inflammasome assembly and inflammatory caspase activation. Based on the modular structure of these proteins, we can distinguish two major types of inflammasome regulators: those containing a CARD domain and those with a PYD domain (**Figure 5**).

Pyrin Domain–Containing Inflammasome Regulators

PYD-containing regulators are believed to interfere with PYD-PYD interaction between NALPs and the adaptor ASC. These PYD regulators include Pyrin, POP1, POP2, and viral PYDs (vPYDs). POPs and the poxviral gene product M13L-PYD (also known as vPYD) are short proteins that contain mainly a PYD (191). Poxviruses deficient in vPYD produce an enhanced activation of caspase-1 and secretion of IL-1β, further strengthening the idea that inflammasomes (that sense viral DNA) are important players in immunity against viruses (192–194). Similarly, POP1 and POP2 modulate inflammasome activity probably by disrupting ASC-NALP interactions (191, 195). The absence in the mouse genome of both POP1 and POP2 makes the evaluation of their physiological importance in vivo challenging. Pyrin was initially identified as the product of the *MEFV* gene, which is mutated in patients with familial Mediterranean fever (FMF) (196), a hereditary autoinflammatory syndrome characterized by episodic fever and serosal or synovial inflammation. Targeted disruption of Pyrin in mice causes increased endotoxin sensitivity and enhanced caspase-1 activation (197). Most of the mutations in Pyrin in FMF patients affect the C terminus, which harbors a PRY-SPRY domain. The function of this domain, which is partially absent in the mouse, is not clear, but a role in the regulation of the inflammasome and caspase-1 was proposed (55, 56, 197). The PYD of Pyrin interacts with the PYD of ASC (197), suggesting that it may be involved in blocking the recruitment of ASC and inflammasome formation. However, artificial overexpression of Pyrin can also be

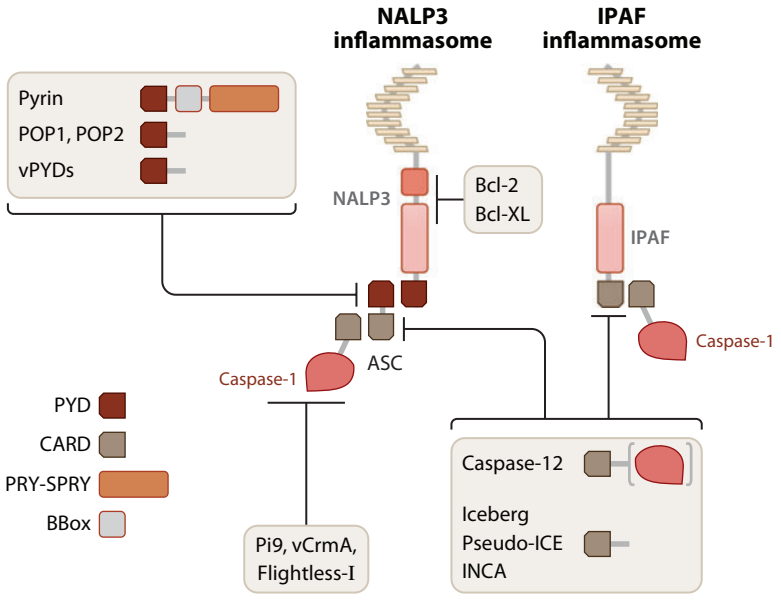

Figure 5

Inflammasome activity is inhibited by PYD-containing proteins that interfere with ASC and NALP interaction and CARD-containing proteins that disrupt caspase-1 interaction with IPAF or ASC. Bcl-2 and Bcl-XL proteins have been suggested to inhibit NALP oligomerization (165). Caspase-1 activity can be directly blocked by Flightless-I (259), and the serpin protease inhibitor 9 (Pi9) (260) or cowpox virus-encoded inhibitor of caspase-1, vCrmA (261).

proinflammatory. This led Fernandes-Alnemri et al. (198) to propose an alternative hypothesis, namely that Pyrin, like NALP3, assembles an inflammasome with ASC and caspase-1. On the other hand, FMF is mainly considered to be an autosomal recessive autoinflammatory disorder, an observation more consistent with the notion that Pyrin loss-of-function may cause the aberrant inflammation in these patients by allowing inflammasome hyperactivation (199). PSTPIP1, a Pyrin-interacting protein, is mutated in PAPA syndrome (pyogenic arthritis, pyoderma gangrenosum, and acne), an autoinflammatory disease associated with overproduction of IL-1β (200). Moreover, mutations in a mouse-related protein, PSTPIP2, cause a macrophage-dependent autoinflammatory syndrome, further delineating the importance of Pyrin and inflammasome regulation in autoinflammatory disorders (201, 202) (see below).

CARD Domain-Containing Regulators

A small family of inflammasome regulators harbor a CARD that is highly similar to the CARD of caspase-1. These proteins most likely emerged from successive gene duplications and include in humans iceberg, INCA, COP, and caspase-12 (33, 203, 204). Through CARD-CARD interactions these proteins presumably inhibit processing of proIL-1β by preventing recruitment and/or activation of the caspase by the adaptor ASC or IPAF. Except for caspase-12, most of these proteins are not present in the mouse or rat genomes, again highlighting considerable differences in the regulation of the inflammasome in diverse species. Caspase-12 is present in two main polymorphic variants in human, resulting in the production of either a truncated protein containing the N-terminal CARD domain (CARD-only) or a full-length variant molecule (caspase-12L) (205).

The full-length variant of caspase-12, which is the less frequent allele, confined to the population of African descent, is linked to hyporesponsiveness to LPS-induced production of cytokines (205, 206). Genetic studies have predicted that the polymorphism generating the caspase-12 short variant was driven by positive selection to complete fixation in the human genome approximately 60,000–100,000 years ago (207, 208). Loss of the caspase-12 C terminus may have conferred a selective benefit, possibly by increasing sepsis resistance in human populations that experienced emergent infectious diseases as geographic expansion occurred in association with increases in human population size and density. In line with this hypothesis, caspase-12-deficient mice clear bacterial infection more efficiently than do wild-type littermates and have an enhanced production of proinflammatory cytokines, including IL-1β and IL-18 (209). Caspase-12 was proposed to be a decoy caspase that blocks caspase-1 activation resulting in enhanced vulnerability to bacterial infection and septic mortality, similar to cFLIP (a decoy caspase-8-like protein), which regulates caspase-8-mediated apoptosis. However, contrary to FLIP, the full-length variant of caspase-12 has autoproteolytic activities, a mechanism that may regulate its anti-inflammasome properties or may underline the possibility that the full-length variant of caspase-12 cleaves specific substrates yet to be identified (210).

INFLAMMASOME SIGNALING AND DISEASE ASSOCIATIONS

Although inflammasomes are involved in both pathways of pathogen and danger signal sensing, their function converges in the activation of inflammatory caspases (mainly caspase-1), which have few known substrates, primarily IL-1β, IL-18, and possibly IL-33. The complexity of inflammasome assembly contrasts with the reductionist vision of its main role as a trigger of IL-1β and IL-18 maturation. Moreover, IL-1β, IL-18, and IL-33 are related cytokines that initiate a MyD88-dependent signaling pathway, very similar to the pathway engaged by TLRs. Therefore, pathogen sensing by inflammasomes can be interpreted as an indirect activation of a TLR-like receptor (IL-1R or IL-18R), a scenario that is comparable to the activation mechanism of the Toll receptor in *Drosophila* (19). Both mammalian and *Drosophila* signaling pathways involve the activation of, in mammals, cytokines IL-1β and IL-18 and, in *Drosophila*, Spätzle through proteolytic processing, which is initiated by microbial sensors that engage and activate specific proteases. Although IL-1-processing inflammasomes in mammals sense pathogens in the cytosol, the proteases that activate Spätzle are localized and activated in the hemolymph of the fruit fly. With this in mind, the complexity and diversity of inflammasomes may reflect the multitude of pathogens and danger signals that are detected in specific locations, whereas the conservation of TLRs and IL-1β/IL-18 receptor signaling cascades emphasizes the importance of MyD88 signaling in innate immunity (19). The discovery of specific activators that signal through an inflammasome-IL-1β pathway brought new interest to the biology of IL-1β. As new caspase-1 substrates are being uncovered, new inflammasome functions will emerge beyond its role in the maturation of IL-1β, IL-18, and possibly IL-33 (171, 211–213).

Inflammasomes, Inflammation, and Inflammatory Diseases

Originally identified as the endogenous pyrogen, exogenous IL-1β triggers fever in experimental animals (214). In addition to fever, IL-1β has multiple other effects on the central nervous system. These include induction of slow-wave sleep, anorexia, and inflammatory pain hypersensitivity, typically associated with infections or injury (214, 215). The important role of IL-1β and the inflammasome in inflammation and fever is strongly supported by genetic evidence that links the inflammasome to

Table 2 Diseases associated with inflammasome activity

Disease	Clinical features	Gene mutated	Etiologic agent	Inflammasome involvement	Anakinra response
Familial cold autoinflammatory syndrome (FCAS)	Fever, arthralgia, cold-induced urticaria	NALP3		overactive	yes
Muckle-Wells syndrome (MWS)	Fever, arthralgia, urticaria, sensorineural deafness, amyloidosis	NALP3		overactive	yes
Chronic infantile neurological cutaneous and articular syndrome (CINCA, NOMID)	Fever, severe arthralgia, urticaria, neurological problems, severe amyloidosis	NALP3		overactive	yes
Familial Mediterranean fever (FMF)	Fever, peritonitis, pleuritis, amyloidosis	Pyrin		overactive	partial
Pyogenic arthritis, pyoderma gangrenosum, and acne syndrome (PAPA)	Pyogenic sterile arthritis	PSTPIP1		overactive	yes
Hyperimmunoglobulin D syndrome (HIDS)	Arthralgia, abdominal pain, lymphadenopathy	Mevalonate kinase		to be demonstrated	yes
Tumor necrosis factor receptor-1-associated syndrome (TRAPS)	Fever, abdominal pain, skin lesions	TNF-R1		to be demonstrated	yes
Systemic juvenile idiopathic arthritis (SOJIA)	Chronic joint inflammation		unknown	to be demonstrated	yes
Adult-onset Still's disease (AOSD)	Arthralgia, fever		unknown	to be demonstrated	yes
Behcet's disease	Arthralgia, uveitis, ulcers		unknown	to be demonstrated	yes
Schnitzler's syndrome	Urticaria, fever arthralgia		unknown	to be demonstrated	yes
Gout	Metabolic arthritis, pain		uric acid (MSU)	activated	yes
Pseudogout	Arthritis		CPPD	activated	yes
Contact dermatitis	Urticaria		irritants	activated	unknown
Fever syndrome	Fever	NALP12		unknown	unknown
Hydatidiform mole	Hydatid mole	NALP7		unknown	unknown
Vitiligo	Skin depigmentation, automimmunity	NALP1		unknown	unknown

a family of hereditary periodic fevers (HPFs) (216) (**Table 2**). HPFs are heritable disorders characterized by unexplained and recurrent episodes of fever and severe inflammation. These patients suffer from rashes and serosal and synovial inflammation with varying degree of neurological involvement. HPFs are part of the expanding family of so-called autoinflammatory diseases that differ from autoimmune disorders in that evidence for adaptive immunity components such as autoreactive T cells or immunoglobulins to self-antigens is lacking (217). Familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and chronic infantile cutaneous neurological articular syndrome (CINCA), also termed neonatal-onset multisystem inflammatory disease (NOMID), are all caused by

mutations in the third exon of NALP3 (218, 219). These diseases have overlapping characteristics and form a clinical continuum. FCAS patients have the less severe symptoms that generally include fever (often triggered by temperature changes), arthralgia, and skin rashes. MWS patients may, in addition, develop amyloidosis together with deafness. NOMID patients have the most severe symptoms that may lead to very serious neurological impairment caused by chronic polymorphonuclear meningitis. The disease-causing mutations in MWS, NOMID, and FCAS are believed to confer gain-of-function to NALP3 leading to a hyperactive inflammasome. Consistent with this model, increased secretion of IL-1β is observed in macrophages from patients with disease-associated NALP3 mutations (78, 220). Moreover, overexpression of mutant NALP3 proteins induces spontaneous IL-1β secretion (221). IL-1β is therefore the likely major mediator of inflammation in this disease. This model is supported by studies demonstrating that treatment of these patients with an inhibitor of IL-1 (IL1-ra) leads to a very striking and dramatic improvement of symptoms in all three conditions (222–225).

Genes responsible for other autoinflammatory diseases may also be linked to inflammasome activity (**Table 2**). FMF is an HPF characterized by recurrent inflammation of serosal surfaces and is associated with mutations in the PYD-containing inflammasome regulator Pyrin (220). The inflammatory disease PAPA is another condition in which impaired IL-1β regulation is caused by mutations in PSTPIP1(CD2BP), a protein that binds to Pyrin and may be involved in scaffolding inflammasome components to the cytoskeleton (200). Other autoinflammatory diseases such as gout, pseudogout, silicosis, and asbestosis are associated with aberrant activation of NALP3. In these cases, aberrant inflammasome activation is not caused by an inherited mutation in inflammasome components or regulators, but by chronic exposure to inflammasome activators such as MSU, CPPD, and inflammation-inducing dust (226).

Inflammasomes, Adjuvanticity, and Autoimmune Diseases

Adjuvants are materials that enhance the immune response to an antigen. Several different types of adjuvants exist, ranging from mineral salts such as alum to oil-based emulsions such as incomplete Freund's adjuvant. On a molecular basis, adjuvants are believed to boost antigen presentation by APCs such as macrophages or DCs. More efficient antigen presentation can be achieved by adding, for example, TLR agonists, which activate the APCs, promote the upregulation of costimulatory molecules, improve antigen presentation, and enhance cytokine induction. Unfortunately, TLR agonists may be too toxic to be used in human vaccines; moreover, immune responses to an antigen can also occur in the absence of TLR signaling (227). Similar to TLR agonists, IL-1β has adjuvant properties. When mice are immunized with protein antigens together with IL-1β, serum antibody production is enhanced. In humans, IL-1 also promotes the expansion of IL-17-secreting memory $CD4^+$ T cells (228, 229). Interestingly, many of the inflammasome activators have adjuvant properties; these include MDP [whose role in enhancing immune responses has been known for more than three decades (230)], MSU (111), and alum (131).

Alum defines particle materials based on aluminum salt precipitates that are the most widely used adjuvants in human vaccines. Alum activates a Th2-biased immunity with elevated Th2-dependent antibody isotypes IgG1 and IgE (131). Similar to MSU, alum triggers an influx of neutrophils when injected in vivo, and both have similar adjuvant properties resulting (in presence of an antigen) in an efficient adaptive immune response (8, 113, 117). Importantly, both MSU and alum depend on an intact inflammasome including NALP3, ASC, and caspase-1 to trigger a Th2-biased response (113, 117, 119). Similarly, silicosis triggered by silica dust (another NALP3 activator) is also characterized by a Th2 response (231). These findings demonstrate the importance of the inflammasome in linking innate immunity to

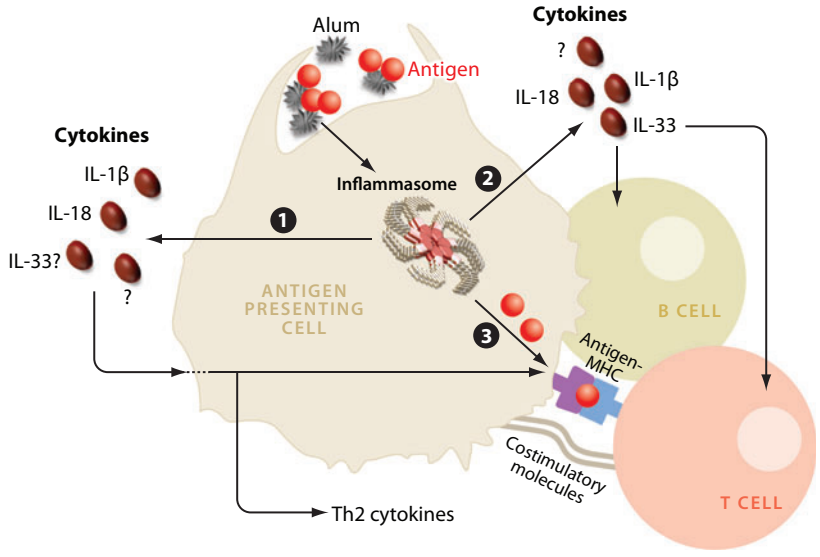

Figure 6

Model of inflammasome-mediated adjuvanticity. Antigen-presenting cells (APCs) can sense antigens (contained within adjuvants, such as alum) through the inflammasome. Inflammasome activation leads to the release of caspase-1-dependent cytokines including IL-1β, IL-18, and IL-33, which may in an autocrine manner trigger their respective receptors to promote antigen presentation, upregulation of costimulatory molecules, and the release of cytokines, which ultimately result in the stimulation of antigen-specific T and B cells (1). Secreted IL-1β, IL-18, and possibly IL-33 may also directly contribute to the activation of T and B cells (2). The question marks illustrate the possibility that the inflammasome may activate an as yet unidentified factor that would signal in a MyD88-independent manner. This could explain the observation that inflammasome-mediated adjuvanticity is MyD88-independent. Yet to be identified substrates and signaling pathways linked to the inflammasome may also directly enhance costimulatory signals (3).

adaptive immunity. A critical question that remains to be addressed is how the inflammasome initiates lymphocyte activation and how it favors Th2 immunity (**Figure 6**). The three cytokines that we know to depend for activity on the NALP3 inflammasome are IL-1β, IL-18, and possibly IL-33, which are all known to trigger various aspects of Th2 immune responses. For example, IL-1 is crucial in regulating Th2 responses during gastrointestinal nematode infection (232) and was suggested more than 20 years ago as mediating Th2 responses upon immunization with an antigen in alum (133). IL-33 induces the expression of IL-4, IL-5, and IL-13 and mediates Th2 polarization by engaging the ST2 receptor (233). IL-18, which is commonly considered to promote Th1 immunity, can amplify Th2 responses and promote Th2-biased pathologies, such as asthma, by triggering the production of IgE antibodies (234). These observations strongly suggest that the inflammasome-generated cytokines play a role in the adjuvant properties of alum and MSU. Surprisingly, no defect in alum adjuvanticity was observed in MyD88-deficient mice (113, 227). MyD88 is a crucial signaling molecule downstream of IL-1 and IL-18. Moreover, MyD88 is downstream of IL-33, suggesting that all three cytokines may not be directly involved in regulating the adjuvant properties of alum, or that MyD88-independent signals, triggered by ST2 or other IL-1R family members, are involved in this process. New inflammasome and caspase-1 substrates have recently been identified, but the role of these proteins in regulating immune responses remains to be investigated in the context of antigen immunization (171, 211–213).

The role of inflammasomes in regulating the adaptive immune response is supported by the findings showing that polymorphisms in NALP1 are associated with vitiligo-associated multiple autoimmune disease (235), a disorder in which the patients, in addition to generalized vitiligo characterized by loss of skin pigments, have increased frequency of several other autoimmune diseases, particularly autoimmune thyroid disease, latent autoimmune diabetes, rheumatoid arthritis, psoriasis, pernicious anemia, systemic lupus erythematosus, and Addison's disease.

Ironically, although the role of the inflammasome in promoting the adaptive immune response and in autoimmunity is now widely accepted, most of the diseases with established inflammasome overactivation lead to autoinflammatory syndromes that, by definition, are devoid of autoreactive T cells and autoreactive antibodies. A better understanding of the role of the various inflammasome mediators in the regulation of the inflammatory (innate) response and the adaptive response may help to solve this paradox.

Inflammasomes and Pyroptosis

Under certain conditions, activation of inflammasomes, and thus inflammatory caspases, leads to cell death. The word pyroptosis—which is derived from the Greek "pyro" (fire), to denote the release of proinflammatory mediators, and "ptosis," which in Greek means falling, a term commonly used to describe cell death—was introduced for this particular type of cell death (236). Pyroptosis is dependent on the activation of caspase-1, is often associated with a high inflammatory state in contrast to the silent apoptotic death, and frequently occurs upon infection with intracellular pathogens. It has been best studied in the context of *Shigella*-infected macrophages. *S. flexneri* is a human intestinal pathogen, causing dysentery by invading the epithelium of the colon. This facultative intracellular pathogen evades the phagosome to enter the cytosol where it can trigger cell death (237). *Shigella*-induced macrophage death requires the inflammatory caspase-1, but not the apoptotic caspase-3 (238, 239). Similarly, caspase-1-dependent cell death has been reported in macrophages infected with *Salmonella typhimurium* (240), *Pseudomonas aeruginosa* (186), *Francisella tularensis* (241), *Legionella pneumophila* (180), and *Listeria monocytogenes* (242).

Surprisingly, inflammasome requirements differ between caspase-1-dependent IL-1β and IL-18 secretion and pyroptosis. Both IPAF and ASC are required for cytokine production by *Salmonella*-infected macrophages; however, only IPAF-deficient macrophages are completely resistant to *Salmonella*-induced pyroptosis, whereas ASC-deficient macrophages are only partially protected (77). Similarly, *S. flexneri* and *P. aeruginosa* require both IPAF and ASC to induce IL-1β secretion, whereas ASC is dispensable for triggering pyroptosis (186–188). In the case of *Bacillus anthracis*, the causative agent of anthrax, pyroptosis depends on NALP1 (176, 243). These findings are in contradiction with an in vitro study proposing that pyroptosis is triggered by an ASC-dependent, but NALP-independent, complex (termed pyroptosome) (198, 244). Overall, these studies support a model whereby ASC as well as NALP and IPAF inflammasomes form distinct complexes, possibly with different cellular localization or different substrate specificity. Detailed biochemical studies are required to shed some light on the various types of inflammasomes formed upon infection of macrophages with live pathogens. It is noteworthy that ASC-dependent inflammasome activation leads to a very rapid secretion of inflammatory caspases including caspase-1 and caspase-5 (70). It is therefore tempting to postulate that the rapid secretion of the inflammatory caspases may be part of a regulatory mechanism aimed at reducing cell death associated with ASC and NALP inflammasomes.

Emerging Inflammasome Functions

Studies on IL-1 and inflammatory caspases have recently highlighted the role of these

Pyroptosis: a form of cell death associated with antimicrobial responses during inflammation and dependent on the activation of an inflammasome and inflammatory caspases such as caspase-1

inflammatory mediators in pathways and pathologies that may involve inflammasome activation, including neurodegenerative disorders, cancer, and fertility-associated conditions (245–247).

IL-1 is a key mediator of experimentally induced neurodegeneration, and its inhibition is neuroprotective in vitro and in vivo (246, 248). Interestingly, NAIP (a NLR associated with the IPAF inflammasome) is partially deleted in individuals with the neurodegenerative disease spinal muscular atrophy (249). The fibrillar peptide amyloid-β has a key function in the pathogenesis of Alzheimer's disease (250). Similar to uric acid crystals, the phagocytosis of amyloid-β was found to activate the NALP3 inflammasome and to be critical for the recruitment of microglia to exogenous amyloid-β in the brain. Thus, the activation of the NALP3 inflammasome may be important for inflammation and tissue damage in Alzheimer's disease.

IL-1β is known to play a role in both ovulation and oocyte maturation (251). In the mare, intrafollicular injection of IL-1β leads to increased ovulation, but also to a very low rate of embryo development, most likely owing to a defect in oocyte maturation (252). Similarly, IL-1β perfusion in the rabbit ovary blocks embryo development at the four-cell stage (253). It is therefore possible that inflammasomes may link some aspects of innate immunity to reproductive biology. Indeed, the expression profiles of NALP4, NALP5, NALP8, and NALP9 in gametes and preimplantation embryos, together with genetic studies, suggest a possible function for these proteins in the biology of reproduction (33). NALP5 (Mater)-deficient female mice are sterile owing to an arrest at the two-cell stage in the development of the embryos (254). Furthermore, mutations in NALP7 cause recurrent hydatidiform moles, an abnormal human pregnancy with no embryo and cystic degeneration of placental villi in humans (255–257). Although it is known that inflammation and bacterial infection cause infertility, ectopic pregnancy, and abortion, the role of NALP7 in this disease is unknown. Similarly, the ability of NALP7, as well as many other NALPs, to form inflammasomes similar to the NALP3 inflammasome has not been investigated.

CONCLUDING REMARKS

In the past decade, our understanding of the cellular and molecular mechanisms by which the innate immune system molecules sense specific molecular patterns from components of invading organisms of both bacterial and viral origin has increased tremendously. The NLRs, together with the TLRs, are now appreciated to be part of this important sensing system that allows the host to mount an effective immune response for elimination of the microbe and for establishment of an effective adaptive immune response for long-lasting immunity. What is fascinating is the realization that at least one NLR member, NALP3, a receptor that was postulated more than 10 years ago, also detects various endogenous, sterile danger signals in the absence of microbial infections. Danger signals include several particles such as uric acid crystals, asbestos, or aluminum, which cause the assembly of the NALP3 inflammasome and the generation of the proinflammatory cytokine IL-1. The remarkable progress in this field offers new hope for many patients. We can anticipate a new generation of IL-1 antagonists in the near future. A new IL-1β antibody is currently in Phase II trials for rheumatoid arthritis, and various inflammatory diseases, such as gout, are now successfully treated with an IL-1 inhibitor. Moreover, the efficiency of aluminum as an adjuvant can now be explained at the molecular level and may help to design effective, but safe, adjuvants in the future.

Yet despite our increased knowledge, many questions remain. The precise roles and needs for molecules such as IPAF and NAIP remain ill defined. The ligands of many other NLRs are unknown and their functions elusive. Although mutations in *NOD2* and *NALP3* genes provide a basis for susceptibility to Crohn's disease and inflammatory diseases, why is a mutation in NALP1 associated with vitiligo? What is the significance of proteins other than IL-1 and

IL-18 cleaved by the inflammasome-activated caspase-1, such as caspase-7 or enzymes of the glycolytic pathway (258)?

From what we have learned about the function of NLRs in the short time since their discovery, it is obvious that a future better understanding of the biology of NLRs will not only help to elucidate their role in host responses to infectious agents and to danger signals, but will certainly contribute to the development of desperately needed novel types of anti-inflammatory drugs.

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