Inflammasomes: mechanism of assembly, regulation and signalling

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Abstract | Inflammasomes are multiprotein signalling platforms that control the inflammatory response and coordinate antimicrobial host defences. They are assembled by pattern-recognition receptors following the detection of pathogenic microorganisms and danger signals in the cytosol of host cells, and they activate inflammatory caspases to produce cytokines and to induce pyroptotic cell death. The clinical importance of inflammasomes reaches beyond infectious disease, as dysregulated inflammasome activity is associated with numerous hereditary and acquired inflammatory disorders. In this Review, we discuss the recent developments in inflammasome research with a focus on the molecular mechanisms that govern inflammasome assembly, signalling and regulation.

Pyroptosis
A specialized form of programmed cell death that requires caspases 1 or 11 in mice and caspases 1, 4 or 5 in humans. It is characterized by cytoplasmic swelling, early plasma membrane rupture, nuclear condensation and internucleosomal DNA fragmentation. The cytoplasmic content is released into the extracellular space, and this is thought to augment inflammatory and repair responses.

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The term inflammasome was coined by Tschopp and co-workers[1] in 2002 to describe a high-molecular-weight complex present in the cytosol of stimulated immune cells that mediates the activation of inflammatory caspases[1]. Since this seminal report, the field has expanded substantially, and multiple distinct inflammasomes have been identified, with the assembly of each being dictated by a unique pattern-recognition receptor (PRR) in response to pathogen-associated molecular patterns (PAMPs) or endogenous danger signals in the cytosol of the host cell. Recognition of the inflammatory ligand results in sensor activation, oligomerization and the recruitment of an adaptor protein known as ASC, which consists of two death-fold domains: a pyrin domain (PYD) and a caspase recruitment domain (CARD). These domains allow ASC to bridge the upstream inflammasome sensor molecule to caspase 1. Proximity-induced autoprocessing results in the formation of the catalytically active protease caspase 1, which initiates downstream responses, including the release of interleukin-1β (IL-1β) and IL-18, and induces pyroptosis, which is a lytic form of cell death.

Inflammasomes have been recognized for their crucial role in host defence against pathogens[2], but dysregulated inflammasome activation is linked to the development of cancer and autoimmune, metabolic and neurodegenerative diseases. Therefore, the tight control of inflammasome assembly and signalling is crucial to allow the immune system to initiate antimicrobial and inflammatory responses while avoiding overt tissue damage.

Recent studies have started to unravel the upstream signalling events that result in the engagement of distinct inflammasome-forming receptors and have also started to shed light on the quaternary structure of these large multiprotein complexes. Furthermore, the identification of the pyroptosis mediator gasdermin D established a new paradigm for understanding pyroptosis and other forms of programmed cell death[3,4]. In this Review, we discuss the latest insights into the activation and assembly mechanisms of inflammasomes, the structure of distinct inflammasome complexes and the mechanisms that regulate inflammasome signalling on a molecular level.

Signals that lead to receptor activation

To date, five receptor proteins have been confirmed to assemble inflammasomes, including the nucleotide-binding oligomerization domain (NOD), leucine-rich repeat (LRR)-containing protein (NLR) family members NLRP1, NLRP3 and NLRC4, as well as the proteins absent in melanoma 2 (AIM2) and pyrin (FIGS 1,2). This set of so-called canonical inflammasomes is complemented by the non-canonical pathway, which targets caspase 11 in mice and caspase 4 and/or caspase 5 in human cells (FIG. 3). The ligands and activation mechanisms of these pathways are well characterized and are discussed in more detail below. There may be other less well-characterized pathways, as NLRP6, NLRP7, NLRP12, retinoic acid-inducible gene I (RIG-I; also known as DDX58) and interferon-γ (IFNγ)-inducible protein 16 (IFI16) have been reported to promote caspase 1 activation[2,5].

The NLRP1 inflammasome. NLRP1 was the first NLR to be shown to form an inflammasome complex[1]. Humans only have one NLRP1 protein, which features an amino-terminal PYD, a NOD, LRRs, a function-to-find domain

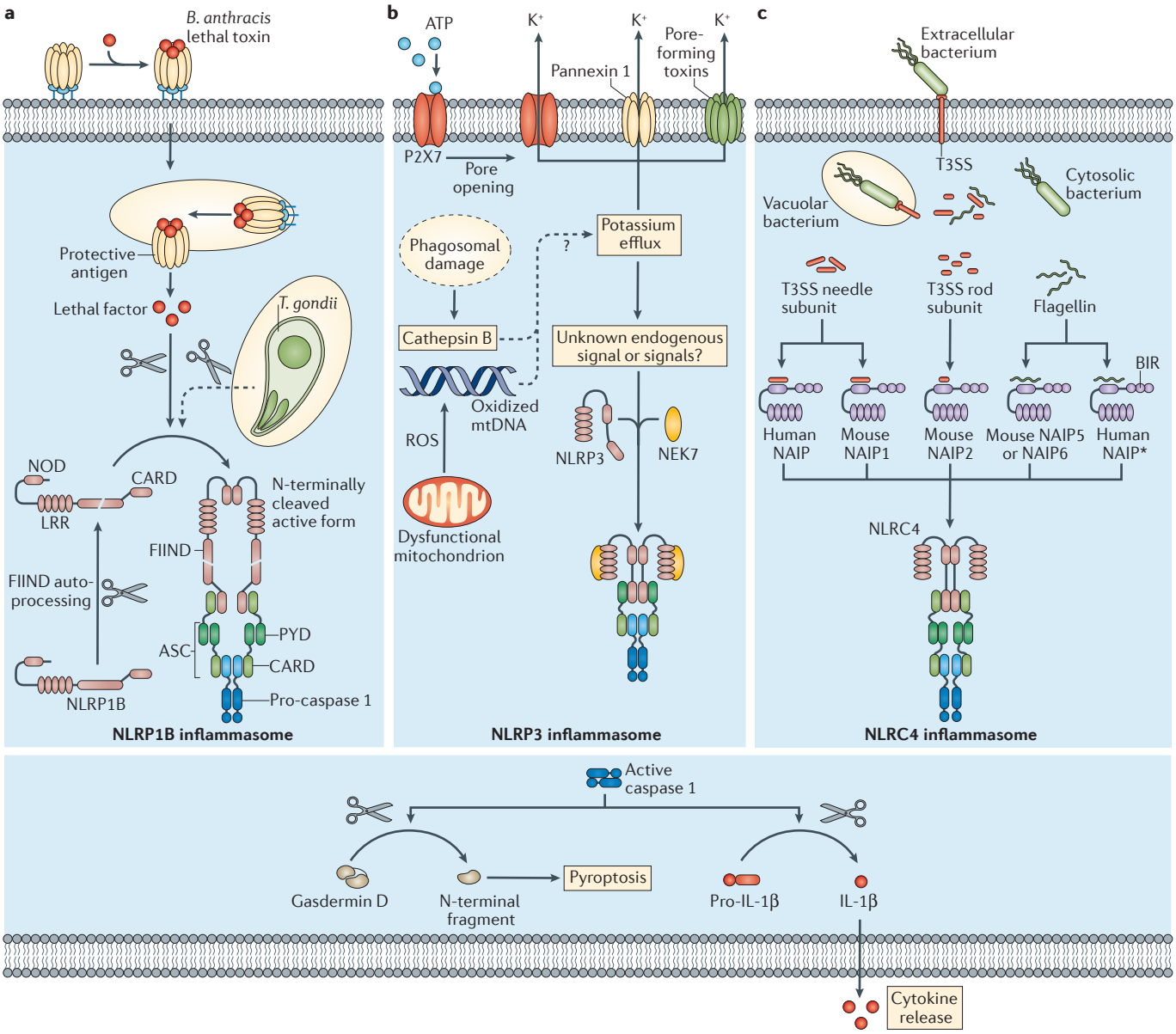

Figure 1 | **The NLR inflammasomes. a** | Mouse NLRP1B and rat NLRP1 respond to *Bacillus anthracis* lethal factor and *Toxoplasma gondii* infection[6,7,11]. Cleavage of NLRP1B or NLRP1 by lethal factor results in the removal of an amino-terminal portion and receptor activation[7,9]. Autoprocessing within the function-to-find domain (FIIND) is also required for activation[10]. **b** | Potassium efflux is a common event that is associated with a diverse array of NLRP3 stimuli[13]. NLRP3 activation also requires NIMA-related kinase 7 (NEK7), which binds to the NLRP3 leucine-rich repeats (LRRs) and is required for its oligomerization[14–16]. **c** | NLR family, apoptosis inhibitory proteins (NAIPs) function as direct receptors for bacterial flagellin and the type 3 secretion system (T3SS) needle and rod subunits[21,22]. Ligand binding results in NAIP activation, allowing it to recruit and activate NLRC4. Activated receptors recruit the bipartite adaptor protein ASC, which consists of a pyrin domain (PYD) and a caspase recruitment domain (CARD), through homotypic PYD–PYD (NLRP3) or CARD–CARD interactions (NLRP1B and NLRC4). The CARD of ASC is necessary to recruit pro-caspase 1 into the complex, although CARD-containing receptors (NLRP1B and NLRC4) might also directly recruit pro-caspase 1. Following its proximity-induced autoproteolytic activation within the complex, caspase 1 processes pro-interleukin-1β (pro-IL-1β) and pro-IL-18 to their mature forms and cleaves gasdermin D[3,4,84]. The N-terminal fragment of gasdermin D drives pyroptosis, which is a lytic type of cell death, and allows the release of mature IL-1β and IL-18 from the cell. mtDNA, mitochondrial DNA; NOD, nucleotide-binding oligomerization domain; P2RX7, P2X purinoceptor 7; ROS, reactive oxygen species.

(FIIND) and a carboxy-terminal CARD. By contrast, multiple paralogues of NLRP1 are found in rodents, such as NLRP1A, NLRP1B and NLRP1C in mice, which share this domain organization but which lack a PYD. Five different alleles of mouse *Nlrp1b* exist and two are associated with responsiveness to lethal toxin of *Bacillus anthracis,* which is an A/B toxin that consists of the protective antigen, a cell-binding protein, oedema

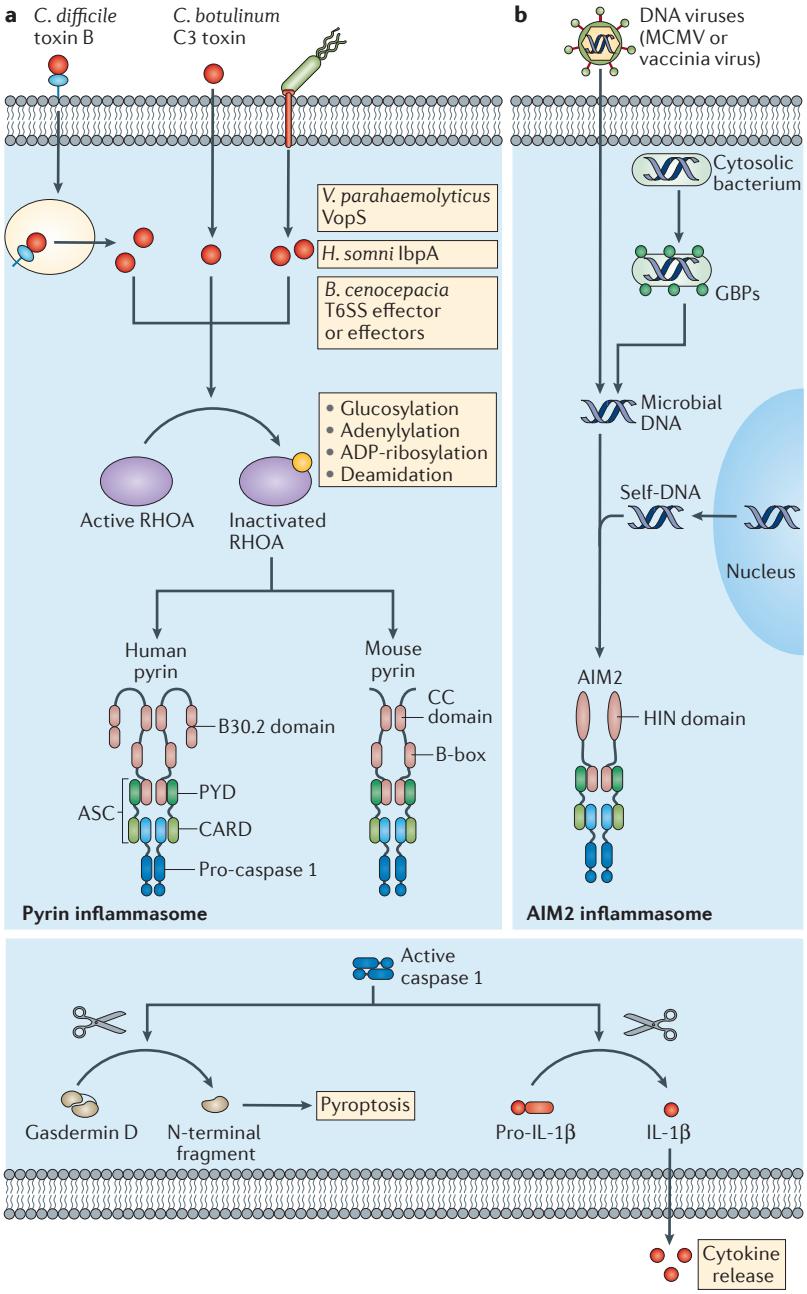

Figure 2 | **The pyrin and AIM2 inflammasomes. a** | Pyrin detects the inactivation of RHOA by bacterial toxins or effector proteins[44]. These toxins target the Switch-I region of RHOA and inactivate it via the glucosylation (by *Clostridium difficile* toxin B), adenylylation (by *Vibrio parahaemolyticus* VopS and *Histophilus somni* IbpA), ADP-ribosylation (by *Clostridium botulinum* C3 toxin) and deamidation (by *Burkholderia cenocepacia* type 6 secretion system (T6SS) effector or effectors) of critical residues. Human pyrin has a B30.2 domain that is not found in mouse pyrin but that responds to RHOA inactivation similarly to mouse pyrin. **b** | Absent in melanoma 2 (AIM2) detects the presence of double-stranded host or microbial DNA in the cytosol[29,30,31]. Murine cytomegalovirus (MCMV), vaccinia virus and several intracellular bacteria activate AIM2, among them *Francisella tularensis* subsp. *novicida*. Recognition of *F. novicida* DNA requires bacteriolysis, which is facilitated by guanylate-binding proteins (GBPs)[117,120]. The recruitment of pro-caspase 1 to activated pyrin and AIM2 relies on the bipartite adaptor protein ASC. Following its proximity-induced autoproteolytic activation within the complex, caspase 1 processes pro-interleukin-1β (pro-IL-1β) and pro-IL-18 to their mature forms and cleaves gasdermin D[3,4,84]. The amino-terminal fragment of gasdermin D drives pyroptosis, which is a lytic type of cell death, and allows the release of mature IL-1β and IL-18 from the cell. CARD, caspase recruitment domain; CC, coiled-coil; PYD, pyrin domain.

factor and lethal factor[6]. The protective antigen forms a channel that translocates the zinc metalloproteinase lethal factor into the host cytosol, where it inactivates immune signalling by cleaving mitogen-activated protein kinase (MAPK) kinases. The initial hypothesis that lethal factor might cleave and activate NLRP1B[6] was confirmed by studies that showed that lethal factor cleaves NLRP1 from lethal toxin-responsive Fisher rats and that cleavage is required for NLRP1-induced caspase 1 activation[7]. Mouse NLRP1B is also activated by lethal factor-mediated cleavage but, unexpectedly, lethal factor cleaves both lethal toxin-responsive and lethal toxin-unresponsive NLRP1B isoforms, which indicates that activation requires an additional event[8,9]. NLRP1B autoprocessing within the FIIND might be such a licensing event, as autoprocessing was shown to be essential for NLRP1B activity and only lethal toxin-responsive alleles undergo autoproteolytic processing[10]. It is likely that lethal factor-mediated cleavage relieves an intramolecular autoinhibition that is conferred by the N terminus and/or that induces conformational changes that allow oligomerization of the receptor. Intriguingly, both rat NLRP1 and mouse NLRP1B confer resistance to *Toxoplasma gondii* infection but the mechanism of activation in this context might be different as no processing of NLRP1B was observed[11]. Nevertheless, the model that has emerged is that rodent NLRP1 proteins act as decoy substrates for the protease lethal factor in response to *B. anthracis*, an ingenious mechanism that was first identified in several plant R (resistance) gene products.

The NLRP3 inflammasome. NLRP3 (also known as cryopyrin) responds to a surprisingly diverse set of stimuli. These include crystalline and particulate matter (such as uric acid crystals, silica, asbestos and alum), extracellular ATP, pore-forming toxins, RNA–DNA hybrids, and several viral, bacterial, fungal and protozoan pathogens[12]. Activation of NLRP3 also requires priming by extracellular inflammatory stimuli, which results in the transcriptional induction of *NLRP3* and controls post-translational modifications that license receptor activation. Given the wide variety of stimuli, it is likely that NLRP3 responds to a common cellular event that is triggered by these activators. However, despite years of research, no unified mechanism for NLRP3 has emerged and many different mechanisms have been proposed, including the release of oxidized mitochondrial DNA, production of reactive oxygen species and mitochondrial dysfunction, lysosomal destabilization, changes in intracellular calcium levels, the formation of large nonspecific membrane pores and potassium efflux (for a detailed review see REF. 12). The meticulous re-examination of these mechanisms has shown that potassium release is associated with all NLRP3 activators and that low potassium medium alone is sufficient to trigger NLRP3 activation[13]. This suggests that a reduction in intracellular potassium levels is the downstream convergence point for NLRP3 stimuli, but whether NLRP3 directly senses this low level of potassium or whether another event correlates with low intracellular potassium concentrations remains to be determined.

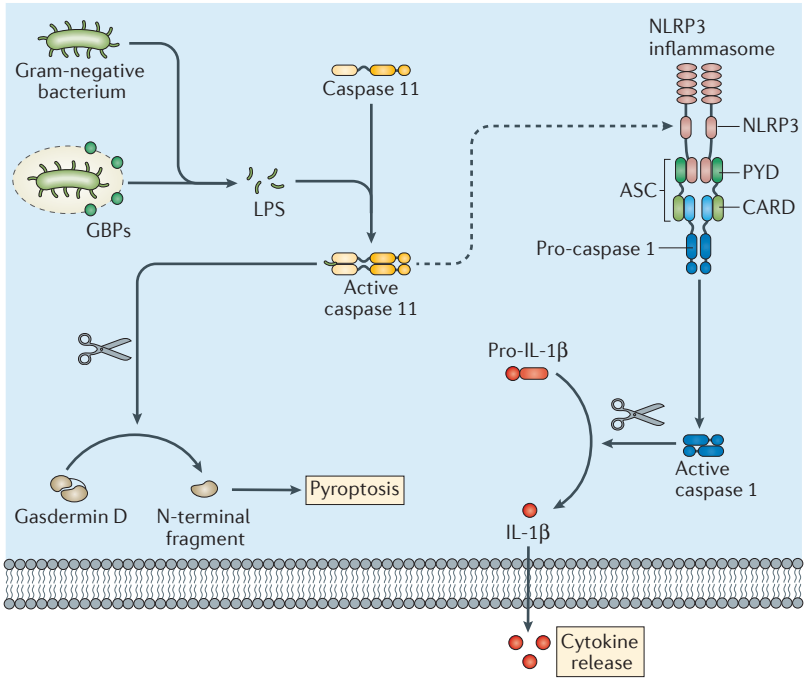

Figure 3 | **The non-canonical inflammasome.** The non-canonical inflammasome pathway results in caspase 11 activation in mice or caspase 4 and caspase 5 activation in humans[49–52]. Lipopolysaccharide (LPS) from intracellular bacteria reaches the cytosol and is directly bound by caspase 11, caspase 4 or caspase 5. Guanylate-binding proteins (GBPs) facilitate the recognition of LPS from vacuolar bacteria, such as *Salmonella enterica* subsp. *enterica* serovar Typhimurium[119]. LPS binding results in the oligomerization and activation of the caspases[50], which then cleave gasdermin D to induce pyroptotic cell death[3,4,84]. Active caspase 11 induces non-canonical activation of NLRP3 possibly by processing pannexin 1 and causing potassium efflux (not shown)[86]. Assembly of the NLRP3 inflammasome results in caspase 1 activation and the processing of pro-interleukin-1β (pro-IL-1β) and pro-IL-18, which are then released by gasdermin D-induced pyroptosis. CARD, caspase recruitment domain; PYD, pyrin domain.

New insights into the mechanism of NLRP3 activation might be provided by three recent reports that have identified an unexpected role for NIMA-related kinase 7 (NEK7) in this process[14–16]. These studies found that NEK7 is essential for NLRP3 activation in response to both canonical and non-canonical stimuli and that it acts downstream of potassium efflux. NEK7 seems to be a component of the NLRP3 complex, as it directly binds NLRP3 and controls NLRP3 oligomerization[14,15]. This interaction requires the catalytic domain of NEK7, but the kinase activity of NEK7 is dispensable for inflammasome activation. Notably, NEK7 was also required for caspase 1 activation by NLRP3(R258W), a gain-of-function mutation that causes Muckle–Wells syndrome[14]. Previous work has linked NEK7 to the formation of mitotic spindles and the separation of centrosomes. Interestingly, mitotic cells show diminished NLRP3–NEK7 interactions and reduced inflammasome activation compared with cells in interphase[15], indicating that NEK7 acts as a cellular switch that enforces mutual exclusivity of the inflammasome response and cell division.

The NAIP–NLRC4 inflammasome. NLRC4 was initially identified on the basis of its similarity to apoptotic-protease activating factor 1 (APAF1) and it was shown to assemble inflammasomes in response to bacterial flagellin[17–19]. Follow-up studies demonstrated that NLRC4 also responds to the rod and needle subunits of bacterial type 3 secretion systems (T3SSs)[20,21]. To detect these distinct ligands, mouse NLRC4 uses NLR family, apoptosis inhibitory proteins (NAIPs) as direct upstream receptors[21,22]. Binding of their cognate ligand — the T3SS rod protein by NAIP2, the T3SS needle protein by NAIP1, and flagellin by NAIP5 and NAIP6 — allows the NAIPs to interact with NLRC4 and initiate inflammasome assembly. Somewhat unexpectedly, ligand sensing is not conferred by the LRR, as was previously assumed, but by the NOD of mouse NAIPs[23]. Humans only have one NAIP, which reportedly responds to the T3SS needle subunit of *Chromobacterium violaceum* and other bacteria[21], but does not respond to flagellin or the T3SS rod protein. However, a recent report showed that two isoforms of human *NAIP* exist and that the extended isoform *NAIP**, which is expressed in primary human monocyte-derived macrophages, confers responsiveness to *Salmonella* flagellin[24].

The AIM2 inflammasome. The existence of a dedicated cytosolic DNA receptor was postulated on the basis of the observation that microbial and host DNA induce caspase 1 activation in an ASC-dependent manner, but independently of NLRP3, Toll-like receptor (TLR) or interferon signalling[25]. Subsequently, several groups showed that this receptor is the PYHIN (pyrin and HIN domain-containing) family member AIM2 (REFS 26–28), which features an N-terminal PYD and a C-terminal HIN domain with tightly packed oligonucleotide or oligosaccharide binding folds. The generation of *Aim2*$^{-/-}$ mice confirmed the importance of AIM2 in coordinating host defence to infections with DNA viruses, such as mouse cytomegalovirus and vaccinia virus, and, surprisingly, also to infections with intracellular bacterial pathogens[29–31]. Follow-up studies have demonstrated that bacterial activation of AIM2 requires the lysis of bacteria, such as *Francisella tularensis* subsp. *novicida* or *Listeria monocytogenes*, in the cytosol[31,32].

A link between AIM2 and several human diseases has been established. Increased AIM2 expression is associated with psoriasis, abdominal aortic aneurysm and systemic lupus erythematosus[33–35]. In the case of psoriasis, autoinflammation could be linked to AIM2-mediated recognition of self-DNA in the cytosol of keratinocytes[34]. By contrast, reduced AIM2 levels correlate with the development of prostate and colorectal cancer[36,37], and it has consistently been shown that *Aim2*-deficient mice are hyper-susceptible to colonic cancer development[38,39]. These reports indicate that besides its role in host defence, AIM2 also has a major role in tumour progression, possibly by sensing self-DNA. Indeed, the release of self-DNA into the cytosol owing to transient nuclear envelope rupture has been reported for cancer cells, but whether AIM2 detects this event remains to be shown.

The pyrin inflammasome. The most recent addition to the group of inflammasome-forming receptors is pyrin (also known as marenostrin and TRIM20), which is encoded

Muckle–Wells syndrome (MWS). A rare autosomal dominant disease caused by mutations in *NLRP3* that lead to the autoactivation of the receptor and increased production of interleukin-1β. The chronic inflammation associated with MWS can lead to deafness and amyloidosis.

Type 3 secretion systems (T3SSs). A virulence-associated specialized molecular machine present in some bacteria that facilitates the translocation of bacterial proteins into host cells.

by the gene *MEFV*. Pyrin features a PYD, two B-boxes and a coiled-coil domain. Unlike mouse pyrin, human pyrin also contains a C-terminal B30.2 domain (also known as a SPRY/PRY domain). Mutations within the B30.2 domain of pyrin are associated with the autoinflammatory disease familial Mediterranean fever (FMF), and result in excessive caspase 1 activation and IL-1β release. Initially, it was postulated that pyrin functions as a negative regulator of other inflammasomes or IL-1β release, and that FMF is caused by loss-of-function mutations in the B30.2 domain[40,41]. However, Chae *et al.*[42] reported that *Mefv*$^{-/-}$ macrophages responded normally to inflammasome activators and they proposed that FMF is instead caused by gain-of-function mutations in *Mefv*, as knock-in mice with mutant B30.2 alleles showed increased caspase 1 activity, which was ASC dependent but NLRP3 independent[42]. Another mutation, which results in the loss of a 14-3-3-binding motif at phosphorylated Ser242, has recently been reported to cause a different type of autoinflammatory disease that is known as pyrin-associated autoinflammation with neutrophilic dermatosis[43].

The physiological function of pyrin in immunity was only recently uncovered when it was shown that bacterial toxins, such as *Clostridium difficile* toxin B and *Clostridium botulinum* C3 toxin, and effector proteins, such as VopS from *Vibrio parahaemolyticus* and IbpA from *Histophilus somni*, trigger inflammasome assembly via pyrin[44]. Interestingly, pyrin seems to specifically recognize the inactivation of RHOA by these proteins but does not recognize the inactivation of the RHO family members RAC and CDC42 and seems to respond irrespective of the type of modification. This might indicate that pyrin does not directly interact with RHOA, but that it senses a downstream event, perhaps related to the modulation of actin dynamics. A direct interaction of pyrin with actin has been reported[45], and a recent study showed that mice homozygous for a polymorphic allele of WD repeat-containing protein 1 (*Wdr1*), which encodes a factor required for actin depolymerization, show pyrin-dependent autoinflammatory disease and thrombocytopaenia[46]. These results support the idea that pyrin detects a specific pathological disturbance of cytoskeleton dynamics. However, the biological relevance of the pyrin inflammasome for host defence has so far only been shown for *Burkholderia cenocepacia*, whereas *C. difficile* infections mainly result in NLRP3 inflammasome activation[47].

Given that only human pyrin contains a B30.2 domain but that both mouse and human pyrin respond to RHOA modification, the role of the B30.2 domain in this process is unclear. Pyrin also belongs to the tripartite motif-containing (TRIM) protein family, although the signature RING domain has been replaced by the PYD. Several TRIMs participate in selective autophagy of protein cargo. Consistent with a role for pyrin in autophagy, a recent study found that it targets NLRP1, NLRP3 and caspase 1 for autophagic degradation, and that the B30.2 domain functions to recognize its cargo[48]. This study supports previous reports that pyrin acts as a regulator of inflammasome signalling, as FMF-associated mutations in the B30.2 domain reduced cargo binding and degradation.

The non-canonical inflammasome. The non-canonical pathway initiates the activation of caspase 11 in mouse cells and caspase 4 and caspase 5 in human cells in response to lipopolysaccharide (LPS; a component of the Gram-negative bacterial cell wall) in the host cell cytosol[49–52]. Upon activation, caspases 4, 5 and 11 initiate pyroptosis similarly to caspase 1, but they do not cleave pro-IL-1β or pro-IL-18 (REFS 49,53). The secretion of mature cytokines is nevertheless observed, as activation of these caspases triggers the assembly of a NLRP3 inflammasome[49,54–56]. Surprisingly, caspase 11-induced pyroptosis was shown to mediate LPS-induced lethality in a mouse model of endotoxaemia and *Escherichia coli*-induced septic shock, which had previously been attributed to TLR4 (REFS 49,51,52). This discrepancy was resolved by the observation that the induction of caspase 11 by injection of poly(I:C) rendered wild-type and *Tlr4*-deficient mice susceptible to LPS-induced lethality, which indicates that caspase 11 drives LPS-induced endotoxaemia, whereas TLR4 is only required to prime this response[51,52]. Therefore, host cells have developed two independent systems to recognize LPS in the extracellular and intracellular spaces.

By analogy to the assembly mechanisms of canonical inflammasomes, a CARD-containing receptor was expected to mediate LPS recognition and caspase 11 recruitment. However, surprisingly, a recent study has suggested that no receptor protein is required; the authors found that caspases 4, 5 and 11 can directly bind LPS, and that this results in the oligomerization and activation of these caspases[50]. Intriguingly, LPS binding was conferred by the caspase 11 CARD, but not by the closely related CARD of caspase 1. The structure of the CARD in complex with LPS is currently unknown and it will be interesting to determine whether there is structural resemblance to other LPS-binding proteins. If confirmed, this mechanism suggests a new activation mode for inflammatory caspases; however, it still remains to be shown whether additional factors are required to promote cytosolic LPS recognition, similarly to LPS-binding protein, CD14 and MD2, which facilitate the detection of extracellular LPS by TLR4.

Other inflammasome complexes. Several other types of inflammasome might also exist, with the best documented being the NLRP6 inflammasome and the IFI16 inflammasome. Evidence for the existence of an NLRP6 inflammasome derived from observations that mice deficient in *Nlrp6*, *Asc* or *Casp1* featured an invasive dysbiotic microbiota that increases their susceptibility to chemically induced colitis and colitis-induced tumorigenesis[57]. This phenotype was linked to a deficiency in basal NLRP6-dependent IL-18 secretion from intestinal epithelial cells, leading to the hypothesis that NLRP6 assembles an inflammasome complex in response to as yet unknown signals. An investigation into the nature of these signals identified several microbiota-derived metabolites that modulate the NLRP6-dependent IL-18 production in colonic explants either positively (taurine) or negatively (spermine and histamine)[58]. Intriguingly, it was also found that a

B30.2 domain
(Also known as SPRY/PRY domain). Defined by a sequence repeat discovered in SplA kinase and ryanodine receptors. B30.2 domains are found in more than 100 human and 70 mouse proteins and are implicated in mediating protein–protein interactions in innate and adaptive immunity.

Familial Mediterranean fever
(FMF). The most common familial inflammatory disease that is characterized by self-limited attacks of fever and serositis. FMF is transmitted in an autosomal recessive pattern and is caused by mutations in the B30.2 domain of *MEFV*, which encodes pyrin.

dysbiotic microbiota is characterized by reduced taurine production and increased production of spermine, and that IL-18 production was required for the expression of antimicrobial peptides, some of which were crucial to maintain microbial diversity. These findings exemplify how the crosstalk between the microbiota and the host maintains intestinal homeostasis and explain why abrogation of IL-18 can lead to commensal dysbiosis. Nevertheless, how NLRP6 controls IL-18 production still needs to be defined, as NLRP6 was also reported to regulate nuclear factor-κB (NF-κB) and MAPK signalling[59], to regulate goblet cell mucus secretion[60], and to participate in the recognition of viral RNA and the subsequent production of type I IFNs and type III IFNs[61].

The human PYHIN family member IFI16 and its mouse orthologue IFI204 have emerged as crucial regulators of STING (stimulator of IFN genes)-dependent IFN production during viral and bacterial infections[62,63]. Conversely, IFI16 was shown to interact with ASC in the nucleus of Kaposi's sarcoma-associated virus (KHSV)-infected cells and to be required for KHSV-induced caspase 1 activation[64]. Another link between IFI16 and caspase 1 activation has been reported for HIV infections[65,66]. In these cases, quiescent 'bystander' $CD4^+$ T cells, which are nonpermissive to HIV infection, accumulate incomplete HIV transcripts in the cytosol. IFI16 was shown to recognize these viral DNAs, leading to caspase 1-dependent death of $CD4^+$ T cells, which is a principle driver of acquired immunodeficiency syndrome[65,66]. However, because IFI16-dependent IFN production might also enhance caspase 1 activation (see below), additional studies will be necessary to unequivocally prove the existence of an IFI16 inflammasome.

Inflammasome assembly and structure

The receptor complex. Whereas the signals that lead to receptor activation are well studied, insights into the structure of the inflammasome complex have only now begun to emerge (FIG. 4). Given the functional relationship between inflammasomes and the apoptosome, it was hypothesized that inflammasomes adopt a comparable wheel-shaped architecture. Negative stain and cryo-electron microscopy studies of purified flagellin–NAIP5–NLRC4 and PrgJ–NAIP2–NLRC4 inflammasomes confirmed this assumption and demonstrated that these complexes adopt a wheel- or disk-like architecture, with 10–12 spokes that correspond to the individual protomers[67–70]. Mechanistic insights into the assembly of these complexes were provided by the observation that a striking ~90° hinge rotation accompanies NLRC4 activation[68,69,71]. This conformational change results in the formation of a new oligomerization surface that interacts with the next protomer in the complex and thus facilitates progressive oligomerization. Importantly, this finding might provide the structural basis for understanding gain-of-function mutations in NLRC4, which cause autoinflammation with recurrent macrophage activation syndrome and which map to this important hinge region[72–74]. Interestingly, NAIPs themselves are precluded from self-oligomerization and thus only a single NAIP is found per complex. These data support a model in which ligand binding activates the NAIPs, which allows them to recruit NLRC4 and to induce the conformational changes that mediate progressive NLRC4 oligomerization. As the studies used CARD-truncated NLRC4, it is currently not known how the CARD of NLRC4 is oriented within the structure and how the complex is connected to downstream signalling components such as ASC and caspase 1.

A fundamentally different complex is assembled by AIM2, which, unlike NLRs, lacks a NOD that could mediate self-oligomerization. Therefore, cytosolic DNA, which is bound by the HIN domain of AIM2 at regular intervals, was proposed to provide an oligomerization template or scaffold[75]. A recent study confirmed that the $AIM2^{HIN}$ forms filaments with double-stranded DNA (dsDNA) but the full-length protein could not be examined, probably owing to $AIM2^{PYD}$-induced aggregation[76]. Modelling based on the crystal structure of human $AIM2^{HIN}$ in complex with DNA suggests that every $AIM2^{HIN}$ occupies four base pairs and is in contact with six adjacent AIM^{HIN} molecules[75,76]. The relatively long linker between the $AIM2^{HIN}$ and the $AIM2^{PYD}$ would then allow the PYDs from several AIM2 molecules to swing around the DNA core and oligomerize, thereby nucleating ASC polymerization.

The ASC speck. Following its assembly, the receptor complex recruits the adaptor ASC and pro-caspase 1 via homotypic PYD–PYD and CARD–CARD interactions. This correlates with the oligomerization of ASC into a single macromolecular aggregate that is known as the ASC speck. Speck formation is observed irrespective of which receptor is activated and ASC specks were even found to be released into the extracellular space, where they enhance inflammatory responses[77,78]. ASC specks appear to be formed by filaments of ASC[77], which is consistent with cryo-electron microscopy and solid-state nuclear magnetic resonance analysis studies that show that human and mouse ASCs oligomerize through their PYDs into long helical filaments[79,80]. The ASC^{CARD} is exposed on the surface of these filaments[80] and acts as a recruitment point for pro-caspase 1. Surprisingly, it also nucleates filaments that are formed by the caspase 1^{CARD} and might be necessary for proximity-induced activation of the caspase[79]. The clustering of ASC filaments into ASC specks also seems to be mediated by the ASC^{CARD}, as filaments (but no specks) are observed in cells expressing only the ASC^{PYD} or full-length ASC with an inactive CARD (M. S. Dick and B.P., unpublished observations).

Although the adaptor ASC is essential for signalling by PYD-containing receptors, CARD-containing receptors (such as NLRP1B and NLRC4) could in theory directly recruit and activate pro-caspase 1. In fact, wild-type and $Asc^{-/-}$ macrophages display comparable levels of pyroptosis in response to NLRC4 or NLRP1B activators[19,81,82]. *Asc*-deficient cells, however, release significantly reduced levels of IL-1β[19,81,82], indicating that cytokine maturation is closely linked to ASC speck formation. Consistently, single point mutations in ASC

Type I IFNs
A multi-gene cytokine family that encodes 13 IFNα subtypes in humans (14 in mice), a single IFNβ and several poorly defined single gene products. Type I IFNs mediate the inhibition of viral replication, activate natural killer cells and macrophages, and increase antigen presentation to T cells.

Type III IFNs
A group of interferons consisting of IFNλ1, IFNλ2 and IFNλ3 (also known as IL-29, IL-28A and IL-28B, respectively), and the recently identified IFNλ4. They have similar functions to cytokines of the type I IFN family.

Apoptosome
A large multimeric protein complex that is formed by apoptotic protease-activating factor 1 (APAF1) following the recognition of cytochrome *c* release from damaged mitochondria and that activates caspase 9.

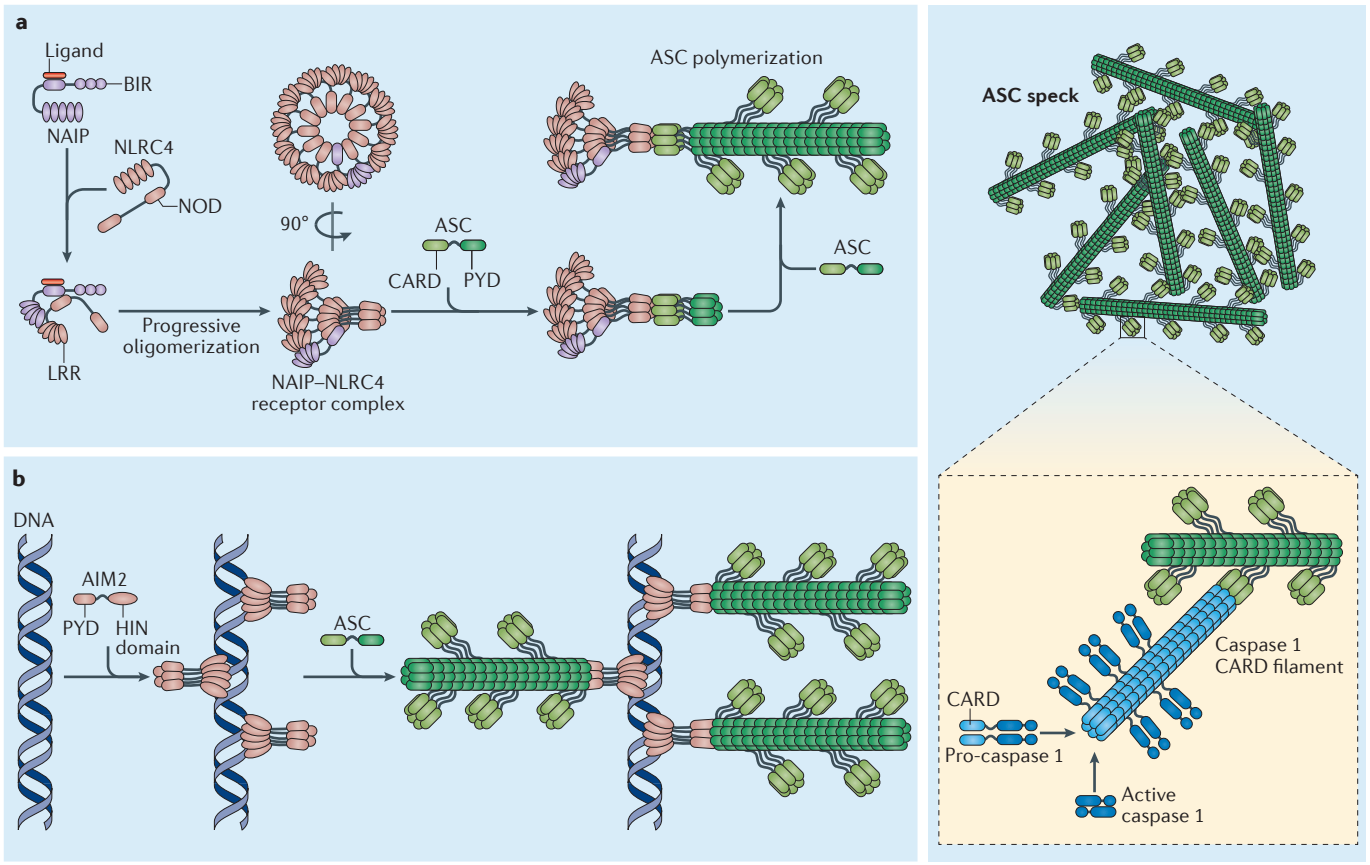

Figure 4 | **Assembly of inflammasome complexes. a** | The NAIP–NLRC4 inflammasome. Binding of their specific ligand activates mouse and human NLR family, apoptosis inhibitory proteins (NAIPs) and allows them to interact with and activate NLRC4 (REFS 21–23). This interaction results in the generation of a new nucleating surface, which in turn allows NLRC4 molecules to recruit and further activate NLRC4 molecules in a domino-like reaction[68,69]. The completed NAIP–NLRC4 complex is a multi-subunit disk-like structure that contains 9–11 molecules of NLRC4, but only one NAIP molecule[68,69]. Following receptor complex assembly, the caspase recruitment domains (CARDs) of NLRC4 cluster and recruit bridging ASC molecules to initiate ASC polymerization. A NAIP–NLRC4 complex can also directly initiate caspase 1 activation in the absence of ASC (not shown), although ASC recruitment seems to be the default situation. **b** | The absent in melanoma 2 (AIM2) inflammasome. DNA is bound in regular intervals by the HIN domain of AIM2, resulting in the clustering of the AIM2 pyrin domains (PYDs)[75,76]. This allows the PYDs of AIM2 to recruit ASC and to initiate ASC polymerization into filaments. ASC filaments aggregate to form a macromolecular assembly that is known as the ASC speck. The CARD of ASC, which is exposed on the surface of the ASC filaments recruits pro-caspase 1 and in turn initiates filaments that are formed by the CARD of caspase 1 and that are necessary for proximity-induced activation of the caspase[79,80]. LRR, leucine-rich repeat; NOD, nucleotide-binding oligomerization domain.

that abrogate ASC oligomerization, but that leave the receptor–ASC interaction intact, resulted in strikingly reduced levels of speck formation, IL-1β maturation and caspase 1 processing after AIM2, pyrin or NLRP3 activation (M. S. Dick and P.B., unpublished observations). Intriguingly, however, gasdermin D processing and pyroptosis were not affected. The formation of ASC filaments thus serves as a signal amplification mechanism for inflammasome-mediated cytokine production by generating a multitude of potential caspase 1 activation sites. As a single ligand-bound NAIP is able to initiate the assembly of a NAIP–NLRC4 complex, which in turn initiates ASC filament formation and caspase 1 activation, inflammasomes can translate the detection of a miniscule amount of ligand into a robust cellular response.

Effector functions of inflammasomes

The use of gene-deficient mice has been essential in determining the role of inflammasomes in host defence and in mouse models of autoinflammatory and inflammatory diseases. These studies have highlighted the importance of inflammasome effector mechanisms, mainly pyroptosis and the release of the cytokines IL-1β and IL-18. However, until recently, the molecular basis of how inflammatory caspases induce these responses was unknown.

Pyroptosis. The pro-inflammatory cell death that is induced by inflammatory caspases (caspase 1 and caspase 11 in mice or caspase 4 and caspase 5 in humans) was named pyroptosis, from the Greek 'pyro' (meaning fire or fever) and 'ptosis' (meaning to fall)[83].

Box 1 | **The gasdermin family: a new class of cell death effectors**

The gasdermin (GSDM) gene family is conserved in vertebrates and comprises four paralogous genes in humans (*GSDMA*, *GSDMB*, *GSDMC* and *GSDMD*)[127]. By contrast, mice lack *Gsdmb* but have three *GSDMA* homologues (*Gsdma1–3*) and four *GSDMC* homologues (*Gsdmc1–4*). Gasdermins are composed of distinct amino-terminal and carboxy-terminal domains, a feature that is shared by the extended gasdermin family members DFNA5 (deafness, autosomal dominant 5) and DFNB59 (deafness, autosomal recessive 59), which have a similar N-terminal domain but a different C terminus.

The physiological functions of gasdermins are poorly understood, except for mouse and human gasdermin D, which are known to function as mediators of pyroptosis[3,4]. Inflammatory caspases cleave gasdermin D, which results in the separation of the N- and C-terminal domains. The N-terminal domain is sufficient to induce pyroptosis, and the C-terminal domain was found to bind the N-terminal domain if the C-terminal domain was overexpressed, thus blocking cell death[3,4]. These results led to the hypothesis that caspase-dependent cleavage releases the N-terminal domain of gasdermin D from an intramolecular inhibitory interaction with its C-terminal domain, thereby allowing the N-terminal domain to initiate pyroptosis, by an as yet undefined mechanism.

Other gasdermin family members have also been linked to the induction of cell death. Gain-of-function mutations in *Gsdma3* cause hyperkeratosis and alopecia in mice that are associated with chronic skin inflammation. These dominant mutations map to the C-terminal domain, and *Shi et al.*[4] recently demonstrated that they abrogate the interaction between the C- and N-terminal domains of GSDMA3, and that the N-terminal domain of this protein also induces pyroptosis. Interestingly, mutations in *DFNA5* that result in exon skipping and premature truncation of the protein are associated with autosomal dominant hearing loss[128]. How truncated DFNA5 causes this pathology is unknown, but it might be linked to the induction of programmed cell death, as the truncated isoform (which contains the entire N-terminal domain) was shown to be cytotoxic[128].

The emergence of gasdermins as a new class of cell death effectors raises many questions. For example, it remains unclear how gasdermins are activated (except gasdermin D none of these proteins features a canonical caspase cleavage site[3,4]) and how they mediate pyroptosis. Do they relay the signal to as yet unknown pyroptosis inducers, or might the N-terminal domain that is shared by all members permeabilize the membrane by itself? Gain-of-function mutations indicate that gasdermins induce cell death and inflammation *in vivo*, but GSDM-deficient animals develop normally and show no abnormal phenotype. Therefore, gasdermin members either are redundant or they control susceptibility to environmental factors, such as allergens, infectious agents and physical stress. Notably, gasdermin expression is frequently suppressed in gastric cancer tissue specimens and cell lines[129], which indicates that they could be involved in the regulation of cell differentiation and proliferation. Further research is thus required to understand how gasdermins function and to identify the physiological context in which they unleash their cytotoxic activity.

Pyroptosis is morphologically distinct from apoptosis and is characterized by cell swelling, lysis and the release of cytoplasmic content, presumably as a result of the formation of membrane pores[83]. Pyroptosis induction was recently shown to require gasdermin D, which is a member of the enigmatic gasdermin protein family[3,4,84] (BOX 1). The generation of *Gsdmd*$^{-/-}$ mice confirmed the essential role of gasdermin D in pyroptosis induction *in vitro* and *in vivo* in a mouse model of endotoxaemia[3]. Gasdermin D is a substrate of inflammatory, but not apoptotic, caspases, and its cleavage results in the generation of an N-terminal fragment that drives pyroptotic cell death. How gasdermin D carries out pyroptosis and whether this is linked to the formation of a membrane pore are currently unknown.

Interestingly, although gasdermin D is essential for caspase 11-induced pyroptosis[3,4], pyroptosis can be observed in *Gsdmd*$^{-/-}$ cells after prolonged caspase 1 activation, indicating that there are additional pro-pyroptotic caspase 1 substrates[3]. Alternatively, prolonged caspase 1 activation might result in secondary necrosis, as *Gsdmd*$^{-/-}$ cells activate apoptotic caspases after canonical inflammasome stimulation in an ASC-dependent manner[84], which is consistent with the ability of ASC specks to activate caspase 8 in the absence of caspase 1 (REF. 85). A recent study has suggested that caspase 11 also cleaves pannexin 1 and that the subsequent release of ATP and the activation of P2X purinoceptor 7 (P2RX7) is required for pyroptosis induction[86]. Because pannexin 1 cleavage also results in the release of intracellular potassium[86], these results might explain previous findings that showed that caspase 11-driven NLRP3 activation is a cell-intrinsic mechanism[3,56]. However, the relevance of this pathway is uncertain, as pannexin 1 and P2RX7 only have a role at early rather than late time points after caspase 11 activation[49,86], and previous work has shown that *Panx1*$^{-/-}$ mice remain susceptible to intraperitoneal challenge with LPS[87].

Cytokine maturation and release. Caspase 1 was initially discovered as interleukin-converting enzyme (ICE), and processing of pro-IL-1β and IL-18 into their mature biologically active forms is still its most well-known effector function. IL-1β and IL-18 are important cytokines that promote inflammation and coordinate innate and adaptive immune responses (for a review see REF. 88). Intriguingly, they both lack a signal sequence and are released in a manner that is independent of the endoplasmic reticulum and Golgi, which is usually referred to as unconventional secretion. However, despite 25 years of research into this mechanism, it is still not understood how IL-1β exits the cell[89]. Microvesicle shedding, exosomes, secretory autophagy and lysosomes have been proposed to release IL-1β, and evidence was brought forward both for and against each model. Other IL-1 family members also lack a signal sequence, but are generally regarded to be alarmins. Recent studies suggest that cell lysis is the main release mechanism, at least in macrophages, as *Gsdmd*$^{-/-}$ cells process IL-1β normally but fail to release it owing to an absence of pyroptosis[4,84]. Thus, IL-1β and IL-18 might be unique alarmins that require caspase-mediated cleavage to become biologically active. Nevertheless, other cell types might release IL-1β differently, and neutrophils, for example, release IL-1β without undergoing pyroptosis[90].

Modulation of inflammasome signalling

Research over the past decade has not only uncovered the essential components of inflammasomes, but has also highlighted many regulatory mechanisms, including transcriptional and post-transcriptional control, post-translational modifications, and a number of proteins that regulate inflammasomes at the level of the receptors, ASC or the caspases. Insight into inflammasome regulation has also come from studies on bacterial or viral inflammasome inhibitors (reviewed in REF. 2). We discuss below the endogenous regulatory mechanisms that act directly at the level of ligand recognition or complex assembly (FIG. 5; TABLE 1).

Secondary necrosis
A process that occurs in apoptotic cells that are not cleared by phagocytes. The integrity of the plasma membrane is lost and the constituents of the cell are released.

Alarmins
Endogenous mediators that are passively released as a result of lytic cell death (for example, necrosis, pyroptosis and necroptosis) in response to infection or injury and that interact with pattern-recognition receptors to activate innate immune cells.

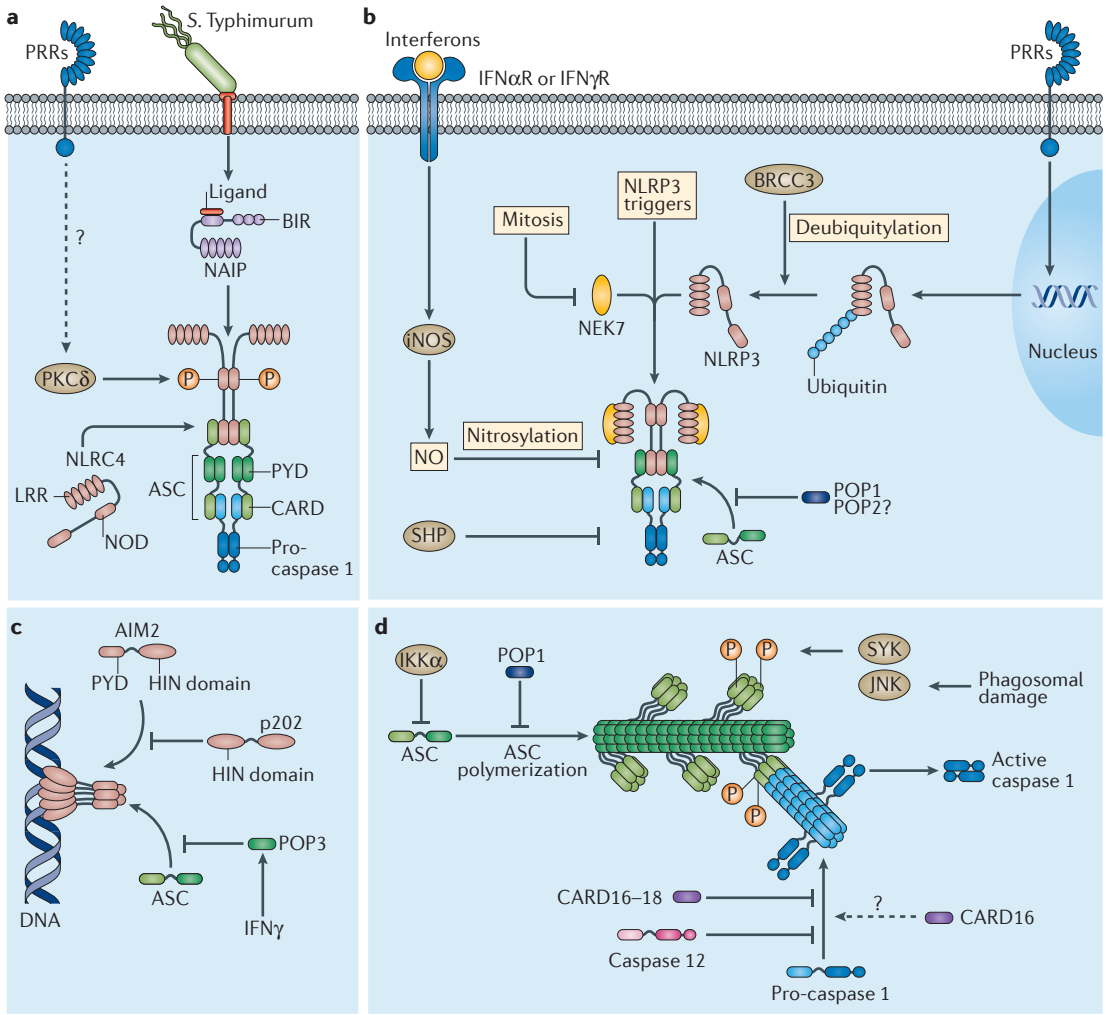

Figure 5 | **Regulation of inflammasome complex assembly and signalling. a** | NLRC4 activation requires phosphorylation within the helical domain 2 (HD2) of the nucleotide-binding oligomerization domain (NOD) module (consisting of the NBD, HD1, WHD and HD2) and is mediated by protein kinase Cδ (PKCδ)[103]. **b** | NLRP3 priming involves pattern-recognition receptor (PRR)-induced upregulation of NLRP3 expression and NLRP3 deubiquitylation by BRCC3 (REF. 106). NIMA-related kinase 7 (NEK7), an essential component of the NLRP3 complex, is necessary for mitotic progression and thus only available for NLRP3 binding during interphase[14–16]. Interferons (IFNs) negatively regulate NLRP3 through the induction of inducible nitric oxide synthase (iNOS) and nitric oxide (NO)-mediated nitrosylation[116]. Orphan nuclear receptor small heterodimer partner (SHP) can bind NLRP3 and negatively regulate inflammasome signalling, but the mechanism is unknown[130]. Pyrin domain (PYD)-only proteins (POPs) regulate NLRP3 inflammasome assembly by sequestering ASC (POP1) or by binding to the PYD of NLRP3 (POP2)[98,99]. **c** | IFN-activable protein 202 (p202) inhibits absent in melanoma 2 (AIM2) signalling by binding to DNA, which results in the spatial separation of the PYDs of AIM2 on the DNA, preventing ASC clustering and signal propagation[28,102]. POP3, an IFNγ-induced protein, directly binds to the PYD of AIM2 and blocks the recruitment of ASC[101]. **d** | IκB kinase-α (IKKα) retains ASC in the nucleus, thus controlling its availability[113]. POP1 binds to the PYD of ASC, thus preventing its recruitment to receptor PYDs and ASC polymerization[98]. Caspase 1 activation requires the phosphorylation of the caspase recruitment domain (CARD) of ASC by spleen tyrosine kinase (SYK) or JUN N-terminal kinase (JNK)[110]. Pro-caspase 1 availability, and thus indirectly its activity, is controlled by CARD16–18 and caspase 12, which bind and sequester pro-caspase 1 (REFS 91–94,96). NAIP, NLR family, apoptosis inhibitory protein; *S.* Typhimurium, *Salmonella enterica* subsp. *enterica* serovar Typhimurium.

CARD- or PYD-containing regulators. Inflammasome assembly largely relies on death-fold domain-mediated interaction of the receptor with ASC, and on interaction of ASC with caspase 1. Modulating these interactions offers a possibility to control inflammasome signalling, mainly through so-called CARD-only proteins (COPs) and PYD-only proteins (POPs) that are found in humans and higher primates, but that are absent from mouse and rat genomes.

Three COPs — CARD16 (also known as COP and PSEUDO-ICE), CARD17 (also known as INCA) and CARD18 (also known as ICEBERG) — are found in the human genome and they are all highly similar to the CARD of caspase 1 (92%, 81% and 52% identity,

respectively)[91–93]. COPs were proposed to act as negative regulators of inflammasomes by sequestering caspase 1, but they might also regulate other signalling pathways, as CARD16 and CARD18 also interact with receptor-interacting serine/threonine protein kinase 2 (RIPK2)[91–93]. The role of CARD16 as a negative regulator was challenged by a recent study that showed that it can enhance caspase 1 activation[94]. Therefore, the role of these proteins might be more complex than previously anticipated and is worth revisiting.

Caspase 12, a mostly uncharacterized member of the inflammatory caspases, was also shown to regulate inflammasome signalling. A polymorphism in the *CASP12* gene results in the expression of a full-length (caspase 12L)

Table 1 | **Endogenous regulators of inflammasomes**

Regulator	Target inflammasome component	Mechanism of action	Experimental evidence	Refs
CARD-containing regulators				
CARD16	Caspase 1	Inhibits caspase 1 activity; enhances caspase 1 oligomerization	Biochemical interaction and overexpression studies	93,94
CARD17	Caspase 1	Inhibits caspase 1 activation; blocks recruitment to ASC	Biochemical interaction and overexpression studies	92,94
CARD18	Caspase 1	Inhibits caspase 1 activity, probably by sequestration	Biochemical interaction and overexpression studies	91,93
Caspase 12	Caspase 1	Inhibits caspase 1 activity, probably by sequestration	Knockout mice and biochemical interaction studies	96
PYD-containing regulators				
POP1	ASC	Binds the PYD of ASC and blocks recruitment to receptor and ASC oligomerization	Knock-in mice, co-localization and biochemical studies	98
POP2	ASC	Binds the PYD of ASC and blocks recruitment to receptor and ASC oligomerization	Immunofluorescence and biochemical studies	99,100
POP3	AIM2	Competes with ASC for the recruitment to AIM2	Knock-in mice, co-localization and biochemical studies	101
ASC splice variants	ASC or caspase 1	Inhibits inflammasome signalling, probably by sequestration	Overexpression, co-localization and biochemical studies	131,132
Additional regulatory proteins				
BRCC3	NLRP3	Licenses receptor by deubiquitylation	Knockdown and biochemical studies	106
PKCδ	NLRC4	Licenses receptor by phosphorylation	Biochemical studies, overexpression, mutagenesis, phosphomimetics, kinase inhibitors and knockout mice	103
SYK	CARD of ASC	Licenses ASC speck assembly by phosphorylation	Knockout mice, inhibitors and biochemical studies	110
TAK1–JNK	CARD of ASC	Licenses ASC speck assembly by phosphorylation	Knockout mice, inhibitors and biochemical studies	110
IKKα	ASC	Controls ASC localization and activation	Knockout mice (kinase domain mutated), inhibitors and biochemical studies	113
PKR	NLRP1, NLRP3, NLRC4 and AIM2	Direct interaction; no function	Activator: knockout mice (kinase domain knocked out) and biochemical studies; No role: knockout mice (kinase and RNA-binding domain knocked out)	108,109
GBP	ASC, NLRP3, caspase 11 and AIM2	Enhances ligand accessibility; enhances oligomerization and enhances pyroptosis	Indirect role: knockout mice, co-localization and biochemical studies; Direct role: knockout mice and biochemical studies	117,119, 120,123, 124
p202	HIN domain of AIM2	Binds DNA and the HIN domain of AIM2, resulting in spatial separation of the PYDs of several AIM2 molecules and preventing ASC clustering	Biochemical studies	102
SHP	NLRP3	Directly binds NLRP3 (mechanism of inhibition unknown)	Knockout mice, co-localization and biochemical studies	130

AIM, absent in melanoma; CARD, caspase recruitment domain; GBP, guanylate-binding protein; JNK, JUN N-terminal kinase; p202, interferon-activable protein 202; PKCδ, protein kinase Cδ; POP, PYD-only protein; PYD, pyrin domain; SHP, small heterodimer partner; SYK, spleen tyrosine kinase; TAK1, TGFβ-activated kinase 1.

and a truncated protein in humans. Caspase 12L is confined to populations of African descent and confers hyporesponsiveness to LPS-induced cytokine production in *ex vivo* whole blood[95]. Consistent with a role as a negative regulator, mouse caspase 12 interacts with caspase 1, and overexpression of caspase 12 reduces caspase 1 activity. *Casp12* deficiency also conferred resistance to sepsis and increased bacterial clearance *in vivo*[96]; however, because the *Casp12*$^{-/-}$ mice used in the study lacked a functional allele of *Casp11* (REF. 97), additional studies with caspase 11-sufficient *Casp12*$^{-/-}$ animals are necessary to confirm this phenotype.

POPs are fairly well characterized and three of these proteins (POP1, POP2 and POP3) interfere with inflammasome signalling at the level of the PYD–PYD interaction. POP1 shows sequence similarity to the ASC^{PYD}, and its overexpression reduces IL-1β release in response to canonical and non-canonical inflammasome activators[98]. Because POP1 binds ASC and prevents ASC^{PYD}–NLRP3^{PYD} interactions, it was hypothesized that POP1 blocks ASC^{PYD} nucleation at the receptor. Consistently, *POP1* gene knockdown increases NLRP3 inflammasome-mediated caspase 1 activation. Although mice do not have POPs, POP1 can be functional in mouse cells, as overexpression of POP1 reduces inflammasome activation, and *POP1* knock-in mice show ameliorated disease outcomes in mouse models of LPS-induced peritonitis and cryopyrin-associated autoinflammatory syndrome[98]. As POP1 expression is induced by TLR or IL-1R signalling, it was proposed that POP1 provides a regulatory feedback loop that shuts down inflammasome signalling, but how this protein would disassemble an already-formed ASC speck is unclear. POP2, the second member of this protein class, resembles the PYDs of NLRP2 and NLRP3. Overexpression studies show that it can inhibit NLRP3 signalling, but its physiological role and regulation are not understood[99,100]. Type I IFNs control the expression of POP3, which shows sequence similarity to the PYD of AIM2 and other HIN-200 family members[101]. It interacts with these proteins, and knock down of *Pop3* results in increased AIM2 inflammasome activation. Reduced AIM2-mediated antiviral immunity to murine cytomegalovirus (MCMV) infections was reported for knock-in mice that express POP3 (REF. 101), which supports the conclusion that POP3 functions as a specific regulator of the DNA-induced response. Interestingly, IFN-activable protein 202 (p202, which is another HIN2 family member) also negatively regulates AIM2 activation, but through binding DNA and interacting with AIM2, which is thought to result in a spatial separation of the PYDs of AIM2, thus preventing ASC clustering[28,102].

Post-translational modifications. Protein phosphorylation or ubiquitylation control the activity of inflammasome receptors and the adaptor ASC. For example, the phosphorylation of NLRC4 by protein kinase Cδ (PKCδ) has been shown to be important for NLRC4 activity during *Salmonella* infections[103,104]. Surprisingly, PKCδ was found to be dispensable for *Shigella flexneri*-induced NLRC4 activation in LPS-primed macrophages[105]. Although other kinases may also phosphorylate the same site of NLRC4, different priming conditions may account for the discrepancy[104]. Our recent findings have shown that Ser533 phosphorylation is mainly required for NLRC4-dependent caspase 1 activation in unprimed macrophages, and that priming induces NLRP3 expression, which associates with NLRC4 independently of Ser533 phosphorylation to enhance caspase 1 activation[104]. The critical residue (Ser533) maps to the helical domain 2 (HD2) of the NLRC4 NOD module, but if and how this controls NLRC4 self-oligomerization into wheel-like assemblies remains to be determined. Interestingly, the post-translational modification only occurred in the presence of NLRC4 activators, but whether this modification is linked to PRR-mediated recognition of *Salmonella enterica* subsp. *enterica* serovar Typhimurium is currently unknown. A role for priming — that is, the engagement of PRRs or cytokine receptors — has been firmly established in the activation mechanism of NLRP3. Priming was initially thought to result in the transcriptional upregulation of NLRP3 expression, but it was recently shown that it also licenses inflammasome signalling by initiating the deubiquitylation of NLRP3 by the Lys63-specific deubiquitinase BRCC3 (REFS 106,107). This step is essential for NLRP3 activity, involves the production of reactive oxygen species and occurs only after TLR stimulation[106,107]. Whether NLRP3 needs to be phosphorylated, similarly to NLRC4, remains to be determined, but it is worth noting that the double-stranded RNA-dependent protein kinase (PKR; also known as EIF2AK2) was proposed to regulate the activity of NLRP3, as well as NLRP1, NLRC4 and AIM2, in a manner that requires its kinase activity[108]. However, a second study did not find a role for PKR in inflammasome signalling[109].

The inflammasome adaptor ASC is phosphorylated in response to inflammasome stimuli at several residues that map to its CARD[110]. These phosphorylation events require the kinases spleen tyrosine kinase (SYK) and JUN N-terminal kinase (JNK) and are necessary for caspase 1 activation[110]. The kinase SYK is also known to promote NLRP3-dependent caspase 1 activation during infections with *C. albicans*, but it remains to be shown whether this is linked to its role in ASC phosphorylation[111]. Notably, the TAK1 (TGFβ-activated kinase 1; also known as MAP3K7)–JNK pathway was found to be activated by lysosomal rupture and to promote NLRP3 activation, which indicates that lysosomal rupture might not be an NLRP3 trigger but is instead required for the priming of the NLRP3–ASC pathway[112]. Another kinase, IκB kinase-α (IKKα), has been implicated in the negative regulation of ASC by controlling its subcellular localization in resting macrophages[113], but it is unclear whether this involves ASC phosphorylation.

Regulation by IFNs. IFNs are an important group of cytokines that activate immune cells and initiate antimicrobial defences. The most well-studied classes are type I and type II IFNs, and both are known to control inflammasome signalling. Type I IFNs were shown to reduce the expression of pro-IL-1β and pro-IL-18, but

Cryopyrin-associated autoinflammatory syndrome
(CAPS). A family of autoinflammatory syndromes, including familial cold autoinflammatory syndrome, Muckle–Wells syndrome and neonatal-onset multisystem inflammatory disease. They are characterized by NLRP3 inflammasome hyperactivity and the excessive release of interleukin-1β, which leads to an autoinflammatory disease phenotype with periodic fever episodes, urticaria and often severe arthritis.

Type II IFNs
Consists of a single gene product, IFNγ, that is predominantly produced by T cells and natural killer cells, and can act on a broad range of cell types that express the IFNγ receptor.

Guanylate-binding proteins (GBPs). A group of interferon-inducible GTPases produced by the host cell that often target pathogen-containing vacuoles, contributing to the release of pathogens from the vacuole and mediating pathogen killing.

Cell-autonomous immunity A defence mechanism used by cells to control infection that is not traditionally considered to be part of the immune system. Examples include compartmentalization to prevent inappropriate entry of bacteria into the cytoplasm within a eukaryotic cell and production of nitric oxide synthases to mediate killing of an invading microorganism.

also to repress NLRP1B and NLRP3 inflammasome activity[114]. Whereas the repression of NLRP1B activity relies on the induction of the anti-inflammatory cytokine IL-10 and signalling through signal transducer and activator of transcription 3 (STAT3), the molecular mechanism of NLRP3 repression is not fully understood, but it involves STAT1-dependent reduction of caspase 1 processing. This mechanism of inflammasome control is also important *in vivo*, as type I IFN induction by poly(I:C) pretreatment reduces the recruitment of inflammatory cells after alum injection in mice and also increases susceptibility to *C. albicans*[114]. During mycobacterial infections, type I and type II IFNs act together to fine-tune IL-1 production by inflammatory monocyte–macrophages and dendritic cells[115]. Type I IFNs inhibited IL-1 production by both subsets, whereas $CD4^+$ T cell-derived IFNγ suppressed IL-1 expression selectively in inflammatory monocytes. How IFNγ modulates IL-1 production in this context is unknown, but T cell-derived IFNγ has been shown to inhibit the NLRP3 inflammasome in a mouse model of tuberculosis through inducible nitric oxide synthase, which resulted in the nitrosylation and inactivation of NLRP3 (REF. 116).

Conversely, IFNs can also enhance inflammasome signalling during microbial infections. AIM2 activation during infections with *F. novicida* but not MCMV requires STING-dependent production of type I IFNs[29–31,117]. Similarly, efficient activation of caspase 11 during Gram-negative bacterial infections requires the TLR4–TRIF (TIR-domain-containing adaptor protein inducing IFNβ)–IRF3 (IFN-regulatory factor 3) axis[53,118]. IFN-induced signalling increases the expression of AIM2 and caspase 11, which partially accounts for the IFN requirement. However, inflammasome activation also requires a group of IFN-inducible GTPases, known as guanylate-binding proteins (GBPs), which are upregulated in an IFNAR–IRF1-dependent manner[117,119,120]. GBPs have so far mainly been shown to function in cell-autonomous immunity and they restrict the replication of intracellular bacterial and protozoan pathogens[121]. They control antimicrobial processes ranging from oxidative and autophagy-based defences, to the destabilization of pathogen-containing vacuoles and direct killing of the pathogen[121,122]. Among the 11 mouse GBPs, GBP2 and GBP5 were shown to be required for *F. novicida*-induced AIM2 activation and GBP2 was required for caspase 11 activation by Gram-negative bacteria, but not for inflammasome activation in response to the purified ligands (such as poly(dA:dT) and LPS)[117,119,120]. In line with their function in cell-autonomous immunity, we and others[117,119,120] have proposed that GBP-mediated destabilization of pathogen-containing vacuoles or direct attack on the pathogen releases PAMPs into the cytosol that are then sensed by AIM2 or caspase 11. However, a direct role for GBPs in inflammasome assembly and signalling has also been described[123,124]. In this model, GBPs enhance inflammasome signalling by promoting NLRP3–ASC oligomerization or by acting further downstream at the level of pyroptosis induction. Although GBPs do not seem to be essential components of inflammasomes, understanding their role in cell-autonomous immunity and inflammasome signalling is an exciting field of future research.

Conclusions and future perspectives

The past couple of years have brought rapid progress in our understanding of inflammasome biology, going far beyond the basic concept that was introduced in the early 2000s. The molecular basis of ligand recognition has been determined for several receptors, and new paradigms for how the innate immune system senses microbial pathogens have been established through the identification and characterization of the pyrin inflammasome and the non-canonical inflammasome pathway. Furthermore, the molecular mechanisms that drive receptor oligomerization, complex assembly and signal propagation within the complex are emerging, and crucial players that mediate downstream signalling have been identified.

These advances have set the stage for the next era of inflammasome studies by allowing unprecedented understanding of inflammasome biology at a structural and biochemical level. Although this progress has raised many new questions, many old mysteries also remain unsolved. For example, the upstream events that control pyrin and NLRP3 activation are not understood, and a role for most of the human and mouse NLRs has not yet been identified. The advances ushered in by new genomic editing technologies such as CRISPR–Cas9, are already transforming immunology research and the field of inflammasome studies[4,16], and will certainly lead to a rapid identification and characterization of such novel regulatory and signalling components.

But finally, and most importantly, how will this newly obtained knowledge be translated into treatments for inflammasome-associated infectious and inflammatory diseases? Recent studies have highlighted the potential of small molecules that block NLRP3 activation in attenuating inflammasome signalling in mouse models of cryopyrin-associated autoinflammatory syndrome and experimental autoimmune encephalomyelitis[125,126]. Therefore, in-depth understanding of the upstream mechanisms and the structural basis of signal recognition and complex assembly are likely to be instrumental in the identification of new therapeutic targets and could foster the development of new anti-inflammatory therapies for inflammasome-associated diseases based on a range of selective inhibitors of individual inflammasomes.

1. Martinon, F., Burns, K. & Tschopp, J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-1β. *Mol. Cell* **10**, 417–426 (2002).
2. von Moltke, J., Ayres, J. S., Kofoed, E. M., Chavarria-Smith, J. & Vance, R. E. Recognition of bacteria by inflammasomes. *Annu. Rev. Immunol.* **31**, 73–106 (2013).
3. Kayagaki, N. *et al.* Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* **526**, 666–671 (2015).
4. Shi, J. *et al.* Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* **526**, 660–665 (2015).
References 3 and 4 show that inflammatory caspases cleave gasdermin D, resulting in the generation of an N-terminal gasdermin D fragment that drives pyroptosis.
5. Broz, P. & Monack, D. M. Newly described pattern recognition receptors team up against intracellular pathogens. *Nat. Rev. Immunol.* **13**, 551–565 (2013).
6. Boyden, E. D. & Dietrich, W. F. Nalp1b controls mouse macrophage susceptibility to anthrax lethal toxin. *Nat. Genet.* **38**, 240–244 (2006).

7. Levinsohn, J. L. *et al.* Anthrax lethal factor cleavage of Nlrp1 is required for activation of the inflammasome. *PLoS Pathog.* **8**, e1002638 (2012).
8. Hellmich, K. A. *et al.* Anthrax lethal factor cleaves mouse Nlrp1b in both toxin-sensitive and toxin-resistant macrophages. *PLoS ONE* **7**, e49741 (2012).
9. Chavarria-Smith, J. & Vance, R. E. Direct proteolytic cleavage of NLRP1B is necessary and sufficient for inflammasome activation by anthrax lethal factor. *PLoS Pathog.* **9**, e1003452 (2013).
10. Frew, B. C., Joag, V. R. & Mogridge, J. Proteolytic processing of Nlrp1b is required for inflammasome activity. *PLoS Pathog.* **8**, e1002659 (2012).
11. Ewald, S. E., Chavarria-Smith, J. & Boothroyd, J. C. NLRP1 is an inflammasome sensor for *Toxoplasma gondii*. *Infect. Immun.* **82**, 460–468 (2014).
12. Latz, E., Xiao, T. S. & Stutz, A. Activation and regulation of the inflammasomes. *Nat. Rev. Immunol.* **13**, 397–411 (2013).
13. Munoz-Planillo, R. *et al.* K^+ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity* **38**, 1142–1153 (2013).
This study establishes that potassium efflux is the common denominator of a wide array of chemically and physically distinct NLRP3 stimuli.
14. He, Y., Zeng, M. Y., Yang, D., Motro, B. & Nunez, G. NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux. *Nature* **530**, 354–357 (2016).
15. Shi, H. *et al.* NLRP3 activation and mitosis are mutually exclusive events coordinated by NEK7, a new inflammasome component. *Nat. Immunol.* **17**, 250–258 (2016).
16. Schmid-Burgk, J. L. *et al.* A genome-wide CRISPR (clustered regularly interspaced short palindromic repeats) screen identifies NEK7 as an essential component of NLRP3 inflammasome activation. *J. Biol. Chem.* **291**, 103–109 (2016).
References 14–16 report that NEK7, a kinase that coordinates microtubule dynamics for spindle formation, is an essential component of the NLRP3 inflammasome.
17. Mariathasan, S. *et al.* Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* **430**, 213–218 (2004).
18. Miao, E. A. *et al.* Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1β via Ipaf. *Nat. Immunol.* **7**, 569–575 (2006).
19. Franchi, L. *et al.* Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1β in salmonella-infected macrophages. *Nat. Immunol.* **7**, 576–582 (2006).
20. Miao, E. A. *et al.* Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. *Proc. Natl Acad. Sci. USA* **107**, 3076–3308 (2010).
21. Zhao, Y. *et al.* The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature* **477**, 596–600 (2011).
22. Kofoed, E. M. & Vance, R. E. Innate immune recognition of bacterial ligands by NAIPs determines inflammasome specificity. *Nature* **477**, 592–595 (2011).
23. Tenthorey, J. L., Kofoed, E. M., Daugherty, M. D., Malik, H. S. & Vance, R. E. Molecular basis for specific recognition of bacterial ligands by NAIP/NLRC4 inflammasomes. *Mol. Cell* **54**, 17–29 (2014).
24. Kortmann, J., Brubaker, S. W. & Monack, D. M. Cutting edge: inflammasome activation in primary human macrophages is dependent on flagellin. *J. Immunol.* **195**, 815–819 (2015).
25. Muruve, D. A. *et al.* The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature* **452**, 103–107 (2008).
26. Hornung, V. *et al.* AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* **458**, 514–518 (2009).
27. Fernandes-Alnemri, T., Yu, J. W., Datta, P., Wu, J. & Alnemri, E. S. AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. *Nature* **458**, 509–513 (2009).
28. Roberts, T. L. *et al.* HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. *Science* **323**, 1057–1060 (2009).
29. Rathinam, V. A. *et al.* The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat. Immunol.* **11**, 395–402 (2010).
30. Fernandes-Alnemri, T. *et al.* The AIM2 inflammasome is critical for innate immunity to *Francisella tularensis*. *Nat. Immunol.* **11**, 385–393 (2010).
31. Jones, J. W. *et al.* Absent in melanoma 2 is required for innate immune recognition of *Francisella tularensis*. *Proc. Natl Acad. Sci. USA* **107**, 9771–9776 (2010).
32. Sauer, J. D. *et al. Listeria monocytogenes* triggers AIM2-mediated pyroptosis upon infrequent bacteriolysis in the macrophage cytosol. *Cell Host Microbe* **7**, 412–419 (2010).
33. Dihlmann, S. *et al.* Increased expression and activation of absent in melanoma 2 inflammasome components in lymphocytic infiltrates of abdominal aortic aneurysms. *Mol. Med.* **20**, 230–237 (2014).
34. Dombrowski, Y. *et al.* Cytosolic DNA triggers inflammasome activation in keratinocytes in psoriatic lesions. *Sci. Transl Med.* **3**, 82ra38 (2011).
35. Javierre, B. M. *et al.* Changes in the pattern of DNA methylation associate with twin discordance in systemic lupus erythematosus. *Genome Res.* **20**, 170–179 (2010).
36. Dihlmann, S. *et al.* Lack of absent in melanoma 2 (AIM2) expression in tumor cells is closely associated with poor survival in colorectal cancer patients. *Int. J. Cancer* **135**, 2387–2396 (2014).
37. Ponomareva, L. *et al.* AIM2, an IFN-inducible cytosolic DNA sensor, in the development of benign prostate hyperplasia and prostate cancer. *Mol. Cancer Res.* **11**, 1193–1202 (2013).
38. Man, S. M. *et al.* Critical role for the DNA sensor AIM2 in stem cell proliferation and cancer. *Cell* **162**, 45–58 (2015).
39. Wilson, J. E. *et al.* Inflammasome-independent role of AIM2 in suppressing colon tumorigenesis via DNA-PK and Akt. *Nat. Med.* **21**, 906–913 (2015).
40. Chae, J. J. *et al.* The B30.2 domain of pyrin, the familial Mediterranean fever protein, interacts directly with caspase-1 to modulate IL-1β production. *Proc. Natl Acad. Sci. USA* **103**, 9982–9987 (2006).
41. Hesker, P. R., Nguyen, M., Kovarova, M., Ting, J. P. & Koller, B. H. Genetic loss of murine pyrin, the Familial Mediterranean Fever protein, increases interleukin-1β levels. *PLoS ONE* **7**, e51105 (2012).
42. Chae, J. J. *et al.* Gain-of-function pyrin mutations induce NLRP3 protein-independent interleukin-1β activation and severe autoinflammation in mice. *Immunity* **34**, 755–768 (2011).
43. Masters, S. L. *et al.* Familial autoinflammation with neutrophilic dermatosis reveals a regulatory mechanism of pyrin activation. *Sci. Transl Med.* **8**, 332ra45 (2016).
This study describes a new mutation in pyrin that causes an autoinflammatory disorder known as pyrin-associated autoinflammation with neutrophilic dermatosis.
44. Xu, H. *et al.* Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. *Nature* **513**, 237–241 (2014).
This study demonstrates that pyrin assembles an inflammasome complex after detecting the inactivation of RHOA by bacterial pathogens.
45. Waite, A. L. *et al.* Pyrin and ASC co-localize to cellular sites that are rich in polymerizing actin. *Exp. Biol. Med. (Maywood)* **234**, 40–52 (2009).
46. Kim, M. L. *et al.* Aberrant actin depolymerization triggers the pyrin inflammasome and autoinflammatory disease that is dependent on IL-18, not IL-1β. *J. Exp. Med.* **212**, 927–938 (2015).
47. Hasegawa, M. *et al.* Protective role of commensals against *Clostridium difficile* infection via an IL-1β-mediated positive-feedback loop. *J. Immunol.* **189**, 3085–3091 (2012).
48. Kimura, T. *et al.* TRIM-mediated precision autophagy targets cytoplasmic regulators of innate immunity. *J. Cell Biol.* **210**, 973–989 (2015).
49. Kayagaki, N. *et al.* Non-canonical inflammasome activation targets caspase-11. *Nature* **479**, 117–121 (2011).
This paper reports the identification of a new non-canonical inflammasome pathway that results in caspase 11 activation in response to Gram-negative bacterial infections.
50. Shi, J. *et al.* Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature* **514**, 187–192 (2014).
This study shows that activation of the non-canonical inflammasome pathway involves the direct binding of LPS to mouse caspase 11 or human caspase 4 and caspase 5.
51. Kayagaki, N. *et al.* Noncanonical inflammasome activation by intracellular LPS independent of TLR4. *Science* **341**, 1246–1249 (2013).
52. Hagar, J. A., Powell, D. A., Aachoui, Y., Ernst, R. K. & Miao, E. A. Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock. *Science* **341**, 1250–1253 (2013).
53. Broz, P. *et al.* Caspase-11 increases susceptibility to Salmonella infection in the absence of caspase-1. *Nature* **490**, 288–291 (2012).
54. Baker, P. J. *et al.* NLRP3 inflammasome activation downstream of cytoplasmic LPS recognition by both caspase-4 and caspase-5. *Eur. J. Immunol.* **45**, 2918–2926 (2015).
55. Schmid-Burgk, J. L. *et al.* Caspase-4 mediates non-canonical activation of the NLRP3 inflammasome in human myeloid cells. *Eur. J. Immunol.* **45**, 2911–2917 (2015).
56. Ruhl, S. & Broz, P. Caspase-11 activates a canonical NLRP3 inflammasome by promoting K efflux. *Eur. J. Immunol.* **45**, 2927–2936 (2015).
57. Elinav, E. *et al.* NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* **145**, 745–757 (2011).
58. Levy, M. *et al.* Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling. *Cell* **163**, 1428–1443 (2015).
59. Anand, P. K. *et al.* NLRP6 negatively regulates innate immunity and host defence against bacterial pathogens. *Nature* **488**, 389–393 (2012).
60. Wlodarska, M. *et al.* NLRP6 inflammasome orchestrates the colonic host-microbial interface by regulating goblet cell mucus secretion. *Cell* **156**, 1045–1059 (2014).
61. Wang, P. *et al.* Nlrp6 regulates intestinal antiviral innate immunity. *Science* **350**, 826–830 (2015).
62. Unterholzner, L. *et al.* IFI16 is an innate immune sensor for intracellular DNA. *Nat. Immunol.* **11**, 997–1004 (2010).
63. Storek, K. M., Gertsvolf, N. A., Ohlson, M. B. & Monack, D. M. cGAS and Ifi204 cooperate to produce type I IFNs in response to *Francisella* infection. *J. Immunol.* **194**, 3236–3245 (2015).
64. Kerur, N. *et al.* IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to Kaposi Sarcoma-associated herpesvirus infection. *Cell Host Microbe* **9**, 363–375 (2011).
65. Doitsh, G. *et al.* Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature* **505**, 509–514 (2014).
66. Monroe, K. M. *et al.* IFI16 DNA sensor is required for death of lymphoid CD4 T cells abortively infected with HIV. *Science* **343**, 428–432 (2014).
67. Halff, E. F. *et al.* Formation and structure of a NAIP5–NLRC4 inflammasome induced by direct interactions with conserved N- and C-terminal regions of flagellin. *J. Biol. Chem.* **287**, 38460–38472 (2012).
68. Hu, Z. *et al.* Structural and biochemical basis for induced self-propagation of NLRC4. *Science* **350**, 399–404 (2015).
69. Zhang, L. *et al.* Cryo-EM structure of the activated NAIP2–NLRC4 inflammasome reveals nucleated polymerization. *Science* **350**, 404–409 (2015).
References 68 and 69 determine the structure of the NAIP–NLRC4 complex and characterize the assembly mechanism.
70. Diebolder, C. A., Halff, E. F., Koster, A. J., Huizinga, E. G. & Koning, R. I. Cryoelectron tomography of the NAIP5/NLRC4 inflammasome: implications for NLR activation. *Structure* **23**, 2349–2357 (2015).
71. Hu, Z. *et al.* Crystal structure of NLRC4 reveals its autoinhibition mechanism. *Science* **341**, 172–175 (2013).
72. Romberg, N. *et al.* Mutation of *NLRC4* causes a syndrome of enterocolitis and autoinflammation. *Nat. Genet.* **46**, 1135–1139 (2014).
73. Kitamura, A., Sasaki, Y., Abe, T., Kano, H. & Yasutomo, K. An inherited mutation in *NLRC4* causes autoinflammation in human and mice. *J. Exp. Med.* **211**, 2385–2396 (2014).
74. Canna, S. W. *et al.* An activating *NLRC4* inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. *Nat. Genet.* **46**, 1140–1146 (2014).
References 72–74 describe gain-of-function mutations in NLRC4 that cause autoinflammation with recurrent macrophage activation syndrome (MAS).
75. Jin, T. *et al.* Structures of the HIN domain:DNA complexes reveal ligand binding and activation mechanisms of the AIM2 inflammasome and IFI16 receptor. *Immunity* **36**, 561–571 (2012).

76. Lu, A. *et al.* Plasticity in PYD assembly revealed by cryo-EM structure of the PYD filament of AIM2. *Cell Discov.* **1**, 15013 (2015).
77. Franklin, B. S. *et al.* The adaptor ASC has extracellular and 'prionoid' activities that propagate inflammation. *Nat. Immunol.* **15**, 727–737 (2014).
78. Baroja-Mazo, A. *et al.* The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. *Nat. Immunol.* **15**, 738–748 (2014).
79. Lu, A. *et al.* Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes. *Cell* **156**, 1193–1206 (2014).
This study used a combination of cryo-electron microscopy and biochemical methods to characterize the structure of the ASC filament and proposed a unified mechanism for how inflammasomes are assembled.
80. Sborgi, L. *et al.* Structure and assembly of the mouse ASC inflammasome by combined NMR spectroscopy and cryo-electron microscopy. *Proc. Natl Acad. Sci. USA* **112**, 13237–13242 (2015).
81. Broz, P., von Moltke, J., Jones, J. W., Vance, R. E. & Monack, D. M. Differential requirement for caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. *Cell Host Microbe* **8**, 471–483 (2010).
82. Guey, B., Bodnar, M., Manie, S. N., Tardivel, A. & Petrilli, V. Caspase-1 autoproteolysis is differentially required for NLRP1b and NLRP3 inflammasome function. *Proc. Natl Acad. Sci. USA* **111**, 17254–17259 (2014).
83. Fink, S. L. & Cookson, B. T. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. *Infect. Immun.* **73**, 1907–1916 (2005).
84. He, W. T. *et al.* Gasdermin D is an executor of pyroptosis and required for interleukin-1β secretion. *Cell Res.* **25**, 1285–1298 (2015).
85. Pierini, R. *et al.* AIM2/ASC triggers caspase-8-dependent apoptosis in *Francisella*-infected caspase-1-deficient macrophages. *Cell Death Differ.* **19**, 1709–1721 (2012).
86. Yang, D., He, Y., Munoz-Planillo, R., Liu, Q. & Nunez, G. Caspase-11 requires the pannexin-1 channel and the purinergic P2X7 pore to mediate pyroptosis and endotoxic shock. *Immunity* **43**, 923–932 (2015).
87. Vanden Berghe, T. *et al.* Passenger mutations confound interpretation of all genetically modified congenic mice. *Immunity* **43**, 200–209 (2015).
88. Dinarello, C. A. Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* **27**, 519–550 (2009).
89. Monteleone, M., Stow, J. L. & Schroder, K. Mechanisms of unconventional secretion of IL-1 family cytokines. *Cytokine* **74**, 213–218 (2015).
90. Chen, K. W. *et al.* The neutrophil NLRC4 inflammasome selectively promotes IL-1β maturation without pyroptosis during acute *Salmonella* challenge. *Cell Rep.* **8**, 570–582 (2014).
91. Humke, E. W., Shriver, S. K., Starovasnik, M. A., Fairbrother, W. J. & Dixit, V. M. ICEBERG: a novel inhibitor of interleukin-1β generation. *Cell* **103**, 99–111 (2000).
92. Lamkanfi, M. *et al.* INCA, a novel human caspase recruitment domain protein that inhibits interleukin-1β generation. *J. Biol. Chem.* **279**, 51729–51738 (2004).
93. Druilhe, A., Srinivasula, S. M., Razmara, M., Ahmad, M. & Alnemri, E. S. Regulation of IL-1β generation by Pseudo-ICE and ICEBERG, two dominant negative caspase recruitment domain proteins. *Cell Death Differ.* **8**, 649–657 (2001).
94. Karasawa, T. *et al.* Oligomerized CARD16 promotes caspase-1 assembly and IL-1β processing. *FEBS Open Bio* **5**, 348–356 (2015).
95. Saleh, M. *et al.* Differential modulation of endotoxin responsiveness by human caspase-12 polymorphisms. *Nature* **429**, 75–79 (2004).
96. Saleh, M. *et al.* Enhanced bacterial clearance and sepsis resistance in caspase-12-deficient mice. *Nature* **440**, 1064–1068 (2006).
97. Skeldon, A. M. *et al.* Caspase-12, but not caspase-11, inhibits obesity and insulin resistance. *J. Immunol.* **196**, 437–447 (2016).
98. de Almeida, L. *et al.* The PYRIN domain-only protein POP1 inhibits inflammasome assembly and ameliorates inflammatory disease. *Immunity* **43**, 264–276 (2015).
99. Bedoya, F., Sandler, L. L. & Harton, J. A. Pyrin-only protein 2 modulates NF-κB and disrupts ASC:CLR interactions. *J. Immunol.* **178**, 3837–3845 (2007).
100. Dorfleutner, A. *et al.* Cellular pyrin domain-only protein 2 is a candidate regulator of inflammasome activation. *Infect. Immun.* **75**, 1484–1492 (2007).
101. Khare, S. *et al.* The PYRIN domain-only protein POP3 inhibits ALR inflammasomes and regulates responses to infection with DNA viruses. *Nat. Immunol.* **15**, 343–353 (2014).
This study shows that POP3 is an IFN-inducible endogenous regulator of the AIM2 inflammasome that inhibits receptor signalling by directly binding to its PYD.
102. Yin, Q. *et al.* Molecular mechanism for p202-mediated specific inhibition of AIM2 inflammasome activation. *Cell Rep.* **4**, 327–339 (2013).
103. Qu, Y. *et al.* Phosphorylation of NLRC4 is critical for inflammasome activation. *Nature* **490**, 539–542 (2012).
104. Qu, Y. *et al.* NLRP3 recruitment by NLRC4 during *Salmonella* infection. *J. Exp. Med.* **213**, 877–885 (2016).
105. Suzuki, S. *et al. Shigella* type III secretion protein MxiI is recognized by Naip2 to induce Nlrc4 inflammasome activation independently of Pkcδ. *PLoS Pathog.* **10**, e1003926 (2014).
106. Py, B. F., Kim, M. S., Vakifahmetoglu-Norberg, H. & Yuan, J. Deubiquitination of NLRP3 by BRCC3 critically regulates inflammasome activity. *Mol. Cell* **49**, 331–338 (2013).
107. Juliana, C. *et al.* Non-transcriptional priming and deubiquitination regulate NLRP3 inflammasome activation. *J. Biol. Chem.* **287**, 36617–36622 (2012).
108. Lu, B. *et al.* Novel role of PKR in inflammasome activation and HMGB1 release. *Nature* **488**, 670–674 (2012).
109. He, Y., Franchi, L. & Nunez, G. The protein kinase PKR is critical for LPS-induced iNOS production but dispensable for inflammasome activation in macrophages. *Eur. J. Immunol.* **43**, 1147–1152 (2013).
110. Hara, H. *et al.* Phosphorylation of the adaptor ASC acts as a molecular switch that controls the formation of speck-like aggregates and inflammasome activity. *Nat. Immunol.* **14**, 1247–1255 (2013).
111. Gross, O. *et al.* Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence. *Nature* **459**, 433–436 (2009).
112. Okada, M., Matsuzawa, A., Yoshimura, A. & Ichijo, H. The lysosome rupture-activated TAK1–JNK pathway regulates NLRP3 inflammasome activation. *J. Biol. Chem.* **289**, 32926–32936 (2014).
113. Martin, B. N. *et al.* IKKα negatively regulates ASC-dependent inflammasome activation. *Nat. Commun.* **5**, 4977 (2014).
114. Guarda, G. *et al.* Type I interferon inhibits interleukin-1 production and inflammasome activation. *Immunity* **34**, 213–223 (2011).
115. Mayer-Barber, K. D. *et al.* Innate and adaptive interferons suppress IL-1α and IL-1β production by distinct pulmonary myeloid subsets during *Mycobacterium tuberculosis* infection. *Immunity* **35**, 1023–1034 (2011).
116. Mishra, B. B. *et al.* Nitric oxide controls the immunopathology of tuberculosis by inhibiting NLRP3 inflammasome-dependent processing of IL-1β. *Nat. Immunol.* **14**, 52–60 (2013).
117. Man, S. M. *et al.* The transcription factor IRF1 and guanylate-binding proteins target activation of the AIM2 inflammasome by *Francisella* infection. *Nat. Immunol.* **16**, 467–475 (2015).
118. Rathinam, V. A. *et al.* TRIF licenses caspase-11-dependent NLRP3 inflammasome activation by Gram-negative bacteria. *Cell* **150**, 606–619 (2012).
119. Meunier, E. *et al.* Caspase-11 activation requires lysis of pathogen-containing vacuoles by IFN-induced GTPases. *Nature* **509**, 366–370 (2014).
120. Meunier, E. *et al.* Guanylate-binding proteins promote activation of the AIM2 inflammasome during infection with *Francisella novicida*. *Nat. Immunol.* **16**, 476–484 (2015).
121. MacMicking, J. D. Interferon-inducible effector mechanisms in cell-autonomous immunity. *Nat. Rev. Immunol.* **12**, 367–382 (2012).
122. Kravets, E. *et al.* Guanylate binding proteins (GBPs) directly attack via supramolecular complexes. *eLife* **5**, e11479 (2016).
123. Pilla, D. M. *et al.* Guanylate binding proteins promote caspase-11-dependent pyroptosis in response to cytoplasmic LPS. *Proc. Natl Acad. Sci. USA* **111**, 6046–6051 (2014).
124. Shenoy, A. R. *et al.* GBP5 promotes NLRP3 inflammasome assembly and immunity in mammals. *Science* **336**, 481–485 (2012).
125. Coll, R. C. *et al.* A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. *Nat. Med.* **21**, 248–255 (2015).
This study describes MCC950, a small molecule that specifically blocks the NLRP3 inflammasome *in vitro* and in mouse models of cryopyrin-associated periodic syndrome and experimental autoimmune encephalomyelitis.
126. Youm, Y. H. *et al.* The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat. Med.* **21**, 263–269 (2015).
127. Tanaka, S., Mizushina, Y., Kato, Y., Tamura, M. & Shiroishi, T. Functional conservation of *Gsdma* cluster genes specifically duplicated in the mouse genome. *G3 (Bethesda)* **3**, 1843–1850 (2013).
128. Op de Beeck, K. *et al.* The *DFNA5* gene, responsible for hearing loss and involved in cancer, encodes a novel apoptosis-inducing protein. *Eur. J. Hum. Genet.* **19**, 965–973 (2011).
129. Saeki, N. *et al. GASDERMIN*, suppressed frequently in gastric cancer, is a target of LMO1 in TGF-β-dependent apoptotic signalling. *Oncogene* **26**, 6488–6498 (2007).
130. Yang, C. S. *et al.* Small heterodimer partner interacts with NLRP3 and negatively regulates activation of the NLRP3 inflammasome. *Nat. Commun.* **6**, 6115 (2015).
131. Matsushita, K. *et al.* A splice variant of ASC regulates IL-1β release and aggregates differently from intact ASC. *Mediators Inflamm.* **2009**, 287387 (2009).
132. Bryan, N. B. *et al.* Differential splicing of the apoptosis-associated speck like protein containing a caspase recruitment domain (ASC) regulates inflammasomes. *J. Inflamm. (Lond.)* **7**, 23 (2010).

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Competing interests statement
The authors declare competing interests: see Web version for details.