Caspase-1 inflammasomes in infection and inflammation

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Abstract: Nucleotide-binding and oligomerization domain-like receptors (NLRs) constitute a family of germline-encoded pattern-recognition receptors, which allow the host to respond rapidly to a wide variety of pathogenic microorganisms. Here, we discuss recent advances in the study of a subset of NLRs, which control the activation of caspase-1 through the assembly of large protein complexes, inflammasomes. The NALP1b inflammasome recognizes anthrax lethal toxin, and flagellin from *Salmonella* and *Legionella* induces assembly of the Ipaf inflammasome. Cryopyrin/NALP3 mediates caspase-1 activation in response to a wide variety of bacterial ligands, imidazoquinolines, dsRNA, and the endogenous danger signal uric acid. The importance of these cytosolic receptors in immune regulation is underscored by the identification of mutations in cryopyrin/NALP3, which are genetically linked to human autoinflammatory disorders. *J. Leukoc. Biol.* 82: 220–225; 2007.

Key Words: *NLR · TLR · ASC · Ipaf · cryopyrin*

INTRODUCTION

To detect and respond rapidly to diverse groups of microorganisms, the host has evolved a number of germline-encoded pattern-recognition receptors, which detect conserved microbial and viral components, pathogen-associated molecular patterns (PAMPs) [1]. Bacterial cell wall components such as peptidoglycan, bacterial flagellin, and nucleic acid structures, unique to bacteria and viruses, are examples of PAMPs [2]. Members of the TLR family sense the presence of PAMPs extracellularly and in phagosomes, whereas the cytosolic nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) recognize PAMPs in intracellular compartments. In humans, the latter family is composed of 23 members, which share remarkable structural similarity to a subset of plant disease-resistance genes (R genes) [3]. The amino-terminal sequence of NLRs generally contains a caspase recruitment domain (CARD), a pyrin domain, baculovirus inhibitor repeat (BIR) domains, or a so-called X domain of unknown function. The central NOD is thought to be involved in self-oligomerization and activation, and the carboxy-terminal leucine-rich repeat (LRR) motifs sense specific PAMPs and autoregulate NLR activity. In this review, we highlight and discuss recent findings, which contributed significantly to our understanding of the role of NLR proteins in the activation of inflammatory caspases.

CASPASES AND THE INFLAMMASOMES

Caspases are cysteinyl aspartate-specific proteases with essential roles in apoptosis and inflammation [4–6]. They are synthesized as zymogens with a prodomain of variable length, followed by a large and a small catalytic subunit. In humans, the caspase family consists of 11 members, which are classified into three phylogenetic groups, correlating with their function [7]. The subclass of the inflammatory caspases consists of caspase-1, -4, and -5 in humans and caspase-1, -11, and -12 in mice, and caspase-4 and -5 are likely paralogues of murine caspase-11. As the prototypical member of the inflammatory caspases, caspase-1 is responsible for the maturation of pro-IL-1β and pro-IL-18, two related cytokines with critical roles in inflammation [8, 9]. Indeed, caspase-1-deficient mice have a defect in the maturation of pro-IL-1β and pro-IL-18 and are resistant to LPS-induced endotoxic shock [9–11]. Recently, the IL-1-related cytokine IL-33 was suggested as an additional caspase-1 substrate [12], although more conclusive evidence for in vivo caspase-1-mediated processing of this cytokine is required. A breakthrough in the identification of the mechanisms controlling activation of inflammatory caspases came from the identification and characterization of the "inflammasomes," large (700 kDa), multiprotein complexes, which recruit inflammatory caspases and trigger their activation [13, 14]. The inflammasomes are often defined by the distinguishing NLR family member that links recognition of specific pathogens to the proximity-induced autoactivation of caspase-1 [4]. Currently, four distinct inflammasomes have been identified: the NALP1 inflammasome [13, 15], the NALP2 inflammasome [13, 16], the NALP3 or cryopyrin inflammasome [13, 17–23], and the IPAF inflammasome [22, 24–26]. The in vitro composition of the human NALP2 inflammasome has been reported [13], but a detailed study of the endogenous protein complex awaits the identification of specific ligands (**Fig. 1**).

The bipartite adaptor protein ASC has been implicated in the NALP1-3 and Ipaf inflammasomes [13, 21]. In these com-

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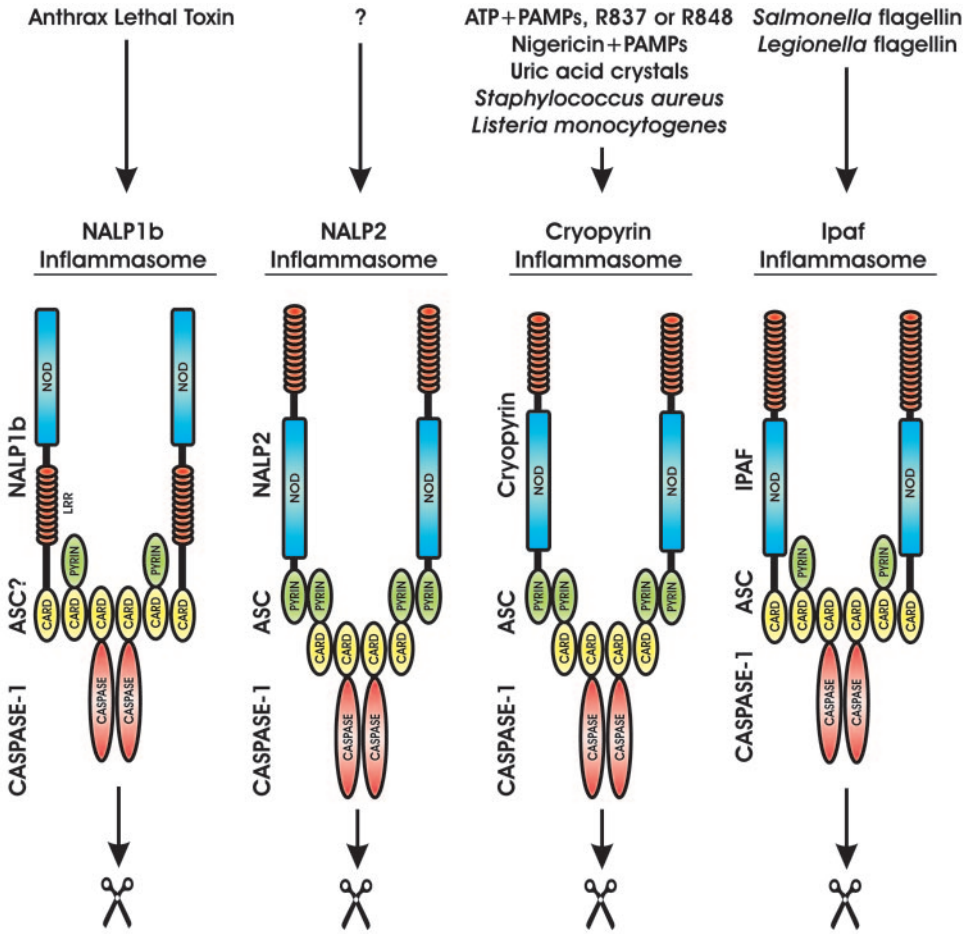

Fig. 1. Ligands and composition of the caspase-1 inflammasomes. The NLR proteins NALP1b, NALP2, cryopyrin, and Ipaf assemble a caspase-1-activating inflammasome complex in response to specific microbial or bacterial factors. The murine NALP1b inflammasome recognizes the cytosolic presence of anthrax lethal toxin (LT), whereas the stimulus that triggers the NALP2 inflammasome remains to be identified. The cryopyrin inflammasome recognizes multiple PAMPs in combination with ATP or nigericin, as well as endogenous danger signals such as uric acid crystals, which may be released by dying cells. Finally, the Ipaf inflammasome senses the cytosolic presence of *Salmonella* and *Legionella* flagellin. The CARD/pyrin-containing inflammasome adaptor apoptosis-associated speck-like protein (ASC) is essential for all inflammasome complexes, although its role in the Nalp1b inflammasome remains to be formally established.

plexes, this 22 kDa adaptor protein bridges the interaction between NLR proteins and inflammatory caspases through homotypic interactions with its own amino-terminal pyrin and carboxy-terminal CARD domains (Fig. 1). As such, ASC plays a central role in the assembly of the inflammasomes and the activation of caspase-1 in response to a broad range of PAMPs and intracellular pathogens [21, 27]. Consistent with this notion, the phenotype of ASC knockout mice mirrors that of caspase-1-deficient animals, including the marked resistance to LPS-induced shock [21, 28]. Moreover, the production of the caspase-1-dependent, proinflammatory cytokines IL-1β and IL-18 was abolished in ASC-deficient macrophages, which were infected with intracellular bacteria or stimulated with bacterial ligands and ATP [21, 27]. In contrast, the secretion of TNF and IL-6 was not affected, suggesting a specific role for ASC in caspase-1 activation.

At least in vitro, another adaptor protein containing a CARD domain, CARDINAL, was shown to interact with caspase-5 and recruit this inflammatory caspase together with caspase-1 to the human NALP1 inflammasome [13]. In contrast, an additional caspase-1 molecule seems to be recruited in the NALP2 and cryopyrin inflammasomes instead of CARDINAL and caspase-5 [13]. Therefore, the role of CARDINAL and caspase-5 in the assembly of inflammasome complexes appears to be more restricted than those of ASC and caspase-1, and its functional contribution to the human NALP1 inflammasome requires further study. Moreover, the gene encoding CARDINAL does not seem to be present in the mouse genome (M. Lamkanfi, unpublished results), and there are no reports currently discussing the roles of the caspase-5 orthologs in the murine NALP1 inflammasomes. In the following sections, we will focus on recent findings that revealed how different pathogens and their ligands activate caspase-1 through the NALP1, Ipaf, and cryopyrin inflammasomes and discuss the possible existence of additional caspase-1-activating inflammasomes.

THE NALP1 INFLAMMASOME RESPONDS TO ANTHRAX LT

LT from *Bacillus anthracis* is believed to be responsible for causing death in systemic anthrax infections [29]. This dimeric toxin consists of the protective antigen subunit, a pore-forming toxin, which delivers the proteolytic subunit lethal factor into the cytosol of infected cells [30]. Recent studies revealed that C57BL/6J macrophages and multiple other inbred mice are resistant, whereas strains such as 129S1 are highly susceptible to LT-induced death [31]. Positional cloning studies indicated that susceptibility maps to mouse chromosome 11 as a single-dominant locus, designated *Ltxs1* [32]. More recently, Boyden and Dietrich [15] identified the presence of a functional *Nalp1b* allele as the key determinant of LT susceptibility in mice. Indeed, macrophages derived from transgenic mice, which

express the susceptible *Nalp1b* allele from 129 S1 mice in a LT-resistant background, became sensitive to LT-induced toxicity [15]. Conversely, inhibition of NALP1b expression using antisense morpholinos rendered LT-sensitive macrophages resistant. These studies suggest that a functional *Nalp1b* allele is required for LT-induced toxicity in mouse macrophages (Fig. 1). In this respect, the *Nalp1b* gene was found to be extremely polymorphic, and at least five distinct *Nalp1b* alleles were identified in the 18 strains analyzed so far [15]. Two alleles were found in susceptible strains, whereas the three remaining alleles correlated with LT-resistant mice. In human macrophages, NALP1 interacts with ASC and the adaptor protein CARDINAL to recruit and activate the inflammatory caspase-1 and -5 [14]. Therefore, the identification of *Nalp1b* as the gene conferring LT sensitivity to mouse macrophages suggested a role for inflammatory caspases in LT-mediated killing of macrophages. Indeed, caspase-1 activation is only detected in LT-sensitive macrophages, and caspase-1-deficient macrophages are resistant to LT-induced death, even in the presence of a sensitive *Nalp1b* allele [15]. However, it is unknown currently whether the mouse orthologs of the remaining components of the human NALP1 inflammasome, i.e., ASC and caspase-11, are also involved in LT-mediated toxicity in mice. In addition, CARDINAL is not encoded by the murine genome. It is noteworthy that in this respect, human NALP1 contains a CARD and pyrin motif to recruit additional inflammasome components, whereas murine NALP1b is devoid of the pyrin domain. The functional mechanism that confers resistance to certain *Nalp1b* alleles and the molecular determinants of the interaction between LT and NALP1b that lead to macrophage killing also warrant further research.

THE IPAF INFLAMMASOME SENSES BACTERIAL FLAGELLIN

The NLR protein Ipaf is critical for sensing and mounting an immune response to intracellular pathogens such as *Salmonella typhimurium* and *Legionella pneumophila* [22, 24–26]. The potent and rapid activation of caspase-1 seen in macrophages infected with these intracellular pathogens is largely abolished in Ipaf-deficient macrophages. Recently, a number of elegant studies have identified flagellin from *S. typhimurium* and *L. pneumophila* as the bacterial ligand, which is sensed by Ipaf [24–26]. Flagellin-deficient bacteria were found to be compromised in their capability to induce caspase-1 activation. Cytosolic delivery of bacterial flagellin requires a functional bacterial secretion system (Type III for *S. typhimurium* and Type IV for *L. pneumophila*), although the molecular mechanism of translocation remains obscure. Direct cytosolic delivery of recombinant purified flagellin in the absence of infecting bacteria induces Ipaf-dependent caspase-1 activation, suggesting that no other bacterial functions but cytosolic delivery of flagellin are required for Ipaf activation [24–26]. It is interesting that detection of flagellin by Ipaf and subsequent caspase-1 activation do not require TLR5, which senses extracellular flagellin [24–26]. These results established a central role for the Ipaf inflammasome in flagellin-induced caspase-1 activation and IL-1β secretion by *S. typhimurium* and *L. pneumophila* (Fig. 1). Furthermore, macrophages are equipped with at least two flagellin receptors (TLR5 and Ipaf), which seem to operate in distinct pathways (NF-κB activation and caspase-1 activation, respectively). In addition to Ipaf, the adaptor protein ASC was reported to be required for *S. typhimurium*-induced caspase-1 activation [21]. It is unclear currently whether Ipaf and ASC also sense bacterial flagellin from other pathogens.

Macrophages from most mouse strains restrict replication of *Legionella*, whereas macrophages derived from the A/J strain are susceptible for *Legionella* growth [33, 34]. Genetic studies in mice identified *NAIP5/Birc1e* as the *Legionella* susceptibility locus [35, 36], delineating the importance of this NLR protein in the control of *Legionella* replication. Whereas Ipaf contains an N-terminal CARD domain, NAIP5 harbors three adjacent BIRs at its N terminus, followed by the centrally located NOD and C-terminal LRRs, which characterize NLR proteins [3]. It is interesting that the studies discussed above showed an increased susceptibility of caspase-1 and Ipaf-deficient macrophages to *Legionella* intracellular replication as a result of an impaired fusion of *Legionella*-containing phagosomes with lysosomes [24], similar to observations in A/J macrophages, carrying a mutated Naip5 allele [37]. A model that links NAIP5 to the caspase-1 pathway has been proposed [38–40]. In this model, infection with *Legionella* activates NAIP5 by delivering flagellin through its Type IV secretion system, which then induces the assembly of the IPAF inflammasome to activate caspase-1 and restrict *Legionella* growth. If this model is correct, caspase-1 activation would be predicted to be abolished in Naip5-deficient macrophages, as occurs in Ipaf-deficient cells. Alternatively, Naip5 may control an additional pathway that restricts *Legionella* growth, independent of Ipaf and caspase-1. Clearly, further studies that address these interesting possibilities are in need.

CRYOPYRIN SENSES MULTIPLE PAMPS AND ENDOGENOUS DANGER SIGNALS

Recent studies revealed an essential role for cryopyrin/NALP3 and the adaptor protein ASC in mediating caspase-1 activation in response to several bacterial ligands, nucleic acids, and synthetic antiviral compounds such as imidazoquinolines (Fig. 1). Indeed, LPS, lipid A, lipoteichoic acid, lipoprotein, and dsRNA were found to induce potent caspase-1 activation through cryopyrin upon stimulation of macrophages with millimolar concentrations of ATP [19, 22, 23]. Bacterial RNA and the imidazoquinoline compounds R837 and R848 alone induce IL-1β secretion [20], but a brief pulse with ATP enhances caspase-1 activation and IL-1β maturation. ATP functions by activating the purinergic $P2X_7$ receptor [41]. Stimulation of the $P2X_7$ receptor with ATP induces a rapid opening of the potassium-selective channel, followed by the gradual opening of a larger pore [42, 43], which is mediated by the hemichannel pannexin-1, recruited upon $P2X_7$ receptor activation [44–46]. Pannexin-1 was found to be critical for caspase-1 activation and IL-1β secretion in LPS-stimulated macrophages pulsed with ATP [45]. The bacterially derived potassium ionophore nigericin and the shellfish toxin maitotoxin can substitute for

ATP to promote caspase-1 activation in a pannexin-1- [44] and cryopyrin-dependent manner [22]. Recent evidence indicates that pannexin-1 mediates the delivery of bacterial molecules into the cytosol, where they are sensed by cryopyrin and activate caspase-1 independently of TLR signaling [47]. Indeed, MyD88; TRIF [Toll/IL-1 receptor (IL-1R) translation initiation region domain-containing adaptor-inducing IFN-β]; and TLR4-deficient macrophages show normal activation of caspase-1 in response to the TLR4 ligand LPS [28, 47]. The repertoire of cryopyrin ligands was broadened further with the discovery that monosodium urate (MSU) and calcium pyrophosphate dehydrate (CPPD) crystals, the causative agents of, respectively, gout and pseudogout, induce cryopyrin-dependent caspase-1 activation in the absence of ATP [18]. It would be interesting to determine at what level the PAMP/ATP and the MSU/CPPD pathways merge to signal through the cryopyrin inflammasome. Finally, Dixit and co-workers [22] reported that cryopyrin is required specifically for the activation of caspase-1 in response to the Gram-positive bacteria *L. monocytogenes* and *S. aureus*.

The importance of cryopyrin in inflammation has become apparent with the discovery that gain-of-function mutations within the NOD of cryopyrin are associated with three hereditary periodic fever syndromes, which are characterized by spontaneous attacks of systemic inflammation, skin rashes, and prolonged episodes of fever in the absence of any apparent infectious or autoimmune etiology. These disorders are known as Muckle-Wells syndrome, familial cold autoinflammatory syndrome, and neonatal-onset multisystem inflammatory disease and are referred to collectively as cryopyrin-associated periodic syndromes (CAPS) [48, 49]. Functional studies revealed that the mutated cryopyrin variants exhibit an enhanced propensity to induce caspase-1 activation and secrete IL-1β [50]. Consistent with these observations, mononuclear cells from CAPS patients secrete IL-1β and IL-18 spontaneously [14, 51]. Administration of an IL-1R antagonist has proven to be an effective treatment for these autoinflammatory syndromes and supports the central roles of caspase-1 and IL-1 in the pathogenesis of these diseases [52]. These findings warrant further study of the mechanisms controlling the activation of the cryopyrin inflammasome.

MORE INFLAMMASOMES?

The Gram-negative coccobacillus *Francisella tularensis*, the causative agent of tularaemia, escapes from the phagosome to replicate in the cytosol of infected macrophages. Phagosomal escape is also linked to the induction of caspase-1 activation as *Franciscella* mutants, which cannot escape the vacuole, are incapable of replicating and activating caspase-1 [53]. The in vivo response to *Franciscella* requires ASC and caspase-1, as bacterial burdens and mortality are increased markedly in ASC and caspase-1-deficient mice [54]. Studies in macrophages derived from these mice confirmed that *Franciscella*-induced caspase-1 activation requires ASC and thus, suggest the involvement of an inflammasome complex. However, cryopyrin and Ipaf-deficient macrophages have no apparent defect in *Franciscella*-induced caspase-1 activation [22, 54]. It remains to be clarified whether the NALP1, NALP2 inflammasome, or another yet-unidentified inflammasome senses the cytosolic presence of *Franciscella*. Moreover, the ligand that triggers caspase-1 activation by this intracellular pathogen is currently obscure, although it is evident that the *Franciscella* genome does not encode flagellin paralogues.

CONCLUSIONS AND PERSPECTIVES

It has now become evident that several NLR family members fulfill important roles in the assembly of inflammasome complexes to induce caspase-1 activation. However, several important questions remain, including the identity of microbial ligands for several inflammasomes and the subcellular localization of inflammasome components. In addition, a better understanding of the mechanisms involved in microbial recognition is required. Indeed, it is unclear currently whether NLR family members such as Ipaf or cryopyrin serve as direct receptors of PAMPs, or instead detect post-translational modifications of host factors induced by the cytosolic activity of bacterial elicitors. It is notable that microbial recognition by NLR homologs in plants has been proposed to proceed largely through an indirect mechanism [55, 56]. Another important question is how the release of endogenous danger signals such as MSU crystals induces inflammasome assembly and caspase-1 activation. Clearly, elucidating the mechanisms involved in microbial recognition through the NLRs will provide a clearer insight into the sophisticated system governing caspase-1 activation and secretion of IL-1β and IL-18. Answering these questions will likely contribute to our knowledge about inflammasome activation and might open up new avenues for therapeutic intervention in inflammatory and infectious diseases.

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REFERENCES

1. Janeway Jr., C. A., Medzhitov, R. (2002) Innate immune recognition. *Annu. Rev. Immunol.* **20,** 197–216.
2. Akira, S., Uematsu, S., Takeuchi, O. (2006) Pathogen recognition and innate immunity. *Cell* **124,** 783–801.
3. Inohara, Chamaillard, McDonald, C., Nunez, G. (2005) NOD-LRR proteins: role in host-microbial interactions and inflammatory disease. *Annu. Rev. Biochem.* **74,** 355–383.
4. Boatright, K. M., Renatus, M., Scott, F. L., Sperandio, S., Shin, H., Pedersen, I. M., Ricci, J. E., Edris, W. A., Sutherlin, D. P., Green, D. R., Salvesen, G. S. (2003) A unified model for apical caspase activation. *Mol. Cell* **11,** 529–541.
5. Nicholson, D. W. (1999) Caspase structure, proteolytic substrates, and function during apoptotic cell death. *Cell Death Differ.* **6,** 1028–1042.

6. Shi, Y. (2002) Mechanisms of caspase activation and inhibition during apoptosis. *Mol. Cell* **9,** 459–470.
7. Lamkanfi, M., Declercq, W., Kalai, M., Saelens, X., Vandenabeele, P. (2002) Alice in caspase land. A phylogenetic analysis of caspases from worm to man. *Cell Death Differ.* **9,** 358–361.
8. Thornberry, N. A., Bull, H. G., Calaycay, J. R., Chapman, K. T., Howard, A. D., Kostura, M. J., Miller, D. K., Molineaux, S. M., Weidner, J. R., Aunins, J., et al. (1992) A novel heterodimeric cysteine protease is required for interleukin-1 β processing in monocytes. *Nature* **356,** 768–774.
9. Ghayur, T., Banerjee, S., Hugunin, M., Butler, D., Herzog, L., Carter, A., Quintal, L., Sekut, L., Talanian, R., Paskind, M., Wong, W., Kamen, R., Tracey, D., Allen, H. (1997) Caspase-1 processes IFN-γ-inducing factor and regulates LPS-induced IFN-γ production. *Nature* **386,** 619–623.
10. Kuida, K., Lippke, J. A., Ku, G., Harding, M. W., Livingston, D. J., Su, M. S., Flavell, R. A. (1995) Altered cytokine export and apoptosis in mice deficient in interleukin-1 β converting enzyme. *Science* **267,** 2000–2003.
11. Li, P., Allen, H., Banerjee, S., Franklin, S., Herzog, L., Johnston, C., McDowell, J., Paskind, M., Rodman, L., Salfeld, J., et al. (1995) Mice deficient in IL-1 β-converting enzyme are defective in production of mature IL-1 β and resistant to endotoxic shock. *Cell* **80,** 401–411.
12. Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., McClanahan, T. K., Zurawski, G., Moshrefi, M., Qin, J., Li, X., Gorman, D. M., Bazan, J. F., Kastelein, R. A. (2005) IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* **23,** 479–490.
13. Martinon, F., Burns, K., Tschopp, J. (2002) The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-β. *Mol. Cell* **10,** 417–426.
14. Agostini, L., Martinon, F., Burns, K., McDermott, M. F., Hawkins, P. N., Tschopp, J. (2004) NALP3 forms an IL-1β-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. *Immunity* **20,** 319–325.
15. Boyden, E. D., Dietrich, W. F. (2006) Nalp1b controls mouse macrophage susceptibility to anthrax lethal toxin. *Nat. Genet.* **38,** 240–244.
16. Bruey, J. M., Bruey-Sedano, N., Newman, R., Chandler, S., Stehlik, C., Reed, J. C. (2004) PAN1/NALP2/PYPAF2, an inducible inflammatory mediator that regulates NF-κB and caspase-1 activation in macrophages. *J. Biol. Chem.* **279,** 51897–51907.
17. Martinon, F., Agostini, L., Meylan, E., Tschopp, J. (2004) Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. *Curr. Biol.* **14,** 1929–1934.
18. Martinon, F., Petrilli, V., Mayor, A., Tardivel, A., Tschopp, J. (2006) Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* **440,** 237–241.
19. Kanneganti, T. D., Body-Malapel, M., Amer, A., Park, J. H., Whitfield, J., Franchi, L., Taraporewala, Z. F., Miller, D., Patton, J. T., Inohara, N., Nunez, G. (2006) Critical role for Cryopyrin/Nalp3 in activation of caspase-1 in response to viral infection and double-stranded RNA. *J. Biol. Chem.* **281,** 36560–36568.
20. Kanneganti, T. D., Ozoren, N., Body-Malapel, M., Amer, A., Park, J. H., Franchi, L., Whitfield, J., Barchet, W., Colonna, M., Vandenabeele, P., Bertin, J., Coyle, A Grant, E. P., Akira, S., Nunez, G. (2006) Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. *Nature* **440,** 233–236.
21. Mariathasan, S., Newton, K., Monack, D. M., Vucic, D., French, D. M., Lee, W. P., Roose-Girma, M., Erickson, S., Dixit, V. M. (2004) Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* **430,** 213–218.
22. Mariathasan, S., Weiss, D. S., Newton, K., McBride, J., O'Rourke, K., Roose-Girma, M., Lee, W. P., Weinrauch, Y., Monack, D. M., Dixit, V. M. (2006) Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* **440,** 228–232.
23. Sutterwala, F. S., Ogura, Y., Szczepanik, M., Lara-Tejero, M., Lichtenberger, G. S., Grant, E. P., Bertin, J., Coyle, A. J., Galan, J. E., Askenase, P. W., Flavell, R. A. (2006) Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1. *Immunity* **24,** 317–327.
24. Amer, A., Franchi, L., Kanneganti, T. D., Body-Malapel, M., Ozoren, N., Brady, G., Meshinchi, S., Jagirdar, R., Gewirtz, A., Akira, S., Nunez, G. (2006) Regulation of Legionella phagosome maturation and infection through flagellin and host Ipaf. *J. Biol. Chem.* **281,** 35217–35223.
25. Franchi, L., Amer, A., Body-Malapel, M., Kanneganti, T. D., Ozoren, N., Jagirdar, R., Inohara, N., Vandenabeele, P., Bertin, J., Coyle, A., Grant, E. P., Nunez, G. (2006) Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1β in salmonella-infected macrophages. *Nat. Immunol.* **7,** 576–582.
26. Miao, E. A., Alpuche-Aranda, C. M., Dors, M., Clark, A. E., Bader, M. W., Miller, S. I., Aderem, A. (2006) Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1β via Ipaf. *Nat. Immunol.* **7,** 569–575.
27. Ozoren, N., Masumoto, J., Franchi, L., Kanneganti, T. D., Body-Malapel, M., Erturk, I., Jagirdar, R., Zhu, L., Inohara, N., Bertin, J., Coyle, A., Grant, E. P., Nunez, G. (2006) Distinct roles of TLR2 and the adaptor ASC in IL-1β/IL-18 secretion in response to Listeria monocytogenes. *J. Immunol.* **176,** 4337–4342.
28. Yamamoto, M., Yaginuma, K., Tsutsui, H., Sagara, J., Guan, X., Seki, E., Yasuda, K., Yamamoto, M., Akira, S., Nakanishi, K., Noda, T., Taniguchi, S. (2004) ASC is essential for LPS-induced activation of procaspase-1 independently of TLR-associated signal adaptor molecules. *Genes Cells* **9,** 1055–1067.
29. Ezzell, J. W., Ivins, B. E., Leppla, S. H. (1984) Immunoelectrophoretic analysis, toxicity, and kinetics of in vitro production of the protective antigen and lethal factor components of Bacillus anthracis toxin. *Infect. Immun.* **45,** 761–767.
30. Leppla, S. H., Arora, N., Varughese, M. (1999) Anthrax toxin fusion proteins for intracellular delivery of macromolecules. *J. Appl. Microbiol.* **87,** 284.
31. Friedlander, A. M. (1986) Macrophages are sensitive to anthrax lethal toxin through an acid-dependent process. *J. Biol. Chem.* **261,** 7123–7126.
32. Roberts, J. E., Watters, J. W., Ballard, J. D., Dietrich, W. F. (1998) Ltx1, a mouse locus that influences the susceptibility of macrophages to cytolysis caused by intoxication with Bacillus anthracis lethal factor, maps to chromosome 11. *Mol. Microbiol.* **29,** 581–591.
33. Yamamoto, Y., Klein, T. W., Newton, C. A., Widen, R., Friedman, H. (1988) Growth of Legionella pneumophila in thioglycolate-elicited peritoneal macrophages from A/J mice. *Infect. Immun.* **56,** 370–375.
34. Yoshida, S., Goto, Y., Mizuguchi, Y., Nomoto, K., Skamene, E. (1991) Genetic control of natural resistance in mouse macrophages regulating intracellular Legionella pneumophila multiplication in vitro. *Infect. Immun.* **59,** 428–432.
35. Diez, E., Lee, S. H., Gauthier, S., Yaraghi, Z., Tremblay, M., Vidal, S., Gros, P. (2003) Birc1e is the gene within the Lgn1 locus associated with resistance to Legionella pneumophila. *Nat. Genet.* **33,** 55–60.
36. Growney, J. D., Dietrich, W. F. (2000) High-resolution genetic and physical map of the Lgn1 interval in C57BL/6J implicates Naip2 or Naip5 in Legionella pneumophila pathogenesis. *Genome Res.* **10,** 1158–1171.
37. Fortier, A., de Chastellier, C., Balor, S., Gros, P. (2007) Birc1e/Naip5 rapidly antagonizes modulation of phagosome maturation by Legionella pneumophila. *Cell. Microbiol.* **9,** 910–923.
38. Molofsky, A. B., Byrne, B. G., Whitfield, N. N., Madigan, C. A., Fuse, E. T., Tateda, K., Swanson, M. S. (2006) Cytosolic recognition of flagellin by mouse macrophages restricts Legionella pneumophila infection. *J. Exp. Med.* **203,** 1093–1104.
39. Ren, T., Zamboni, D. S., Roy, C. R., Dietrich, W. F., Vance, R. E. (2006) Flagellin-deficient Legionella mutants evade caspase-1- and Naip5-mediated macrophage immunity. *PLoS Pathog* **2,** e18.
40. Zamboni, D. S., Kobayashi, K. S., Kohlsdorf, T., Ogura, Y., Long, E. M., Vance, R. E., Kuida, K., Mariathasan, S., Dixit, V. M., Flavell, R. A., Dietrich, W. F., Roy, C. R. (2006) The Birc1e cytosolic pattern-recognition receptor contributes to the detection and control of Legionella pneumophila infection. *Nat. Immunol.* **7,** 318–325.
41. Solle, M., Labasi, J., Perregaux, D. G., Stam, E., Petrushova, N., Koller, B. H., Griffiths, R. J., Gabel, C. A. (2001) Altered cytokine production in mice lacking P2X(7) receptors. *J. Biol. Chem.* **276,** 125–132.
42. Khakh, B. S., North, R. A. (2006) P2X receptors as cell-surface ATP sensors in health and disease. *Nature* **442,** 527–532.
43. Surprenant, A., Rassendren, F., Kawashima, E., North, R. A., Buell, G. (1996) The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X7). *Science* **272,** 735–738.
44. Pelegrin, P., Surprenant, A. (2007) Pannexin-1 couples to maitotoxin- and nigericin-induced interleukin-1β release through a dye uptake-independent pathway. *J. Biol. Chem.* **282,** 2386–2394.
45. Pelegrin, P., Surprenant, A. (2006) Pannexin-1 mediates large pore formation and interleukin-1β release by the ATP-gated P2X7 receptor. *EMBO J.* **25,** 5071–5082.
46. Locovei, S., Scemes, E., Qiu, F., Spray, D. C., Dahl, G. (2007) Pannexin1 is part of the pore forming unit of the P2X(7) receptor death complex. *FEBS Lett.* **581,** 483–488.
47. Kanneganti, T-D., Lamkanfi, M., Kim, Y-G., Chen, G., Park, J-H., Franchi, L., Vandenabeele, P., Nunez, G. (2007) Pannexin-1-mediated recognition of bacterial molecules activates the cryopyrin inflammasome independent of Toll-like receptor signaling. *Immunity*, In press.

48. Martinon, F., Tschopp, J. (2004) Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* **117,** 561–574.
49. McDermott, M. F. (2002) Genetic clues to understanding periodic fevers, and possible therapies. *Trends Mol. Med.* **8,** 550–554.
50. Dowds, T. A., Masumoto, J., Zhu, L., Inohara, N., Nunez, G. (2004) Cryopyrin-induced interleukin 1β secretion in monocytic cells: enhanced activity of disease-associated mutants and requirement for ASC. *J. Biol. Chem.* **279,** 21924–21928.
51. Rosengren, S., Mueller, J. L., Anderson, J. P., Niehaus, B. L., Misaghi, A., Anderson, S., Boyle, D. L., Hoffman, H. M. (2007) Monocytes from familial cold autoinflammatory syndrome patients are activated by mild hypothermia. *J. Allergy Clin. Immunol.*, **119,** 991–996.
52. Hoffman, H. M., Rosengren, S., Boyle, D. L., Cho, J. Y., Nayar, J., Mueller, J. L., Anderson, J. P., Wanderer, A. A., Firestein, G. S. (2004) Prevention of cold-associated acute inflammation in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist. *Lancet* **364,** 1779–1785.
53. Gavrilin, M. A., Bouakl, I. J., Knatz, N. L., Duncan, M. D., Hall, M. W., Gunn, J. S., Wewers, M. D. (2006) Internalization and phagosome escape required for Francisella to induce human monocyte IL-1β processing and release. *Proc. Natl. Acad. Sci. USA* **103,** 141–146.
54. Mariathasan, S., Weiss, D. S., Dixit, V. M., Monack, D. M. (2005) Innate immunity against Francisella tularensis is dependent on the ASC/caspase-1 axis. *J. Exp. Med.* **202,** 1043–1049.
55. Mackey, D., Belkhadir, Y., Alonso, J. M., Ecker, J. R., Dangl, J. L. (2003) Arabidopsis RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. *Cell* **112,** 379–389.
56. Mackey, D., Holt III, B. F., Wiig, A., Dangl, J. L. (2002) RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. *Cell* **108,** 743–754.