The transcription factor IRF1 and guanylate-binding proteins target activation of the AIM2 inflammasome by Francisella infection

Si Ming Man1,2, Rajendra Karki1, R K Subbarao Malireddi1, Geoffrey Neale3, Peter Vogel4, Masahiro Yamamoto5, Mohamed Lamkanfi6,7 & Thirumala-Devi Kanneganti1

Inflammasomes are critical for mounting host defense against pathogens. The molecular mechanisms that control activation of the AIM2 inflammasome in response to different cytosolic pathogens remain unclear. Here we found that the transcription factor IRF1 was required for activation of the AIM2 inflammasome during infection with the Francisella tularensis subspecies novicida (F. novicida), whereas engagement of the AIM2 inflammasome by mouse cytomegalovirus (MCMV) or transfected double-stranded DNA did not require IRF1. Infection of F. novicida detected by the DNA sensor cGAS and its adaptor STING induced type I interferon–dependent expression of IRF1, which drove the expression of guanylate-binding proteins (GBPs); this led to intracellular killing of bacteria and DNA release. Our results reveal a specific requirement for IRF1 and GBPs in the liberation of DNA for sensing by AIM2 depending on the pathogen encountered by the cell.

Inflammasomes are cytosolic multimeric protein complexes that provide host defense against microbial pathogens and have a role in the development of autoinflammatory and metabolic diseases. Formation of the inflammasome is initiated by sensors of the innate immune system, including nucleotide-binding oligomerization domain (Nod)-like receptors (NLRs) or AIM2-like receptors (ALRs), following detection of microbe-associated or damage-associated molecular patterns in the cytoplasm of a cell. In most cases, NLRs and ALRs engage the adaptor ASC, whose caspase-recruitment domain is critical for subsequent interaction with the caspase-recruitment domain of procaspase-1 (ref. 3). Activation of caspase-1 induces proteolytic cleavage and release of mature interleukin 1β (IL-1β) and IL-18, as well as the induction of a type of pro-inflammatory cell death called ‘pyroptosis’. Therefore, inflammasomes are able to mount innate immunological effector functions in response to a range of stimuli encountered by the cell.

AIM2 is a member of the ALR family that contributes to the host defense against bacterial pathogens and viral pathogens, such as Francisella tularensis and cytomegalovirus, respectively. AIM2 contains a HIN-200 domain that directly binds double-stranded DNA (dsDNA) released into the cytosol of a cell during a microbial infection. Similarly, self DNA that is released into and accumulates in keratinocytes also activates the AIM2 inflammasome to drive the release of IL-1β in lesions of patients with psoriasis, which indicates that AIM2 also responds to endogenous dsDNA released during cellular damage. Structural analysis has demonstrated that the positively charged HIN-200 domain of AIM2 embraces the dsDNA, whereas the pyrin domain of AIM2 facilitates the recruitment of ASC.

The molecular mechanisms that govern assembly of the AIM2 inflammasome are less clear than those for the NLRP3 inflammasome. NLRP3 is activated by a plethora of microbe-associated and damage-associated molecular patterns. Activation of the NLRP3 inflammasome requires two signals. The first signal, or ‘priming’, is provided by the engagement of Toll-like receptors (TLRs), the receptor Nod2 or the receptor for tumor-necrosis factor to activate the transcription factor NF-kB and induce NLRP3 expression. The second signal is provided by an NLRP3 activator; these include ATP, uric acid crystals, silica, asbestos, bacterial messenger RNA, bacterial DNA-RNA hybrids and muramylidipeptide. In addition, signaling via type I interferons is critical for activation of the so-called ‘non-canonical’ NLRP3 inflammasome. In this context, extracellular lipopolysaccharide (LPS) from Gram-negative bacteria activates TLR4 and its adaptor TRIF to induce the expression of type I interferons and pro-caspase-11 (refs. 12–15). LPS released into the cytosol by means of vacuolar lysis binds caspase-11, which leads to caspase-11-dependent cell death and activation of the non-canonical NLRP3 inflammasome. In contrast, AIM2 is constitutively expressed and does not require NF-kB-mediated priming for its activation. Instead, signaling via type I interferons contributes to activation of
the AIM2 inflammasome in response to certain pathogens, such as the *F. tularensis* subspecies novicida (*F. novicida*), but not in response to the DNA virus mouse cytomegalovirus (MCMV)\(^8,9,21\). Therefore, the downstream mechanism that leads to activation of the AIM2 inflammasome in response to different pathogens is probably different and remains to be resolved\(^3\).

Here we identified two distinct pathways that led to activation of the AIM2 inflammasome. We found that engagement of the AIM2 inflammasome by infection with *F. novicida* required the transcription factor IRF1 but not TRIF or caspase-11. IRF1 was required for robust expression of small interferon-inducible GTPases known as guanylate-binding proteins (GBP)s, which killed cytosolic *F. novicida* and mediated the release of DNA for access by AIM2. In addition, IRF1-deficient (IRF1\(--/-\)) mice failed to control infection with *F. novicida*. In contrast, engagement of AIM2 via MCMV or transfected dsDNA did not require IRF1. Therefore, we have identified a specific requirement for IRF1 and GBP s in *F. novicida*-induced activation of the AIM2 inflammasome.

**RESULTS**

**IRF1 mediates AIM2 activation by *F. novicida***

*F. tularensis*, a pathogen that causes a rapid and lethal infections in humans and mice, is recognized by the AIM2 inflammasome\(^8,9,22\). In unprimed primary mouse bone marrow–derived macrophages (BMDMs), *F. novicida* induced AIM2-dependent activation of caspase-1, release of IL-1β and IL-18 and cell death in a manner that required type I interferon receptor 1 (IFNAR1) and IFNAR2 and components of the transcription factors ISGF3, STAT1 and IFR9 (refs. 8,9,21,22) (Fig. 1a and Supplementary Fig. 1a). In contrast, activation of the AIM2 inflammasome by transfection of the dsDNA ligand poly(dA:dT) occurred independently of signaling via type I interferons (Fig. 1a). The type I interferon signature that leads to activation of the AIM2 inflammasome in response to infection with *F. novicida* is unknown. The production of type I interferons induced by the TLR adaptor TRIF is required for robust activation of the caspase-11–NLRP3 inflammasome in macrophages infected with *Citrobacter rodentium*, *Escherichia coli* or *Vibrio cholerae*\(^12,13,15\), however, we found that TRIF and caspase-11 were dispensable for activation of the AIM2 inflammasome by *F. novicida* (Fig. 1b). Indeed, *F. novicida* synthesizes a tetra-acylated lipid A that differs to the hexa-acylated species of many enteric bacteria that normally activates caspase-11, which explains the ability of *F. novicida* to evade caspase-11–mediated detection\(^18\). These findings suggested that an as-yet undefined type I interferon signature independent of TRIF and caspase-11 was needed to engage the AIM2 inflammasome by infection with *F. novicida* but not in response to transfected dsDNA.

We performed microarray analysis to identify genes regulated differentially in wild-type and IFNAR1-deficient (Ifnar1\(--/-\)) BMDMs that had been infected with *F. novicida*. The expression of many members of the IFR family of interferon-regulatory transcription factors, including IRF1, IRF7 and IFR9, was significantly reduced in the absence of IFNAR1 (Fig. 1c). IRF9, a subunit of the ISGF3 complex, contributed to activation of AIM2 inflammasome by infection with *F. novicida* (Fig. 1a and Supplementary Fig. 1a), and we excluded the possibility of a role for IRF7 in activation of the AIM2 inflammasome upon infection with *F. novicida* (Supplementary Fig. 1b). Confirmation of IRF1 protein expression indicated that it was robustly upregulated by infection with *F. novicida* through a mechanism that required IFNAR1, IFNAR2, STAT1 and IFR9 (Fig. 1d). Consistent with that, BMDMs stimulated with recombinant mouse interferon-β (IFN-β) also robustly upregulated IRF1 expression, which was critically dependent on type I interferon receptors (Supplementary Fig. 1c).

To investigate whether IRF1 is an upstream molecule that leads to activation of the AIM2 inflammasome, we infected unprimed wild-type and IRF1\(--/-\) BMDMs with *F. novicida* and analyzed inflammasome responses 20 h later. Notably, unprimed IRF1\(--/-\) BMDMs failed to respond to *F. novicida*-induced, AIM2-dependent activation of caspase-1, release of IL-1β and IL-18 and cell death, whereas their...
Figure 2  IRF1 is essential for activation of the AIM2 inflammasome by infection with *F. novicida*. (a,b) Immunoblot analysis of caspase-1 (as in Fig. 1a) (a) and the release of IL-1β and IL-18 (b) of unprimed BMDMs left untreated or assessed 20 h after infection with *F. novicida* (MOI, 100) or 5 h after transfection of poly(dA:dT). (c,d) Microscopic analysis (c) and quantification (b) of the death of unprimed BMDMs 20 h after infection with *F. novicida*, 5 h after transfection of poly(dA:dT) or 10 h after infection with MCMV (MOI, 10). Arrowheads (e) indicate dead cells. Original magnification (c), ×40. (e,f) Immunoblot analysis of caspase-1 (as in Fig. 1a) (e) and release of IL-18 and death (f) of unprimed BMDMs left untransfected (med) or assessed 5 h after transfection of plasmid DNA (pcDNA). (g,h) Immunoblot analysis of caspase-1 (as in Fig. 1a) (g) and release of IL-1β and IL-18 (h) of unprimed BMDMs left uninfected (Med) or assessed 10 h after infection with MCMV (MOI, 10). (i) Immunoblot analysis of caspase-1 (as in Fig. 1a) in BMDMs left uninfected (−) or infected (+) with *F. novicida* with (+ or wedge) or without (−) co-stimulation with recombinant mouse IFN-γ (at a concentration of 500 U/ml or at increasing concentrations (wedges) of 25, 250 and 500 U/ml. *P < 0.01, **P < 0.001 and ***P < 0.0001 (two-tailed t-test). Data are representative of three (a−f,i) or two (g,h) independent experiments (mean and s.e.m. in b,d,f,h).

Wild-type counterparts exhibited robust AIM2 inflammasome responses (Fig. 2a−d). In contrast, *Irf1*−/− BMDMs responded normally to the transfected dsDNA ligands poly(dA:dT) and plasmid pcDNA3.1 DNA and induced AIM2-dependent maturation of caspase-1, release of IL-18 and cell death (Fig. 2a−f). Consistent with that, *Irf1*−/− BMDMs had an impaired ability to generate an AIM2 inflammasome speck in response to infection with *F. novicida* but not in response to transfected poly(dA:dT) (Supplementary Fig. 1d,c). Analysis of mRNA transcripts of *Aim2* and *Il1b* by quantitative RT-PCR revealed similar expression of these genes in wild-type and *Irf1*−/− BMDMs infected with *F. novicida* (Supplementary Fig. 2a). The levels of the pro-inflammatory cytokines tumor-necrosis factor and IL-6 were slightly lower in *Irf1*−/− BMDMs than in wild-type BMDMs (P > 0.05), whereas the level of the chemokine CXCL1 (KC) was similar in *Irf1*−/− and wild-type BMDMs (Supplementary Fig. 2b). Accordingly, the level of IL-12 was lower in *F. novicida*-infected *Irf1*−/− BMDMs than in *F. novicida*-infected wild-type BMDMs (Supplementary Fig. 2e).

We next investigated whether IRF1 was required for activation of the AIM2 inflammasome in response to MCMV7. Notably, engagement of the AIM2 inflammasome with MCMV did not require IRF1 (Fig. 2c,d,g,h). This indicated that detection of bacteria and viruses by AIM2 occurred via distinct pathways governed by the requirement for IRF1.

IRF1 has been identified as a transactivator of IFN-β23, which suggests that defective production of IFN-β might lead to impaired activation of the AIM2 inflammasome. Although we detected reduced expression of the gene encoding IFN-β in *Irf1*−/− BMDMs infected with *F. novicida* (Supplementary Fig. 2d), priming of *Irf1*−/− BMDMs with recombinant IFN-β failed to ‘rescue’ activation of the AIM2 inflammasome by *F. novicida*, whereas stimulation with IFN-β increased AIM2-dependent maturation of caspase-1 in wild-type cells in a dose-dependent way (Fig. 2i). These findings suggested that the defective activation of the AIM2 inflammasome in *Irf1*−/− BMDMs was not due solely to reduced production of IFN-β but largely reflected defects in additional IRF1-mediated processes.

**IRF1 is dispensable for the NLRP3 and NLRC4 inflammasomes**

To investigate whether IRF1 is required for activation of additional inflammasomes, we stimulated unprimed wild-type and *Irf1*−/− BMDMs with activators of the canonical NLRP3 inflammasome (ATP and nigericin) and activators of the non-canonical NLRC4 inflammasome (*C. rodentium* and *Salmonella enterica* serovar Typhimurium (S. Typhimurium) lacking the flagellin subunits flIC and flIB)17. We found that *Irf1*−/− BMDMs underwent normal activation of caspase-1, release of IL-1β and IL-18 and cell death in response to activators of the canonical or non-canonical NLRC4 inflammasomes (Fig. 3a,b). In addition, wild-type and *Irf1*−/− BMDMs infected with S. Typhimurium grown to log phase, which activates the NLRC4 inflammasome via an SPI-1 type III secretion system23, showed similar levels of activation of caspase-1, release of IL-1β and IL-18 and cell death (Fig. 3c,d), which indicated that IRF1 did not interfere with activation of the NLRC4 inflammasome.
GBP-encoding genes require IRF1

We further investigated the IRF1 signature that led to activation of the AIM2 inflammasome by *F. novicida*. For this we performed microarray analysis to identify genes expressed differentially in *F. novicida*-infected wild-type, *Irf1*−/−, *Ifnar1*−/− and AIM2-deficient (*Aim2*−/−) BMDMs. Analysis of the expression of genes encoding molecules involved in macrophage-mediated immunity revealed that the gene set with reduced expression in both *Irf1*−/− BMDMs and *Ifnar1*−/− BMDMs relative to their expression in wild-type BMDMs was dominated by those encoding GBPs, including Gbp2, Gbp3, Gbp4, Gbp5, Gbp6, Gbp8, Gbp9 and Gbp10 (Fig. 4a). *Aim2*−/− BMDMs, in contrast, showed elevated expression of genes encoding GBPs and other interferon-inducible genes, relative to their expression in wild-type BMDMs (Fig. 4a,b), an observation supported by the negative regulatory effect of AIM2 on interferon responses8,9. We further confirmed by real-time quantitative RT-PCR analysis the downregulation of GBP-encoding genes in the absence of IRF1 or IFNAR1 (Fig. 4b).

Activation of the AIM2 inflammasome requires GBPs

Genes encoding GBPs are located on mouse chromosomes 3 and 5 (ref. 24). Those located on chromosome 3 (Gbp1, Gbp2, Gbp3, Gbp5 and Gbp7) encode GBPs linked to the control of bacterial and parasitic replication19,24. To investigate the role of these GBPs in the activation of the AIM2 inflammasome, we obtained BMDMs from mice lacking the GBP-encoding locus on chromosome 3 (called ‘Gbpchr3’ here)19,24 and infected the cells with *F. novicida*. We observed significantly decreased levels of activation of caspase-1, release of IL-1β and IL-18 and induction of cell death in *F. novicida*-infected BMDMs in which Gbpchr3 was deleted, relative to their levels in *F. novicida*-infected wild-type BMDMs (Fig. 4c). Gbpchr3-deficient BMDMs, however, displayed normal levels of activation of caspase-1, release of IL-18 and induction of cell death in response to transfected poly(dA:dT)19 (Fig. 4c).

Of the GBP-encoding genes located on chromosome 3, IRF1 controlled upregulation of the expression of Gbp2, Gbp3 and Gbp5 (Fig. 4a,b). Gbp1 was not identified in our microarray data set (Fig. 4a), consistent with published studies showing that Gbp1 is not expressed in mice on the C57BL/6 background25. Given the importance of IRF1 for activation of the AIM2 inflammasome, we set out to identify which of the remaining GBPs was responsible for this activity.

For this, we individually knocked down each of the remaining GBP-encoding genes in the chromosome 3 locus (Gbp2, Gbp3, Gbp5 and Gbp7) in primary wild-type BMDMs by an approach involving small interfering RNA and found that silencing of Gbp2 or Gbp5 led to a significant reduction in the activation of caspase-1 and secretion of IL-1β in response to infection with *F. novicida* (Supplementary Fig. 3a–c).

To confirm the role of GBP2 and GBP5 in activation of the AIM2 inflammasome by infection with *F. novicida*, we stimulated GBP2-deficient (Gbp2−/−) and GBP5-deficient (Gbp5−/−) BMDMs with *F. novicida*, poly(dA:dT), LPS plus ATP (to activate the NLRP3 inflammasome) or *S. Typhimurium* grown to log phase (to activate the NLRC4 inflammasome). Both Gbp2−/− and Gbp5−/− BMDMs showed significantly reduced activation of caspase-1, secretion of IL-1β and IL-18 and cell death following infection with *F. novicida* relative to that in their wild-type counterparts (Fig. 4d–f), which confirmed a role for both GBP2 and GBP5 in activation of the AIM2 inflammasome. In contrast, stimulation of wild-type, Gbp2−/− and Gbp5−/− BMDMs with poly(dA:dT), LPS plus ATP or *S. Typhimurium* grown to log phase resulted in levels of activation of caspase-1, secretion of IL-1β and IL-18 and cell death similar to those in their wild-type counterparts19 (Fig. 4d–f). These results indicated a non-redundant role for GBP2 and GBP5 in activation of the AIM2 inflammasome in response to infection with *F. novicida*.

GBPps mediate killing of *F. novicida* to induce AIM2 activation

To investigate whether the expression of GBP2 and GBP5 is entirely under the control of IRF1, we infected wild-type and *Irf1*−/− BMDMs with *F. novicida* and monitored the dynamics of the expression of GBP2 and GBP5 over the course of infection. We found that the expression of GBP2 and GBP5 was delayed compared with the expression of IRF1 in wild-type BMDMs and was reduced in the absence of IRF1 (Fig. 5a). However, the residual expression of GBP2 and GBP5 in *Irf1*−/− BMDMs indicated that an alternative IRF1-independent pathway might exist to induce the expression of these GBPs. It is possible that signaling via type I interferons might trigger an alternative
pathway that leads to GBP expression independently of IRF1. To investigate this, we infected wild-type and Irf1−/− BMDMs with F. novicida and found that a lack of signaling via type I interferons completely abolished the expression of GBP2 and GBP5 (Fig. 5a). Together these findings provided evidence demonstrating that two different members of the GBP family, GBP2 and GBP5, were under the control of IRF1 and signaling via type I interferons and were expressed to specifically engage the AIM2 inflammasome.

GBPs can target vacuolar bacteria, such as Salmonella, and induce the recruitment of antimicrobial peptides to kill the bacteria19,38. Whether GBPs mediate killing of cytosolic bacteria such as F. novicida has not been investigated. We therefore used confocal microscopy to observe the spatial distribution of GBP5 relative to that of F. novicida in infected BMDMs. GBP5 was recruited to and engulfed F. novicida bacteria in wild-type BMDMs (Fig. 5b and Supplementary Fig. 4). The absence of IRF1 led to a significantly reduced prevalence of GBP5-associated bacteria (Fig. 5c). BMDMs lacking IRF1 or Gbp5−/− also failed to control bacterial replication over time (Fig. 5d).

To investigate whether GBP5 directly affected the viability of bacteria in macrophages, we infected wild-type and Gbp5−/− BMDMs with F. novicida expressing green fluorescent protein (GFP) and quantified bacteria in these cells over time. Single-cell analysis revealed that wild-type BMDMs restricted bacterial replication from 4 h to 16 h after infection, whereas Gbp5−/− BMDMs failed to control bacterial replication at 16 h and harbored a significantly larger number of bacteria than wild-type BMDMs had (Fig. 5e and Supplementary Fig. 4). In addition, GBP5-associated bacteria tended to lose their GFP expression, in contrast to non–GBP5-associated bacteria (Supplementary Fig. 4), which might suggest a loss of bacterial viability following recruitment of GBP5 (ref. 19). To investigate the hypothesis that IRF1 mediates the AIM2-dependent killing of intracellular bacteria, we infected unprimed wild-type, Irf1−/− and Aim2−/− BMDMs with F. novicida and quantified bacteria in these cells over time. In agreement with that hypothesis, Irf1−/− and Aim2−/− BMDMs failed to suppress bacterial replication over 24 h of infection (Fig. 5f).

For AIM2 to detect dsDNA, F. novicida or its DNA must be able to escape the vacuole and enter the cytoplasm. Indeed, co-staining of the inflammasome and DNA in F. novicida-infected BMDMs revealed colocalization of DNA with the inflammasome speck (Supplementary Fig. 5a). To investigate whether F. novicida that has escaped from the vacuole engages IRF1- and GBP-dependent activation of the AIM2 inflammasome, we infected wild-type BMDMs with wild-type F. novicida or an isogenic mutant form of F. novicida that fails to disrupt the vacuole as required for escape into the cytoplasm (lacking
the gene encoding MglA, which regulates various pathogenicity island proteins, including IgIC (F. novicida ΔmglA)22. F. novicida ΔmglA retained the ability to induce IRF1 and the expression of GBP2 and GBP5, although at a lower capacity relative to that of wild-type F. novicida (Supplementary Fig. 5b). However, F. novicida ΔmglA failed to activate the AIM2 inflammasome to trigger the release of IL-1β and IL-18 and cell death (Supplementary Fig. 5c,d). The production of other proinflammatory cytokines, tumor-necrosis factor, IL-6 and KC by wild-type BMDMs infected with F. novicida ΔmglA was similar to that of wild-type BMDMs infected with wild-type F. novicida (Supplementary Fig. 5c), which demonstrated that the inability of F. novicida ΔmglA to activate the AIM2 inflammasome was not due to a general defect in its ability to stimulate cytokines in macrophages. These results suggested that escape from the vacuole into the cytosol was essential for activation of the AIM2 inflammasome and that such escape enhanced but was not indispensable for the induction of IRF1-mediated expression of GBPs. It is possible that additional processes that complement GBP expression are required for full engagement of the AIM2 inflammasome. Indeed, deficiency in IRF1 did not completely abolish the expression of GBP2 and GBP5 (Fig. 5a), which suggested that IRF1 might direct additional processes other than driving GBP expression to activate the AIM2 inflammasome.

We next investigated whether the absence of IRF1 prevented escape of F. novicida into the cytosol, which might be an additional mechanism that governs the IRF1-dependent activation of the AIM2 inflammasome. Cytosolic pathogens such as F. novicida escape the vacuole and can be used as a mechanism for delivering ligands into the cytosol of a cell18. We took advantage of this cytosolic delivery method and infected wild-type, Irf1−/− and Aim2−/− BMDMs with F. novicida in the presence of ultrapure LPS from Salmonella to investigate whether Salmonella LPS can be efficiently introduced into the cytosol to activate the inflammasome in the absence of IRF1. We found that wild-type, Irf1−/− and Aim2−/− BMDMs all underwent induction of the activation of caspase-1 in response to infection with F. novicida in the presence of Salmonella LPS (Supplementary Fig. 6a); this indicated that the LPS was delivered into the cytosol, which probably activated the non-canonical NLRP3 inflammasome. These findings suggested that in the absence of IRF1, escape into the cytosol was not fully compromised at 20 h after infection.

DNA sensor cGAS mediates activation of the AIM2 inflammasome Although signaling via type I interferons that engages the AIM2 inflammasome requires IRF1, the pattern-recognition receptors that induce the production of type I interferons in response to infection with F. novicida have remained largely unexplored. A major pathway that mediates the recognition of cytosolic bacteria for the generation of IFN-β production is the signaling axis of the DNA sensor cyclic GMP-AMP synthase (cGAS) and the adaptor STING12,22. In agreement with that, both cGAS-deficient BMDMs and STING-deficient BMDMs (with nonfunctional ‘Gt’ alleles encoding STING that result in a lack of detectable protein)28 had impaired ability to robustly activate caspase-1 or induce the release of IL-1β and IL-18 or cell death in response to infection with F. novicida (Fig. 5g and Supplementary Fig. 6b,c). In addition, cGAS-deficient BMDMs failed to robustly induce IRF1 expression (Supplementary Fig. 6d). The residual inflammasome responses in cGAS-deficient and STING-deficient BMDMs infected with F. novicida could have been due to a minor source of type I interferons induced by TLRs, RLRs or other PRRs. Collectively, these findings indicated that the cGAS signaling axis induced type I interferon–dependent expression of IRF1 necessary for the engagement GBP-mediated killing of F. novicida, which ultimately resulted in the release of bacterial DNA for activation of the AIM2 inflammasome (Supplementary Fig. 6e).

IRF1 provides protection against infection with F. novicida We extended our findings to an in vivo setting and infected wild-type mice, Irf1−/− mice, Aim2−/− mice and mice deficient in both caspase-1
and caspase-11 (Casp1/−/−Casp4/−/−, called ‘Casp1/−/−Casp11/−/−’ here) with F. novicida and monitored their susceptibility to infection. Ifri1−/−, Aim2−/− and Casp1/−/−Casp11−/− mice lost more body weight than wild-type mice did, and all succumbed to infection within 6 d, whereas 75% of the wild-type mice survived beyond day 6 (Fig. 6a,b). Analysis of bacterial burdens showed that Ifri1−/− mice harbored a significantly larger burden of F. novicida in the liver and spleen than did wild-type mice (Fig. 6c). Similarly, Aim2−/− mice were significantly more susceptible to infection with F. novicida than were wild-type mice8,22 (Fig. 6d). The greater susceptibility of Ifri1−/− mice than Aim2−/− mice to infection with F. novicida might suggest that Ifri1 is important in multiple antimicrobial defense mechanisms. Analysis of serum IL-18 showed that Ifri1−/− mice had an impaired ability to produce this cytokine following infection with F. novicida; Aim2−/− and Casp1/−/−Casp11−/− mice had a similar phenotype for this (Fig. 6e). Histopathology analysis of liver tissues showed that Ifri1−/− mice failed to control the dissemination of F. novicida (Fig. 6f,g). In contrast, granulomas that surround infected particles were present in the liver of wild-type mice (Fig. 6g). In addition, Ifri1−/− mice had more dead cells than wild-type mice had, but their granulocyte recruitment in the liver was similar to that of wild-type mice, as revealed by TUNEL staining and staining of myeloperoxidase, respectively (Fig. 6h,i). The greater prevalence of TUNEL staining in Ifri1−/− mice was probably the result of more damage from their greater bacterial burden, which leads to the death of many cell types in the liver. Together these results highlighted a role for Ifri1 in the host defense against F. novicida infection via engagement of the AIM2 inflammasome.

**DISCUSSION**

Various pathways exist for the recognition of DNA for triggering innate immune responses in the cell. TLR9 recognizes CpG dinucleotide DNA in the endosomal compartment to activate the transcription of genes encoding pro-inflammatory cytokines29, and STING responds directly to cyclic dinucleotides or serves as an adaptor for a range of DNA sensors to induce type I interferon responses30. AIM2 mediates the sensing of cytosolic dsDNA to initiate assembly of the inflammasome. However, the precise mechanisms by which different pathogens trigger AIM2 have remained unclear. In addition, the function of signaling via type I interferons in activation of the AIM2 inflammasome has remained unclear, given that such signaling is important for mediating activation of the AIM2 inflammasome in response to infection with F. novicida but not in response to infection with MCMV.

Here we have identified an IFR1-dependent pathway downstream of signaling via type I interferons that was responsible for activation of the AIM2 inflammasome by F. novicida. The existence of this ‘non-canonical’ AIM2 inflammasome pathway defined by its requirement for IFR1, GBPs and signaling via type I interferons is analogous to the non-canonical NLRP3 inflammasome and its requirement for signaling via type I interferons and caspase-11 (refs. 12,13,15,17). Ifri1 was first identified as a virus-induced transcription factor that interacts with regulatory DNA elements of the gene encoding human IFN-β, and that contributes to the transcription of the gene encoding IFN-β31. Subsequent studies have revealed that both human and mouse Ifri1 are involved in the expression of IFN-α, major histocompatibility class I, the GTPase MX (in mice), 2′-5′-oligo A synthetase (in mice) and the regulator ISG54 (in humans)32. We have shown here that Ifri1 was not absolutely required for the production of IFN-β and certain subtypes of IFN-α in F. novicida–infected macrophages. Instead, we found that STING were probably the upstream sensors that initiated the production of type I interferons.

Notably, Ifri1 and type I interferons induce GBPs to kill and lyse cytosolic bacteria to drive the release of bacterial DNA for access by AIM2. Although our microarray analysis suggested that Ifri1 and IFNAR induced a similar expression profile of interferon-stimulated genes, protein-expression analysis revealed that the expression of GBP2 and GBP5 was completely abolished in the absence of IFNAR, whereas we observed some residual expression of these proteins in the absence of IFR1. This suggested that IFR1 and IFNAR1 probably each regulated their own set of genes in...
F. novicida–infected macrophages, some of which might be commonly regulated by both IRF1 and IFNAR1.

Herpesviruses, including MCMV, enter the host cell via a mechanism that requires binding and fusion of the viral envelope with the host cell membrane, which results in release of the viral capsid into the host cell. Under these circumstances, viral DNA may have access to the cytoplasm directly for detection by AIM2. As shown before, we found that mutant F. novicida that fails to escape the vacuole did not activate the AIM2 inflammasome. In addition, we showed that escape of F. novicida from the vacuole into the cytoplasm enhanced the expression of IRF1 and GBP, which suggested that this process might provide an additional signal for enhancing GBP-mediated killing of the bacteria in the cytosol. F. tularensis live vaccine strain escapes the vacuole as early as 1 h after infection, suggesting that escape from vacuoles precedes GBP expression, which occurs mostly beyond 16–24 h after infection, following signaling via type 1 interferons. However, the precise mechanism by which GBPp9 kill F. novicida is unknown. Published studies have found that GBP1 or GBP7 can recruit additional effector proteins to mediate the killing of pathogens. GBP7 interacts with and recruits p22phox and other components of the NADPH oxidase to the mycobacteria-containing vacuole to mediate the killing of bacteria, whereas GBP1 facilitates delivery of ubiquitinylated cargo (for example, bacteria decorated with ubiquitin) to autolysosomes to mediate killing. GBP1, GBP2 and GBP5 share a common feature in that they harbor a carboxy-terminal 'CaaX' motif (a cysteine residue followed by two alpha-helical residues and then any amino acid residue) that is targeted by prenylation, a post-translational modification process that results in the attachment of a lipid hydrophobic moiety to mediate docking of the protein to cellular membranes. It is possible that GBP2 and GBP5 might directly target cytosolic F. novicida to induce recruitment of an antimicrobial process to mediate the killing of this bacteria. Indeed, IRF1 controls the expression of inducible nitric oxide synthase. The contribution of inducible nitric oxide synthase and its connection with GBPs in the activation of the AIM2 inflammasome warrants further investigation.

We observed that macrophages lacking either IRF1 or AIM2 failed to restrict F. novicida replication, whereas mice lacking IRF1 were more susceptible to infection with F. novicida than were mice lacking AIM2. These results suggested that IRF1 controls multiple antimicrobial pathways to suppress the bacterial burden in vivo, which ultimately leads to lysis of the bacterial pathogen for activation of the AIM2 inflammasome. IRF1 has been linked to the production of IL-12. However, published studies have shown that at 3 d after infection with F. tularensis live vaccine strain, mice lacking the IL-12 subunit p40 or p30 or mice treated with antibodies to IL-12 have a bacterial burden similar that of their wild-type or PBS-treated counterparts. In our study, mice lacking IRF1 had a significantly higher bacterial burden than did wild-type mice on day 2, and by day 3, more than 80% of the mice lacking IRF1 succumbed to infection. Thus, we speculate that IL-12 probably does not account for the substantial difference between wild-type mice and Irf1−/− mice in their bacterial burden.

Additional mechanisms have been identified that control assembly and degradation of the AIM2 inflammasome. Polyubquitination of ASC recruits the autophage marker p62 to target the AIM2 inflammasome for degradation by autophagy. The human pyrin domain–containing protein POP3 interacts with the pyrin domain of AIM2 and competes with ASC to inhibit activation of the AIM2 inflammasome in response to poly(dA:dT) and MCMV. The existence of these pathways indicates that controlling activation of the AIM2 inflammasome is important for maintaining homeostasis of the cell. Indeed, aberrant activation of the AIM2 inflammasome in response to dsDNA could lead to considerable physiological consequences. Increased expression of AIM2 is associated with the development of psoriasis, an abdominal aortic aneurysm and systemic lupus erythematosus, whereas reduced expression of AIM2 is linked to colorectal and prostate cancer. Our findings have identified a previously unknown layer of regulation that governs cytoplasmic sensing of DNA after infection with intracellular pathogens. Therapies that modulate IRF1 activities might lead to enhanced protection against rapid and lethal infection with F. tularensis.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. GEO: microarray data, GSE66461.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

We thank V.M. Dixit (Genentech) and N. Kayagaki (Genentech) for Aim2-deficient mice and Gbp5−/− mice; K.A. Fitzgerald (University of Massachusetts Medical School) for femurs from c-GAS-deficient mice; P. Broz (University of Basel) for femurs from Gbp3−/− mice; K. Pfeffer (Heinrich-Heine-University Duesseldorf) for femurs from Gbp2−/− mice; P.G. Thomas (St. Jude Children’s Research Hospital) for the MCMV Smith MSGV strain; and X. Qi and M. Barr for technical assistance. Supported by the US National Institutes of Health (AI056296, CA163507 and AI101995), the American Lebanese Syrian Associated Charities (T.-D.K.), European Research Council (281600), the Fund for Scientific Research-Flanders (G030212N to M.L.), St. Jude Children’s Research Hospital and the National Health and Medical Research Council (Neoma Roadway Endowed Fellowship and R.G. Menzies Early Career Fellowship to S.M.M.).

AUTHOR CONTRIBUTIONS

S.M.M., R.K. and T.-D.K. designed the study; S.M.M., R.K., R.K.S.M., G.N. and P.V. performed experiments and analyzed the data; M.Y. contributed reagents; and S.M.M., M.L. and T.-D.K. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

1. Stroug, T., Henao-Mejia, J., Elina, E. & Flavell, R. Inflammasomes in health and disease. Nature 481, 278–286 (2012).
2. Lamkanfi, M. & Dixit, V.M. Mechanisms and functions of inflammasomes. Cell 157, 1013–1022 (2014).
3. Rathinam, V.A.K., Vanaja, S.R. & Fitzgerald, K.A. Regulation of inflammasome signaling. Nat. Immunol. 13, 333–342 (2012).
4. Bürckstümmer, T. et al. An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. Nat. Immunol. 10, 266–272 (2009).
5. 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).
6. Hornung, V. et al. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature 458, 514–518 (2009).
7. Roberts, T.L. et al. HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. Science 323, 1057–1060 (2009).
8. Fernandes-Alnemri, T. et al. The AIM2 inflammasome is critical for innate immunity to Francisella tularensis. Nat. Immunol. 11, 385–393 (2010).
9. Rathinam, V.A.K. et al. The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. Nat. Immunol. 11, 395–402 (2010).
10. Dombrowski, Y. et al. Cytosolic DNA triggers inflammasome activation in keratinocytes in psoriatic lesions. Sci. Transl. Med. 3, 82ra38 (2011).
11. 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).
12. Rathinam, V.A.K. et al. TRIF licenses caspase-11-dependent NLRP3 inflammasome activation by Gram-negative bacteria. Cell 150, 606–619 (2012).
13. Broz, P. et al. Caspase-11 increases susceptibility to Salmonella infection in the absence of caspase-1. Nature 490, 288–291 (2012).
14. Sander, L.E. et al. Detection of prokaryotic mRNA signifies microbial viability and promotes immunity. Nature 474, 385–389 (2011).
15. Gurung, P. et al. Toll or interleukin-1 receptor (TIR) domain-containing adaptor inducing interferon-β (TRIF)-mediated caspase-11 protease production integrates Toll-like receptor 4 (TLR4) protein- and Nlrp3 inflammasome-mediated host defense against enteropathogens. J. Biol. Chem. 287, 34474–34483 (2012).
16. Kayagaki, N. et al. Noncanonical inflammasome activation by intracellular LPS independent of TLR4. Science 341, 1246–1249 (2013).
17. Kayagaki, N. et al. Non-canonical inflammasome activation targets caspase-11. Nature 479, 117–121 (2011).
18. 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).
19. Meunier, E. et al. Caspase-11 activation requires lysis of pathogen-containing vacuoles by IFN-induced GTPases. Nature 514, 187–192 (2014).
20. Shi, J. et al. Inflammatory caspses are innate immune receptors for intracellular LPS. Nature 514, 7403–7408 (2014).
21. Henry, T., Brotcke, A., Weiss, D.S., Thompson, L.J. & Monack, D.M. Type I interferon action: mouse strain distribution and inheritance of an induced protein β-inducing interferon-γ. J. Exp. Med. 204, 987–994 (2007).
22. 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).
23. Man, S.M. et al. Inflammasome activation causes dual recruitment of NLRCA and NLRP3 to the same macromolecular complex. Proc. Natl. Acad. Sci. USA 111, 7403–7408 (2014).
24. Yamamoto, M. et al. A cluster of interferon-γ-inducible p65 GTPases plays a critical role in host defense against Toxoplasma gondii. Immunity 37, 302–313 (2012).
25. Staeheli, P., Prochazka, M., Steigmeier, P.A. & Hailer, O. Genetic control of interferon action: mouse strain distribution and inheritance of an induced protein with guanylate-binding property. Virology 137, 135–142 (1984).
26. Kim, B.H. et al. A family of IFN-γ-inducible 65-kD GTPases protects against bacterial infection. Science 332, 717–721 (2011).
27. Santic, M., Molmeret, M., Kloet, K.E., Jones, S. & Kwak, Y.A. The Francisella tularensis pathogenicity island protein IglC and its regulator MglA are essential for modulating phagosome biogenesis and subsequent bacterial escape into the cytoplasm. Cell. Microbiol. 7, 969–979 (2005).
28. Sauer, J.D. et al. The N-ethyl-N-nitrosourea-induced Goldenticket mouse mutant reveals an essential function of Sting in the in vivo interferon response to Listeria monocytogenes and cyclic dinucleotides. Infect. Immun. 79, 688–694 (2011).
29. Hemmi, H. et al. A Toll-like receptor recognizes bacterial DNA. Nature 408, 740–745 (2000).
30. Ishikawa, H., Ma, Z. & Barber, G.N. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature 461, 788–792 (2009).
31. Fujita, T. et al. Evidence for a nuclear factor(s), IRF-1, mediating induction and silencing properties to human IFN-β gene regulatory elements. EMBO J. 7, 3397–3405 (1988).
32. Miyamoto, M. et al. Regulated expression of a gene encoding a nuclear factor, IRF-1, that specifically binds to IFN-beta gene regulatory elements. Cell 54, 903–913 (1988).
33. Mariathasan, S., Weiss, D.S., Dixit, V.M. & Monack, D.M. Innate immunity against Francisella tularensis is dependent on the ASC/caspase-1 axis. J. Exp. Med. 202, 1043–1049 (2005).
34. Checroun, C., Wehrly, T.D., Fischer, E.R., Hayes, S.F. & Celli, J. Autophagy-mediated reentry of Francisella tularensis into the endocytic compartment after cytoplasmic replication. Proc. Natl. Acad. Sci. USA 103, 14578–14583 (2006).
35. Britzen-Laurent, N. et al. Intracellular trafficking of guanylate-binding proteins is regulated by heterodimerization in a hierarchical manner. PLoS ONE 5, e14246 (2010).
36. Kamijo, R. et al. Requirement for transcription factor IRF-1 in NO synthase induction in macrophages. Science 263, 1612–1615 (1994).
37. Elkins, K.L., Cooper, A., Colombini, S.M., Cowley, S.C. & Kieffer, T.L. In vivo clearance of an intracellular bacterium, Francisella tularensis LV5, is dependent on the p40 subunit of interleukin-12 (IL-12) but not on IL-12 p70. Infect. Immun. 70, 1936–1948 (2002).
38. Shi, C.S. et al. Activation of autophagy by inflammatory signals limits IL-1β production by targeting ubiquitinated inflammasomes for destruction. Nat. Immunol. 13, 255–263 (2012).
39. 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).
40. Connolly, D.J. & Bowie, A.G. The emerging role of human PYHIN proteins in innate immunity: implications for health and disease. Biochem. Pharmacol. 92, 405–414 (2014).
Animal studies were conducted under protocols approved by the St. Jude Children’s Research Hospital on the Use and Care of Animals.

Cell culture and stimulation. Primary BMDMs were grown for 5–6 d in IMDM (Gibco) supplemented with 1% non-essential amino acids (Gibco), 10% FBS (Atlanta Biologicals), 30% medium conditioned by L929 mouse fibroblasts, and 1% penicillin and streptomycin (Sigma). BMDMs in antibiotic-free incubation (for expression of IRF1 or GBP) or for activation of caspase-1) and an MOI of 50 and 2, 8, 16 and 24 h of infection with C. rodentium or S. Typhimurium strain SL1344, an isogenic mutant lacking flcC and fliB (ΔflcCΔfliB STM) and C. rodentium (51459; American Type Culture Collection) were inoculated into LB broth and were incubated overnight under aerobic conditions at 37 °C. S. Typhimurium strain SL1344 was subcultured (1:10) for 3 h at 37 °C in fresh LB broth to generate bacteria grown to log phase. The following conditions were used: F. novicida ΔmglA were grown overnight under aerobic conditions at 37 °C in BBL Trypticase Soy Broth (211768; BD) supplemented with 0.2% l-cysteine (Fisher). Bacteria were subcultured (1:10) for 4 h in fresh Trypticase Soy Broth supplemented with 0.2% l-cysteine and resuspended in PBS. S. Typhimurium strain SL1344, an isogenic mutant lacking flcC and fliB (ΔflcCΔfliB STM) and C. rodentium (51459; American Type Culture Collection) were inoculated into LB broth and were incubated overnight under aerobic conditions at 37 °C. S. Typhimurium SL1344 was subcultured (1:10) for 3 h at 37 °C in fresh LB broth to generate bacteria grown to log phase. The following conditions were used: F. novicida or F. novicida ΔmglA, an MOI of 100 and 20 h of incubation (for activation of caspase-1) and an MOI of 50 and 2, 8, 16 and 24 h of incubation (for expression of IRF1 or GBP); S. Typhimurium, an MOI 1 and 4 h of incubation; ΔflCΔfljB STM, an MOI of 20 and 20 h of incubation; and C. rodentium, an MOI 20 and 20 h of incubation. Gentamicin (50 µg/ml Gibco) was added at 2 h (S. Typhimurium), 4 h (C. rodentium and ΔflCΔfljB STM) or 8 h (F. novicida) after infection to kill extracellular bacteria. The MCMV Smith MSGV strain (VR-1399; American Type Culture Collection) was obtained from P.G. Thomas. Virus was used to unprimed BMDMs at an MOI of 10, followed by 10 h of incubation, for activation of the AIM2 inflammasome.

For activation of the canonical NLRP3 inflammasome, BMDMs were primed for 4 h with 500 ng/ml ultrapure LPS from Salmonella minnesota R595 (InvivoGen) and were stimulated for 45 min with 5 mM ATP or 10 µg/ml ultrapure LPS from P. G. Thomas. Virus was added to unprimed BMDMs at an MOI of 100, followed by 20 h of incubation, for activation of the AIM2 inflammasome. Levels of lactate dehydrogenase released by cells were measured with a CytoTox 96 Non-Radioactive Cytotoxicity Assay according to the manufacturer’s instructions (Promega). Cell culture supernatants were collected for enzyme-linked immunosorbent assay (Millipore) or enzyme-linked immunosorbent assay for cytokines measured by multiplex enzyme-linked immunosorbent assay (Millipore) or enzyme-linked immunosorbent assay for IL-18 (MBL international) according to the manufacturer’s instructions.

Microarray. Transcripts were profiled for F. novicida–infected unprimed BMDMs obtained from wild-type and mutant mice. Total RNA (100 ng) was converted into biotin-labeled cRNA with an Ambion Wild-Type Expression Kit (Life Technologies). The RNA isolated was reverse-transcribed into cDNA with a First-Strand cDNA Synthesis Kit (Life Technologies). Real-time quantitative PCR was performed on an ABI 7500 real-time PCR instrument with 2x SYBR Green (Applied Biosystems) and the appropriate primers (sequences, Supplementary Table 2).

Cytokine analysis. Cytokines were measured by multiplex enzyme-linked immunosorbent assay (Millipore) or enzyme-linked immunosorbent assay for IL-18 (MBL international) according to the manufacturer’s instructions.

Histopathology. Formalin-preserved tissues were embedded in paraffin according to standard procedures. Sections 5 µm in thickness for immunoblot analysis of signaling via IRF1 or GBP, supernatants were removed and BMDMs were washed once with PBS, followed by lysis in RIPA buffer and sample loading buffer containing SDS and 100 mM DTT. Proteins were separated by electrophoresis through 8–12% polyacrylamide gels. Following electrophoretic transfer of proteins onto PVDF membranes, nonspecific binding was blocked by incubation with 5% skim milk; then membranes were incubated with primary antibodies (antibody to caspase-1 (anti-caspase-1) (1:3,000 dilution; Casper-1; AG-20B-0042; Adipogen), anti-IRF1 (1:1,000 dilution; DSE4; 8478; Cell Signaling Technologies), anti-GBP5 (1:1,000 dilution; 11854-1-AP); Proteintech), anti-GBP5 (1:1,000 dilution; 13220-1-AP; Proteintech) or anti-GAPDH (1:10,000 dilution; D16H11; S74, Cell Signaling Technologies), followed by incubation with secondary antibodies (horseradish peroxidase–conjugated anti-rabbit (111-035-047) or anti-mouse (315-035-047) (1:5,000 dilution; Jackson Immuno Research Laboratories).
were stained with hematoxylin and eosin and were examined by a pathologist ‘blinded’ to experimental sample identity. For immunohistochemistry, formalin-fixed, paraffin-embedded livers were cut into sections 4 µm in thickness. For staining of granulocytes, samples were incubated for 30 min with anti-MPO (1:500 dilution; A0398; Dako) followed by incubated for 30 min with Rabbit-on-Rodent HRP-Polymer (RMR622L; BioCare Medical). TUNEL (terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling) staining was performed with the Dead End kit (PRG7130) according to the manufacturer’s instructions (Promega).

**Statistical analysis.** GraphPad Prism 6.0 software was used for data analysis. Statistical significance was determined by t-tests (two-tailed) for two groups or one-way ANOVA (with Dunnett’s or Tukey’s multiple comparisons tests) for three or more groups. Survival curves were compared with the log-rank test. P values of <0.05 were considered statistically significant.

41. Matsuyama, T. et al. Targeted disruption of IRF-1 or IRF-2 results in abnormal type I IFN gene induction and aberrant lymphocyte development. *Cell* **75**, 83–97 (1993).

42. Honda, K. et al. IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature* **434**, 772–777 (2005).

43. Kimura, T. et al. Essential and non-redundant roles of p48 (ISGF3 γ) and IRF-1 in both type I and type II interferon responses, as revealed by gene targeting studies. *Genes Cells* **1**, 115–124 (1996).

44. Müller, U. et al. Functional role of type I and type II interferons in antiviral defense. *Science* **264**, 1918–1921 (1994).

45. Durbin, J.E., Hackenmiller, R., Simon, M.C. & Levy, D.E. Targeted disruption of the mouse *Stat1* gene results in compromised innate immunity to viral disease. *Cell* **84**, 443–450 (1996).

46. Kanneganti, T.D. et al. Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. *Nature* **440**, 233–236 (2006).

47. Mariathasan, S. et al. Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* **430**, 213–218 (2004).

48. Yamamoto, M. et al. Role of adaptor TRIF in the MyD88-independent Toll-like receptor signaling pathway. *Science* **301**, 640–643 (2003).

49. Irizarry, R.A. et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4**, 249–264 (2003).

50. Huang da, W., Sherman, B.T. & Lempicki, R.A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* **4**, 44–57 (2009).