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The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses

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Inflammasomes regulate the activity of caspase-1 and the maturation of interleukin 1β (IL-1β) and IL-18. AIM2 has been shown to bind DNA and engage the caspase-1-activating adaptor protein ASC to form a caspase-1-activating inflammasome. Using *Aim2*-deficient mice, we identify a central role for AIM2 in regulating caspase-1-dependent maturation of IL-1β and IL-18, as well as pyroptosis, in response to synthetic double-stranded DNA. AIM2 was essential for inflammasome activation in response to *Francisella tularensis*, vaccinia virus and mouse cytomegalovirus and had a partial role in the sensing of *Listeria monocytogenes*. Moreover, production of IL-18 and natural killer cell–dependent production of interferon-γ, events critical in the early control of virus replication, were dependent on AIM2 during mouse cytomegalovirus infection *in vivo*. Collectively, our observations demonstrate the importance of AIM2 in the sensing of both bacterial and viral pathogens and in triggering innate immunity.

Optimal protection from infection requires complex immune responses involving both the innate and adaptive immune systems. The innate immune system has a key role in initiating and orchestrating host defenses by regulating the production of proinflammatory cytokines, type I interferons and antimicrobial effectors[1]. Several distinct classes of germline-encoded pattern-recognition receptors have been discovered and have been linked to the sensing of microbial products. These include the Toll-like receptors (TLRs)[2], the C-type lectin receptors[3], the RIG-like helicases[4], the Nod-like receptors (NLRs)[5], and the cytosolic DNA sensors DAI[6], RNA polymerase III (refs. 7,8) and AIM2 (refs. 9–12). These receptors recognize microbial products from bacteria, viruses, fungi and parasites and in most cases trigger signaling pathways that culminate in the transcription of genes involved in the immune response[1].

The proinflammatory cytokine interleukin 1β (IL-1β) is in the arsenal of defense measures deployed by the innate immune system[13]. The activity of IL-1β is regulated at the level of expression, processing, secretion and antagonism by its naturally occurring inhibitor, IL-1 receptor antagonist[13]. An initial microbial stimulus acting through innate pattern-recognition receptors results in the accumulation of intracellular stores of pro-IL-1β. A second stimulus then controls the activation of caspase-1, a proteolytic enzyme, which in turn leads to cleavage of pro-IL-1β, followed by release of the active, mature, 17-kilodalton cytokine. IL-18 is structurally similar to IL-1β and, like IL-1β, is first synthesized as a leaderless precursor that requires caspase-1 for cleavage into an active molecule[14].

Multiprotein complexes containing one or more NLR(s), commonly referred to as 'inflammasomes', regulate caspase-1 activity. The NLRP1 inflammasome is activated by anthrax lethal toxin[15]. The NLRP3 inflammasome is activated by a broad range of stimuli, including bacteria, bacterial pore-forming toxins, viruses, endogenous danger signals such as ATP and monosodium urate, and indigestible particulates such as silica, asbestos, alum and amyloid-β[16]. The IPAF inflammasome is activated by flagellin or by the basal body rod component of the type 3 secretion system found in *Salmonella typhimurium* and other bacteria[17]. A fourth such complex, the AIM2 inflammasome, has also been identified. AIM2 is a member of the IFI20X-IFI16 (PYHIN) protein family[18]. AIM2 binds to DNA via a HIN-200 domain, whereas the pyrin domain associates with the adaptor molecule ASC to activate caspase-1 (refs. 9–12). The role of AIM2 in orchestrating immune responses to DNA or in regulating immune responses to viral or bacterial pathogens *in vivo* has not been examined further.

Here we investigate the role of AIM2 *in vivo* by generating mice lacking *Aim2*. We show that AIM2 regulated caspase-1-dependent processing of pro-IL-1β and pro-IL-18 in response to double-stranded DNA (dsDNA) in both dendritic cells (DCs) and macrophages. AIM2 did not mediate type I interferon responses to dsDNA but instead acted to negatively regulate this pathway. We also show a central role for AIM2 in the sensing of the cytosolic bacterial pathogen *Francisella tularensis* live vaccine strain (LVS), as well as vaccinia virus and mouse cytomegalovirus (mCMV). Additionally, we show a partial role for AIM2 in the sensing of *Listeria monocytogenes*. *In vivo*, AIM2-deficient mice ($Aim2^{-/-}$) and ASC-deficient mice ($Pycard^{-/-}$; called '$Asc^{-/-}$' here) infected with mCMV had lower serum concentrations of

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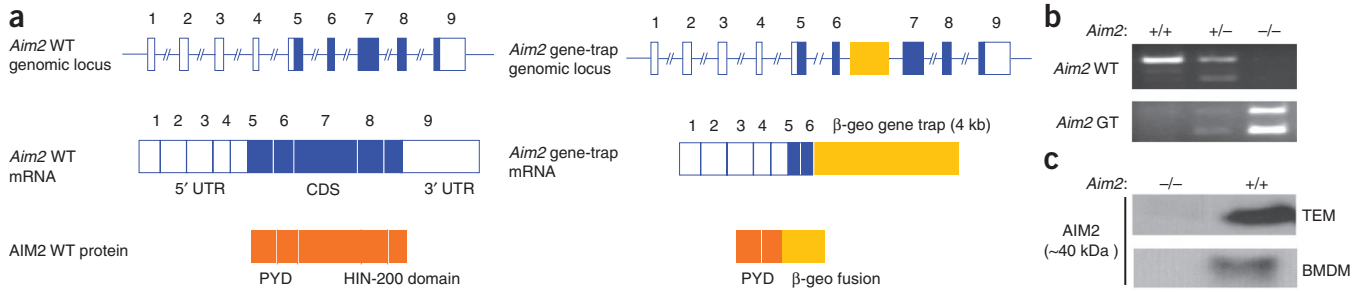

Figure 1 Characterization of *Aim2* gene-trap mice. (**a**) Wild-type *Aim2* (left) and *Aim2* with the gene-trap insertion (right). Exons are numbered above diagrams. UTR, untranslated region; CDS, coding sequence; PYD, pyrin domain; β-geo, β-galactosidase and neomycin-resistance cassette; kb, kilobase. (**b**) RT-PCR analysis of *Aim2* transcripts in wild-type mice (top) and mice with the *Aim2* gene-trap (GT) insertion (bottom). (**c**) Immunoblot analysis of AIM2 protein in $Aim2^{+/+}$ and $Aim2^{-/-}$ TEMs and BMDMs. Equal amounts of protein were loaded in each lane; AIM2 was detected with a rabbit polyclonal antibody. kDa, kilodaltons. Data are representative of at least three experiments.

IL-18 and considerably attenuated production of interferon-γ (IFN-γ) by natural killer (NK) cells, events critical for the early control of viral replication. Collectively, our observations establish AIM2 as an important antimicrobial sensor and a key determinant of protective immunity to viral pathogens.

RESULTS

Generation of $Aim2^{-/-}$ mice

To study the role of AIM2 *in vivo*, we generated AIM2-deficient mice through the use of a gene-trap embryonic stem cell line carrying a pGT0lxf vector insertion (**Fig. 1a**). In this cell line, the pGT0lxf gene-trap vector is inserted into intron 6 of *Aim2*. The fusion transcript generated by insertion of the gene-trap vector directs the expression of a truncated AIM2 protein carrying only the N-terminal pyrin domain fused to a cassette of β-galactosidase and neomycin-resistance proteins (**Fig. 1a**). We confirmed insertion of the pGT0lxf vector into *Aim2* in the gene-trap embryonic stem cells by RT-PCR (**Supplementary Fig. 1**) and mapped the site of the pGT0lxf integration by long-range PCR and sequencing and found it resided in intron 6 (**Supplementary Fig. 2**). We bred mice with two copies of the trapped *Aim2* to homozygosity and confirmed deletion of *Aim2* by RT-PCR (**Fig. 1b**) and immunoblot analysis (**Fig. 1c**). AIM2 was absent from thioglycollate-elicited macrophages (TEMs), bone marrow–derived macrophages (BMDMs) and splenocytes from $Aim2^{-/-}$ mice (**Fig. 1c** and data not shown). Mutant mice were born at the expected Mendelian ratios, and they developed and bred normally and showed no apparent abnormalities. Throughout our studies we compared $Aim2^{+/+}$ and $Aim2^{-/-}$ mice generated from breeding of heterozygous parents.

AIM2 is critical for responses to cytosolic dsDNA

Consistent with published studies identifying human AIM2 as a cytosolic sensor for dsDNA and a key regulator of IL-1β production[9–12], transfection of mouse *Aim2* also led to the formation of a functional inflammasome complex. As an initial screen for the ability of AIM2 to associate with ASC and elicit signaling, we tested the ability of transfected *Aim2* to trigger ASC-dependent signaling by the transcription factor NF-κB. Overexpression of mouse *Aim2* led to ASC-dependent activation of an NF-κB reporter gene (**Fig. 2a**). We next examined whether the mouse AIM2-ASC complex led to the caspase-1-dependent maturation of pro-IL-1β by using transient transfection of *Aim2* and *Asc* in the presence of caspase-1 and Flag-tagged pro-IL-1β in human embryonic kidney (HEK293T) cells. AIM2 led to the maturation of pro-IL-1β in an ASC-dependent manner (**Fig. 2b**).

To evaluate the role of AIM2 in the innate response to DNA, we generated BMDMs from $Aim2^{+/+}$ and $Aim2^{-/-}$ littermates. We examined macrophage differentiation by staining cells for the common myeloid marker CD11b and the macrophage marker F4/80. $Aim2^{+/+}$ and $Aim2^{-/-}$ macrophages had equivalent F4/80 and CD11b staining patterns (**Fig. 2c**), indicative of normal macrophage differentiation. Synthetic B-form dsDNA (poly(dA:dT)) induced a robust IL-1β response in BMDMs, as measured by enzyme-linked immunosorbent assay (ELISA), and this response was abolished in $Aim2^{-/-}$ macrophages (**Fig. 2d**). We obtained similar results with TEMs and bone marrow–derived DCs (BMDCs; **Fig. 2e**). DNA treatment also led to the proteolytic cleavage of caspase-1 and production of the mature 17-kilodalton IL-1β cytokine, and in both cases these responses were impaired in $Aim2^{-/-}$ cells (**Fig. 2f**). Macrophages lacking ASC or caspase-1 had a similar phenotype (data not shown). Poly(dA:dT) also induced IL-18 production and this was also AIM2 dependent (**Fig. 2g**). Finally, poly(dA:dT) induced an inflammatory form of cell death (pyroptosis) in $Aim2^{+/+}$ macrophages, and this response was also attenuated in $Aim2^{-/-}$ cells (**Fig. 2h**). Collectively, these data indicate that AIM2 is a nonredundant sensor that regulates the caspase-1-dependent maturation of IL-1β, IL-18 and pyroptosis in response to DNA[19].

AIM2-deficient cell responses to other inflammasome activators

To define the specificity of AIM2 for DNA, we examined inflammasome activity in response to agonists of other NLRs. $Aim2^{+/+}$ and $Aim2^{-/-}$ BMDMs responded equivalently to anthrax lethal toxin, which signals via NLRP1 (ref. 15; **Fig. 3a**). The release of IL-1β in response to ATP and nigericin, activators of the NLRP3 inflammasome, was also normal (**Fig. 3b**). Analysis of caspase-1 cleavage and IL-1β cleavage produced similar results (**Fig. 3c**). AIM2-deficient cells also responded normally to *S. typhimurium*, which activates the NLRC4 (Ipaf) inflammasome (**Fig. 3d**). RIG-I regulates caspase-1-dependent processing of IL-1β in response to some RNA viruses[20]. To evaluate this pathway, we monitored the release of IL-1β from $Aim2^{+/+}$ and $Aim2^{-/-}$ BMDCs infected with Sendai virus, a paramyxovirus that signals through RIG-I. The Sendai virus–induced IL-1β response was not impaired in $Aim2^{-/-}$ DCs (**Fig. 3e**). Collectively, these data indicate that AIM2 is a receptor for cytosolic dsDNA and does not contribute to inflammasome activation in response to agonists of NLRP1, NLRP3, NLRC4 or RIG-I inflammasomes.

Interferon responses in AIM2-deficient macrophages

Cytosolic DNA is also a potent trigger of the production of type I interferons. To evaluate the role of AIM2 in this response, we measured

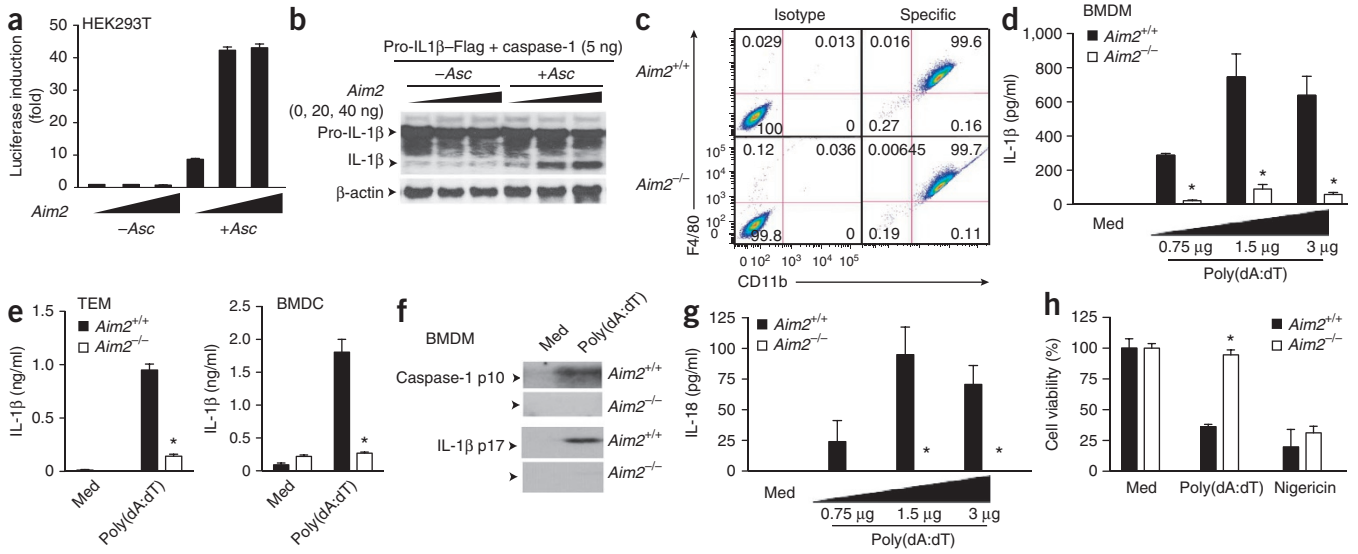

Figure 2 AIM2 is essential for inflammasome activation by DNA. (**a**) Luciferase activity in HEK293T cells transfected for 48 h with increasing concentrations (wedges) of an *Aim2* plasmid with (right) or without (left) an *Asc* plasmid, plus an NF-κB–luciferase reporter gene. (**b**) Immunoblot analysis of Flag-tagged pro-IL-1β and IL-1β, and β-actin (loading control), in lysates of HEK293T cells transfected for 24 with an *Aim2* plasmid with (right) or without (left) an *Asc* plasmid, together with plasmids encoding Flag-tagged pro-IL-1β–gaussia luciferase (Pro-IL-1β–Flag) and caspase-1. (**c**) Expression of F4/80 and CD11b by $Aim2^{+/+}$ and $Aim2^{-/-}$ BMDMs. Numbers in quadrants indicate percent cells in each. Isotype (left), isotype-matched control antibody; Specific (right), specific antibody (anti-F4/80 and anti-CD11b). (**d**–**f**) IL-1β secretion (**d**,**e**) and cleavage of IL-1β and caspase-1 (**f**) by $Aim2^{+/+}$ and $Aim2^{-/-}$ BMDMs (**d**,**f**) and TEMs and BMDCs (**e**) primed with LPS (200 ng/ml) and transfected for 6 h with poly(dA:dT) (0.75–3 μg per 1×10^6 cells). Med, medium alone; p10, cleaved form of caspase-1; p17, cleaved form of IL-1β. (**g**) ELISA of IL-18 in supernatants of $Aim2^{+/+}$ and $Aim2^{-/-}$ BMDMs treated as described in **d**–**f**. (**h**) Viability of $Aim2^{+/+}$ and $Aim2^{-/-}$ TEMs treated for 24 h with poly(dA:dT) or nigericin, presented relative to the viability of medium-treated cells. $^*P < 0.05$, $Aim2^{+/+}$ versus $Aim2^{-/-}$ (two-way analysis of variance followed by Bonferroni's post-test). Data are from one experiment representative of three (mean and s.d. in **a**,**d**,**e**,**g**,**h**).

IFN-β production in response to poly(dA:dT) in $Aim2^{+/+}$ and $Aim2^{-/-}$ mice. Poly(dA:dT) induced IFN-β in $Aim2^{+/+}$ splenocytes and TEMs, and this response was enhanced in cells from $Aim2^{-/-}$ mice (**Fig. 4a**,**b**). These observations indicate that AIM2 is not a sensor of IFN-β production but may exert a negative regulatory effect on interferon responses. We also examined the responsiveness of $Aim2^{-/-}$ BMDMs to type I interferons themselves. We stimulated $Aim2^{+/+}$ and $Aim2^{-/-}$ macrophages with IFN-β and measured by immunoblot analysis the induction of viperin, which is encoded by a well-characterized interferon-stimulated gene. IFN-β induced high viperin expression in $Aim2^{+/+}$ cells, and this response was also induced in $Aim2^{-/-}$ cells (**Fig. 4c**).

AIM2 is encoded by a type I interferon–inducible gene[21]. Although human monocytes do not express AIM2, except after interferon induction[10], mouse macrophages and splenocytes expressed AIM2 constitutively (**Fig. 4d** and data not shown), and the expression of AIM2 was even higher after treatment of TEMs with IFN-β or poly(dA:dT) or after infection with Sendai virus (**Fig. 4d**). Published work has suggested that type I interferon signaling is essential for inflammasome activation in response to *F. tularensis* and *L. monocytogenes*[22,23]. To define the requirement for interferon production and/or signaling in AIM2 inflammasome activation, we monitored DNA-induced IL-1β responses under conditions in which IFN-β was not induced.

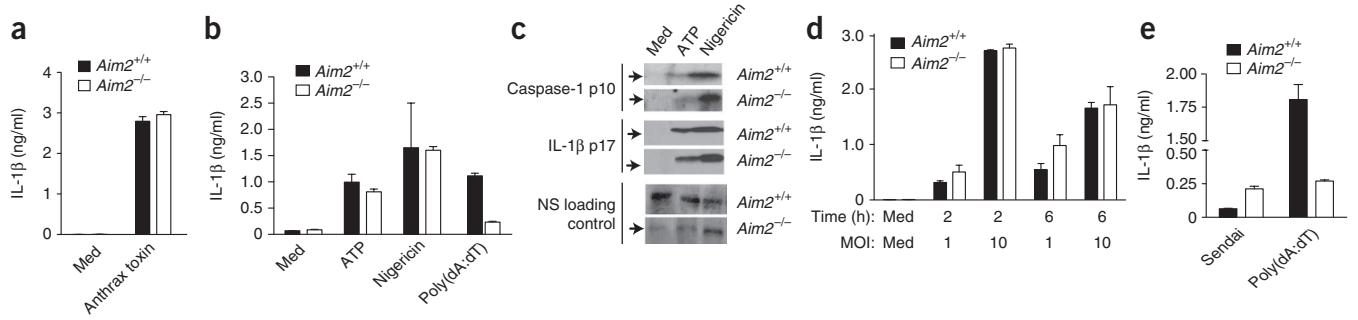

Figure 3 AIM2-deficient cells respond normally to stimuli that activate other inflammasomes. (**a**) ELISA of IL-1β in supernatants of $Aim2^{+/+}$ and $Aim2^{-/-}$ BMDMs primed for 3 h with LPS (200 ng/ml) and stimulated with anthrax toxin, assessed after 6 h. (**b**,**c**) Secretion of IL-1β (**b**) and cleavage of IL-1β and caspase-1 (**c**) by LPS-primed $Aim2^{+/+}$ and $Aim2^{-/-}$ TEMs treated for 1 h with ATP (5 mM) or nigericin (5 μM) or for 6 h with poly(dA:dT). NS, nonspecific band. (**d**) ELISA of IL-1β in supernatants of $Aim2^{+/+}$ and $Aim2^{-/-}$ BMDMs primed for 3 h with LPS (200 ng/ml) and infected for 2 or 6 h with *S. typhimurium* at a multiplicity of infection (MOI) of 1 or 10. (**e**) ELISA of IL-1β in supernatants of BMDCs treated for 18 h with Sendai virus (200 hemagglutinating units per ml) or poly(dA:dT). Data are from one experiment representative of two or three (mean and s.d. in **a**,**b**,**d**,**e**).

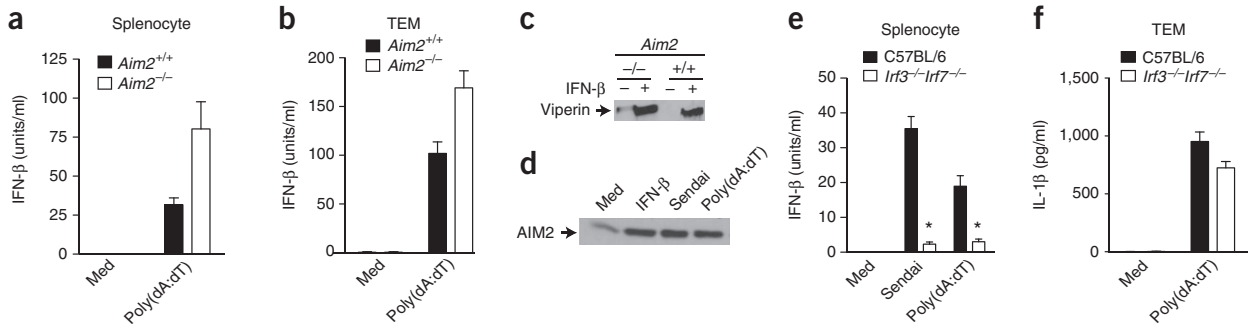

Figure 4 Production and signaling of type I interferons remain intact in AIM2-deficient cells. (**a**) IFN-β in supernatants of *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ splenocytes treated for 24 h with poly(dA:dT). (**b**) IFN-β in supernatants of *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ TEMs treated for 6 h with poly(dA:dT). (**c**) Immunoblot analysis of viperin in lysates of *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ BMDMs left untreated (–) or treated (+) for 18 h with IFN-β (1,000 units/ml). (**d**) Immunoblot analysis of AIM2 in lysates of C57BL/6 TEMs treated for 18 h with IFN-β (1,000 units per ml), Sendai virus (200 hemagglutinating units per ml) or poly(dA:dT) (1.5 μg per 1 × 10^6 cells). (**e**,**f**) IFN-β (**e**) and IL-1β (**f**) in supernatants of C57BL/6 and *Irf3*$^{-/-}$*Irf7*$^{-/-}$ splenocytes and TEMs treated for 24 h with Sendai virus or for 6 h with poly(dA:dT). *$P < 0.05$, *Aim2*$^{+/+}$ versus *Aim2*$^{-/-}$ (two-way analysis of variance followed by Bonferroni's post-test). Data are from one experiment representative of two or three (mean and s.d. in **a**,**b**,**e**,**f**).

Poly(dA:dT) induced a robust IFN-β response that was completely dependent on the transcription factors IRF3 and IRF7 (**Fig. 4e**). To determine if IFN-β production after poly(dA:dT) treatment was required for activation of the AIM2 inflammasome, we monitored the release of IL-1β from these cells. In contrast to IFN-β production, IL-1β production was not defective in response to poly(dA:dT) in cells doubly deficient in IRF3 and IRF7 (**Fig. 4f**). These observations indicate that although DNA treatment leads to the production of type I interferons and higher expression of AIM2, such events are dispensable for AIM2 inflammasome activity.

AIM2-dependent IL-1β responses to bacteria

We next examined the role of AIM2 in regulating inflammasome activation in response to microbial pathogens. *F. tularensis* is a pathogenic bacterium whose virulence is linked to its ability to replicate in the host cell cytosol[24]. Entry of the organism into the cytosol from the phagosome triggers the release of type I interferons, IL-1β and IL-18, as well as caspase-1–dependent cell death[22,23,25]. Although the production of inactive pro-IL-1β in response to *F. tularensis* LVS is dependent on TLR2 (refs. 22,26), cleavage of pro-IL-1β into its active mature form requires both entry into the cytoplasm and engagement of a previously unknown cytosolic receptor[22,23,25]. These events are dependent on ASC[25] but independent of NLRP1 (refs. 27,28), NLRC4 (ref. 25) and NLRP3 (refs. 27,28). Additionally, an intact type I interferon response has been shown to be critical for this response[22,23]. To assess AIM2 as a candidate mediator of this response, we infected *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ macrophages with *F. tularensis* LVS and assayed cleavage of caspase-1 and release of IL-1β. Although *F. tularensis* LVS strongly activated caspase-1 and the release of IL-1β from *Aim2*$^{+/+}$ cells, the loss of AIM2 ablated these responses (**Fig. 5a**,**b**). Priming of macrophages with lipopolysaccharide (LPS) or other TLR ligands was not required for *F. tularensis* LVS–induced activation of caspase-1, as the recognition of *F. tularensis* by TLR2 regulates induction of the gene encoding pro-IL-1β[22,26,29]. To confirm that the lack of IL-1β secretion by *Aim2*$^{-/-}$ cells was due to impaired inflammasome activation and not lower production of pro-IL-1β, we also evaluated the induction of IL-1β mRNA by quantitative PCR. Infection with *F. tularensis* LVS led to equivalent induction of pro-IL-1β mRNA in *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ cells (**Fig. 5c**).

F. tularensis is known to induce IFN-β, and published work has shown that IFN-β–deficient macrophages[22] and IFN-α/β receptor–deficient macrophages[23] fail to undergo inflammasome activation induced by *F. tularensis*, which indicates a requirement for an intact interferon response. We therefore also examined *F. tularensis*–induced cleavage of caspase-1 and release of IL-1β in macrophages lacking IRF3 and IRF7. These cells had a blunted but not defective response (**Fig. 5a**,**b**), in contrast to published studies of IFN-β- or IFN-α/β receptor–deficient mice[22,23]. Although these combined data indicate that *F. tularensis* LVS–mediated activation of the inflammasome is dependent on type I interferon signaling and AIM2, they also suggest that in the absence of IRF3 and IRF7, *F. tularensis* can still induce some IFN-β production that is sufficient to trigger AIM2. Indeed, *F. tularensis*

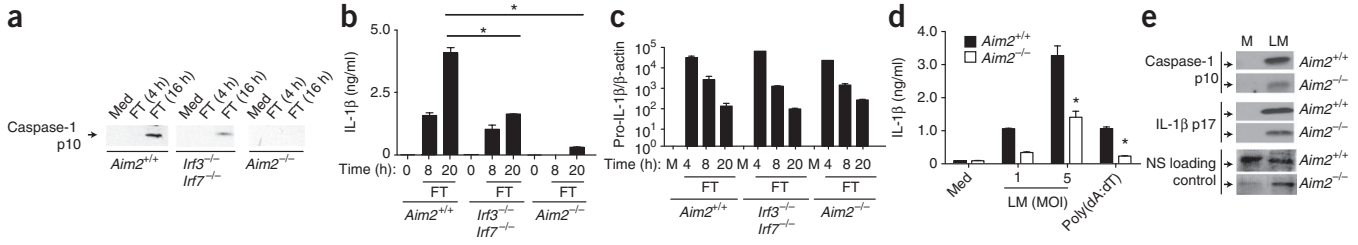

Figure 5 AIM2 mediates inflammasome activation in response to bacterial pathogens. (**a**,**b**) Cleavage of caspase 1 (**a**) and IL-1β (**b**) by *Aim2*$^{+/+}$, *Irf3*$^{-/-}$*Irf7*$^{-/-}$ and *Aim2*$^{-/-}$ TEMs infected for various times (above lanes (**a**) and along horizontal axis (**b**)) with *F. tularensis* LVS (FT) at an MOI of 50. (**c**) Pro-IL-1β mRNA in the cells in **a**,**b**, presented relative to β-actin mRNA. M, medium. (**d**,**e**) ELISA of secreted IL-1β (**d**) and immunoblot analysis of the cleavage of caspase-1 and IL-1β (**e**) in LPS-primed *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ TEMs infected with *L. monocytogenes* (LM) at an MOI of 1 or 5, or treated with poly(dA:dT). *$P < 0.05$, *Aim2*$^{+/+}$ versus *Aim2*$^{-/-}$ (two-way analysis of variance followed by Bonferroni's post-test). Data are from one experiment representative of two or three (mean and s.d. in **b**–**d**).

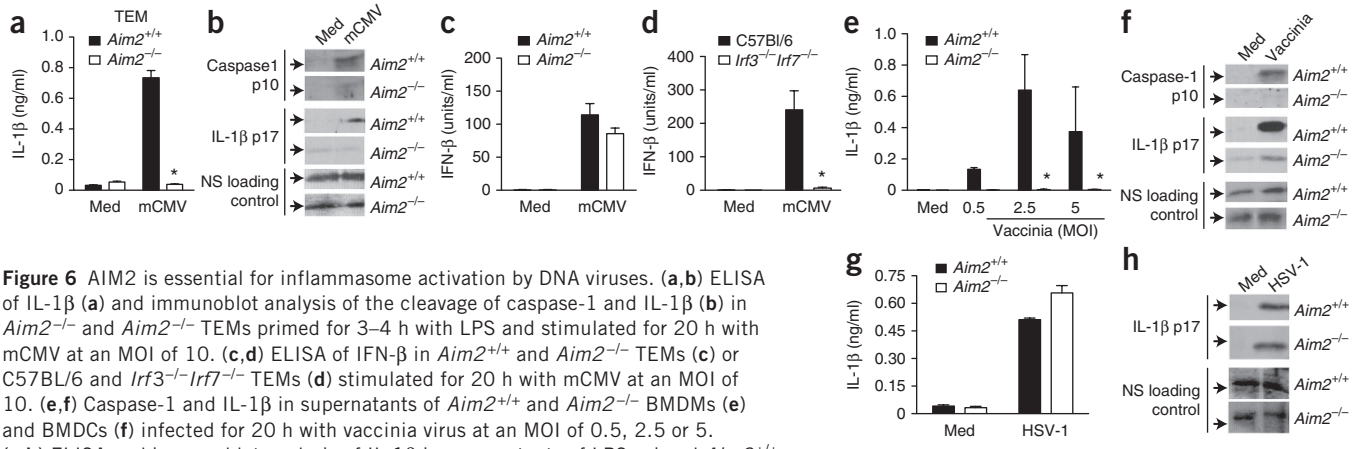

Figure 6 AIM2 is essential for inflammasome activation by DNA viruses. (**a**,**b**) ELISA of IL-1β (**a**) and immunoblot analysis of the cleavage of caspase-1 and IL-1β (**b**) in *Aim2*$^{-/-}$ and *Aim2*$^{-/-}$ TEMs primed for 3–4 h with LPS and stimulated for 20 h with mCMV at an MOI of 10. (**c**,**d**) ELISA of IFN-β in *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ TEMs (**c**) or C57BL/6 and *Irf3*$^{-/-}$*Irf7*$^{-/-}$ TEMs (**d**) stimulated for 20 h with mCMV at an MOI of 10. (**e**,**f**) Caspase-1 and IL-1β in supernatants of *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ BMDMs (**e**) and BMDCs (**f**) infected for 20 h with vaccinia virus at an MOI of 0.5, 2.5 or 5. (**g**,**h**) ELISA and immunoblot analysis of IL-1β in supernatants of LPS-primed *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ TEMs treated for 18 h with HSV-1 at an MOI of 40. *$P < 0.05$, *Aim2*$^{+/+}$ versus *Aim2*$^{-/-}$ (two-way analysis of variance followed by Bonferroni's post-test). Data are from one experiment representative of three (mean and s.d., **a**,**c**,**e**,**g**).

LVS still induced IFN-β production, albeit lower, in macrophages lacking IRF3 and IRF7 (data not shown). Precisely how *F. tularensis* induces type I interferons is still unclear, although DNA-sensing mechanisms have been proposed. The sensor responsible for these events has not been identified.

L. monocytogenes can also trigger inflammasome activity, although the mechanisms involved are not well established. Like *F. tularensis*, *L. monocytogenes* also triggers type I interferon responses and IL-1β after entering the cytosol. The NLRP3 and NLRC4 inflammasomes and another as-yet-unidentified ASC-dependent inflammasome have all been linked to this[30–33]. To define the role of AIM2 in response to *L. monocytogenes*, we infected macrophages from *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ mice and measured the cleavage of caspase-1 as well as the maturation and release of IL-1β. In all cases, these responses were lower but not abrogated in AIM2-deficient cells (**Fig. 5d**,**e**). We obtained similar results with DCs (data not shown). These data therefore indicate that inflammasome activation by *L. monocytogenes* is only partially dependent on AIM2 signaling and that additional pathways also contribute to this activation, consistent with published studies[30–33].

AIM2-dependent IL-1β responses to DNA viruses

To examine the role of AIM2 in inflammasome activation after DNA virus infection, we first examined responses in cells infected with mCMV. We found that mCMV induced the release of IL-1β from TEMs and that this response was entirely dependent on AIM2 (**Fig. 6a**). We obtained similar data with BMDCs (data not shown). Activation of caspase-1 and maturation of IL-1β in response to mCMV were also AIM2 dependent (**Fig. 6b**). We found that mCMV also induced IFN-β and this response was not altered in AIM2-deficient TEMs (**Fig. 6c**) but was ablated in TEMs doubly deficient in IRF3 and IRF7 (**Fig. 6d**).

Vaccinia virus also triggered the AIM2 inflammasome. Vaccinia virus–induced release of IL-1β, cleavage of caspase-1 and maturation of IL-1β were all attenuated in macrophages and BMDCs lacking AIM2 (**Fig. 6e**,**f**). Herpes simplex virus type 1 (HSV-1) is also a strong inducer of IL-1β in macrophages; however, in contrast to the response to vaccinia virus and mCMV, maturation and release of IL-1β in response to HSV-1 was normal in cells from *Aim2*$^{-/-}$ mice (**Fig. 6g**,**h**). Collectively, these data indicate that AIM2 is important in driving the maturation and release of IL-1β in response to DNA viruses. How AIM2 senses mCMV and vaccinia virus differently, but not HSV-1, is unclear.

AIM2-dependent responses to mCMV infection *in vivo*

IL-18 has biochemical similarities to IL-1β but is functionally more similar to IL-12. IL-18 and IL-12 act in synergy to stimulate IFN-γ production from NK cells[34], events that are crucial in the early defense against mCMV infection. IL-18 receptor–deficient mice have much less IFN-γ in both the systemic circulation and spleen after mCMV infection[35]. To define the role of AIM2 *in vivo*, we infected C57BL/6, *Asc*$^{-/-}$, *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ mice with mCMV and first monitored IL-18 production. In agreement with published reports[35], IL-18 was readily detectable in the serum of wild-type C57BL/6 mice at 36 h after infection with mCMV (**Fig. 7a**). We could not detect IL-18 in the serum of mCMV-infected mice deficient in ASC (serum concentrations in *Asc*$^{-/-}$ mice were below the limit of detection of this assay, 25.6 pg/ml). The serum concentration of IL-18 during mCMV infection was significantly lower in *Aim2*$^{-/-}$ mice than in their *Aim2*$^{+/+}$ littermates (**Fig. 7b**), whereas systemic concentrations of tumor necrosis factor were unaffected (*Aim2*$^{+/+}$, 66 ± 30 pg/ml; *Aim2*$^{-/-}$, 56 ± 16 pg/ml ($P = 0.56$; $n = 4$ mice per group); data not shown). These results suggest that AIM2 and ASC are required for the systemic induction of IL-18 but not of tumor necrosis factor during mCMV infection *in vivo*.

Given the role of IL-18 in the induction of systemic and splenic IFN-γ production during mCMV infection, we next evaluated *ex vivo* IFN-γ production by NK cells in the spleens of mCMV-infected mice[36]. The frequency of NK1.1$^+$NKp46$^+$CD3ε$^-$ NK cells in the spleens of C57BL/6, *Asc*$^{-/-}$, *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ mice was similar after mCMV infection (**Fig. 7c** and **Supplementary Fig. 3**). However, C57BL/6 and *Aim2*$^{+/+}$ mice had significantly more IFN-γ-producing NK cells *ex vivo*, as determined by intracellular staining, and *Asc*$^{-/-}$ and *Aim2*$^{-/-}$ mice had considerably fewer IFN-γ-producing NK cells (29% and 52% less than controls, respectively; **Fig. 7d**). The lower number of IFN-γ-producing NK cells could not be attributed to less NK cell activation, as similar proportions of *Asc*$^{-/-}$ and *Aim2*$^{-/-}$ splenic NK cells expressed the activation marker CD69 in response to infection, relative to their C57BL/6 and *Aim2*$^{+/+}$ controls, respectively (**Fig. 7e**). Of note, NK cell CD69 expression was enhanced in *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ mice (on a mixed 129 × C57BL/6 background) relative to that of mice on a pure C57BL/6 background (**Supplementary Fig. 3**).

In C57BL/6 mice, which are resistant to mCMV, NK cell control of early virus replication is mediated in large part through recognition of the mCMV m157 protein by the activating NK cell receptor Ly49H. Although Ly49H expression was slightly

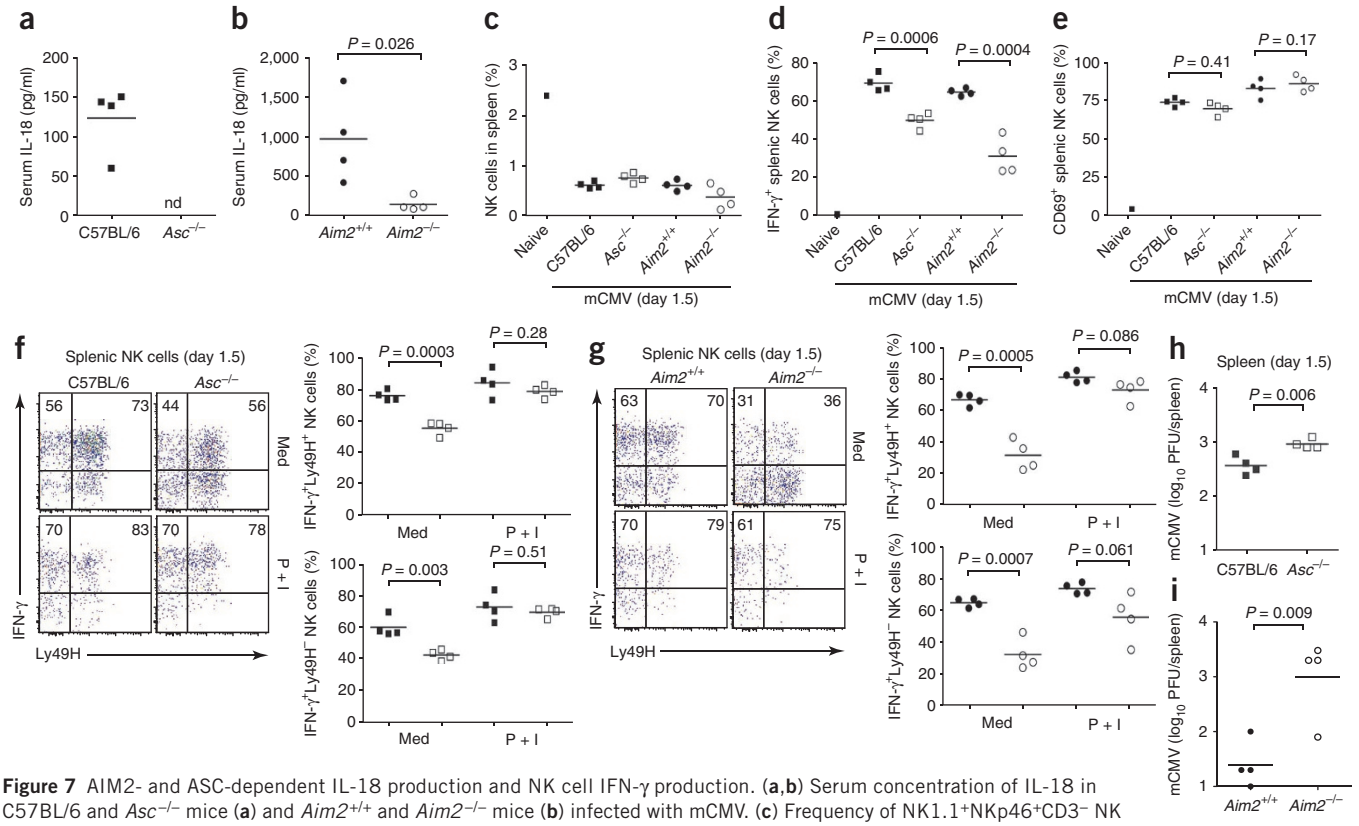

Figure 7 AIM2- and ASC-dependent IL-18 production and NK cell IFN-γ production. (**a**,**b**) Serum concentration of IL-18 in C57BL/6 and *Asc*$^{-/-}$ mice (**a**) and *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ mice (**b**) infected with mCMV. (**c**) Frequency of NK1.1^{+}NKp46^{+}CD3^{-} NK cells in the spleens of mCMV-infected mice. Naive (far left), uninfected C57BL/6 mouse (control). (**d**,**e**) Intracellular expression of IFN-γ (**d**) and surface expression of CD69 (**e**) by splenic NK cells obtained from mice at 36 h after mCMV infection and cultured for 4 h *ex vivo* in medium containing brefeldin A with no additional stimuli. (**f**,**g**) Surface Ly49H and intracellular IFN-γ of C57BL/6 and *Asc*$^{-/-}$ splenocytes (**f**) or *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ splenocytes (**g**) cultured in medium alone without additional stimuli (Med) or in medium containing PMA (50 ng/ml) and ionomycin (500 ng/ml; P + I); gated NK cells were stained. Numbers in quadrants indicate the proportion of Ly49H^{-} (left) or Ly49H^{+} (right) NK cells that are IFN-γ^{+}. Right graphs, frequency of Ly49H^{-} or Ly49H^{+} NK cells expressing IFN-γ: filled squares, C57BL/6; open squares, *Asc*$^{-/-}$; filled circles, *Aim2*$^{+/+}$; open circles, *Aim2*$^{-/-}$. (**h**,**i**) Viral titers in the spleen at 36 h after mCMV infection. Each symbol represents an individual mouse; small horizontal lines indicate the mean. *P* values, unpaired two-tailed Student's *t*-test. Data are from one experiment representative of two ($n = 4$ per group).

lower in both *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ littermates on a mixed 129 × C57BL/6 background relative to that of C57BL/6 and *Asc*$^{-/-}$ mice (**Supplementary Fig. 3**), there was no significant difference in the proportion of Ly49H^{+} NK cells in paired groupings of 129 × C57BL/6 littermates (*Aim2*$^{+/+}$, 48 ± 9; *Aim2*$^{-/-}$, 52 ± 4 ($P = 0.45$, $n = 4$)) or mice on the C57BL/6 background (*Aim2*$^{+/+}$, 68 ± 4; *Aim2*$^{-/-}$, 68 ± 1 ($P = 0.98$, $n = 4$)). Notably, both the Ly49H^{+} and Ly49H^{-} subsets of splenic NK cells from mCMV-infected *Asc*$^{-/-}$ mice (**Fig. 7f**) and *Aim2*$^{-/-}$ mice (**Fig. 7g**) showed less spontaneous *ex vivo* IFN-γ production relative to that of cells from C57BL/6 and *Aim2*$^{+/+}$ mice, respectively. Thus, the lower IFN-γ responses of NK cells in AIM2 inflammasome–defective (*Asc*$^{-/-}$ and *Aim2*$^{-/-}$) mice could not be attributed to the lower number of cells that could be activated by mCMV-encoded ligands for Ly49H. After *in vitro* stimulation with the phorbol ester PMA and ionomycin, NK cells (both Ly49H^{+} and Ly49H^{-}) from spleens of mCMV-infected C57BL/6 and *Asc*$^{-/-}$ mice (**Fig. 7f**), as well as those from their *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ littermates (**Fig. 7g**), produced similarly large amounts of IFN-γ. These data indicate that NK cells in *Asc*$^{-/-}$ and *Aim2*$^{-/-}$ mice are not intrinsically defective but instead that their impaired IFN-γ responses are probably due to the less systemic IL-18 production during mCMV infection.

As NK cell–derived IFN-γ is critical for early control of mCMV replication *in vivo*, we next determined viral loads in the spleens of infected mice at 36 h after infection. *Asc*$^{-/-}$ mice had higher viral titers than those of C57BL/6 mice at this early time point (**Fig. 7h**). As mice of mixed background have higher viral titers than those of C57BL/6 mice, to evaluate the role of AIM2 in viral control, we used a lower dose of mCMV (1×10^5 rather than 1×10^6 plaque-forming units). Like *Asc*$^{-/-}$ mice, *Aim2*$^{-/-}$ mice had much higher viral titers at this early time point (**Fig. 7i**). Notably, IL-18 in the serum and NK cell–dependent IFN-γ production were lower in *Aim2*$^{-/-}$ mice than in *Aim2*$^{+/+}$ mice at this lower dose (data not shown). Collectively, these data demonstrate the essential role of the AIM2-ASC inflammasome in early control of mCMV infection through IL-18 and NK cell–dependent production of IFN-γ.

DISCUSSION

Considerable progress has been made in defining the molecular basis of the immune-stimulatory activity of microbial DNA. TLR9 recognizes CpG DNA and resides in the endosomal compartment[37]. Additional TLR9-independent pathways have also emerged. These include an interferon-inducing pathway and a pathway leading to conversion of the zymogens pro-IL-1β and IL-18 into the active mature cytokines. The interferon-inducing pathway is complex and involves RNA polymerase III and one or more redundant sensors, which collectively converge on a signaling axis involving the interferon gene–stimulator STING[38], the kinase TBK1 (ref. 39) and IRF3

and IRF7 (ref. 37). The IL-1–IL-18 pathway has been shown to involve AIM2, which binds its ligand DNA directly and engages ASC to form a caspase-1-activating inflammasome[9–12]. Whether AIM2 is the sole sensor for this pathway is unknown.

Here we have characterized the role of AIM2 in DNA responses by generating mice lacking *Aim2*. Our studies monitoring IL-1β responses demonstrated an essential and nonredundant role for AIM2 in DNA-dependent activation of caspase-1 and maturation of IL-1β–IL-18 in both macrophages and DCs. Although overproduction of IL-1β can have deleterious consequences for the host if not well regulated[13], the importance of IL-1β in antimicrobial defenses is highlighted by the enhanced susceptibility of IL-1 receptor–deficient mice to various pathogens[40,41] and by the ability of viruses to evade the production and/or action of these cytokines[42–44]. Our studies of IL-1β responses in AIM2-deficient mice have identified a central role for AIM2 in regulating IL-1β in response to *F. tularensis* LVS. Published work has shown the importance of type I interferons, ASC and caspase-1 in innate responses to *F. tularensis*[25]. However, the precise mechanisms by which *F. tularensis* trigger caspase-1 activation have remained unclear[22,45]. Our studies have identified AIM2 as the sole mediator of inflammasome activation by *F. tularensis*. *F. tularensis* also elicits IFN-β production, an event that is not mediated by AIM2. The sensor that regulates IFN-β induction and whether DNA is also the activating ligand remain unknown. Our data have also demonstrated the contribution of the AIM2 pathway to the sensing of *L. monocytogenes*. In this case, the role of AIM2 is partial, consistent with redundant NLR sensing mechanisms. Indeed, AIM2 and NLRP3 seem to act together for the sensing of *L. monocytogenes*, as knockdown of AIM2 in NLRP3-deficient macrophages abolished all inflammasome activity (S. Kim, F. Bauernfeind, A. Ablasser, G. Hartmann, K.A.F., E.L. and V.H., data not shown).

Our most notable finding was that the AIM2-ASC pathway was crucial for caspase-1-dependent signaling and innate immunity to DNA viruses, particularly mCMV. Using a mouse model of mCMV, we found a critical role for AIM2 and ASC in regulating IL-18 production and NK cell–dependent production of IFN-γ. These events are critical for the early innate response to infection, as reflected by the higher viral titers in AIM2- and ASC-deficient mice than in their wild-type counterparts. It is important to note that the $Aim2^{-/-}$ and $Aim2^{-/-}$ mice were on a mixed 129 × C57BL/6 background and thus were more susceptible to mCMV than were C57BL/6 mice, which have an autosomal dominant gene on chromosome 6 (Cmv-1 locus) that confers resistance to mCMV[46]. Defects associated with DAP12 and possibly other signaling pathways on the 129 background may also contribute to the greater susceptibility of 129 × C57BL/6 mice to mCMV[47]. In our studies, the use of $Aim2^{+/+}$ littermate controls (derived from the breeding of heterozygous parents), which had expression of the NK cell receptors NK1.1 and Ly49H (encoded by the Cmv-1 locus[36,48,49]) similar to that of their $Aim2^{-/-}$ littermates, permitted us to properly control for the influence of genetic background on resistance to mCMV and to assess the importance of AIM2 in early antiviral responses. Moreover, the similarly lower systemic IL-18 and NK cell–dependent production of IFN-γ as well as the enhanced viral burden in $Asc^{-/-}$ mice fully backcrossed onto the C57BL/6 background support our argument that the AIM2- and ASC-dependent induction of IL-18 contributes to early control of mCMV replication. Moreover, these data are consistent with published studies of IL-18 receptor–deficient mice[35]. A key question arising from these studies relates to how AIM2, which is located in the cytosolic compartment, gains access to mCMV DNA, as mCMV replicates in the nucleus. Whether the incoming virion or newly synthesized virions are being sensed is unclear at present. One possibility is that as virions are disassembled or assembled, DNA may gain access to the cytosol if the capsid proteins fail to perfectly protect and/or sequester the DNA.

Although it is not unexpected that organisms have evolved mechanisms to sense microbial DNA and trigger innate immunity, understanding of how cells discriminate between self and non-self DNA is still limited. The normal defense strategies that discriminate between host and pathogenic nucleic acids are not perfect and fail under certain circumstances. This perturbation in normal control may contribute to the pathogenesis of autoinflammatory and autoimmune diseases such as systemic lupus erythematosis[37]. Normally, mammalian DNA is sequestered in the nucleus or mitochondria of cells. However, there is an exception to this during replication or cell death, when the integrity of the nuclear envelope is compromised, thereby exposing the cytosolic compartment to free DNA. DNA found free outside cells, in endosomes or in the cytosol is degraded by DNase I, DNase II and DNase III (TREX1). Failure of these systems to function (because of mutation, deletion and so on) can lead to aberrant accumulation of DNA and inappropriate immune activation. Whether AIM2 senses aberrant host DNA and thus contributes to the pathogenesis of systemic lupus erythematosis awaits investigation. Further characterization of the AIM2 inflammasome will probably yield new insights into microbial pathogenesis as well as autoinflammatory and autoimmune diseases and enable the rational design of new therapies and treatments for many debilitating conditions.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureimmunology/.

Note: Supplementary information is available on the Nature Immunology website.

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AUTHOR CONTRIBUTIONS

K.A.F. oversaw the entire project; K.A.F., V.A.K.R. and S.N.W. conceived of the research with assistance from S.N.V., E.L. and E.S.-T.; V.A.K.R., B.G.M. and V.H. characterized the gene-trap integration; V.A.K.R. and L.W. did all the genotyping; V.A.K.R., Z.J. and S.N.W. designed and did the experiments with help from S.S., L.W., S.K.V. and S.G.; L.E.C. did the *F. tularensis* experiments; and K.A.F., V.A.K.R., S.S. and S.N.W. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Kawai, T. & Akira, S. Toll-like receptor and RIG-I-like receptor signaling. *Ann. NY Acad. Sci.* **1143**, 1–20 (2008).
2. Takeda, K. & Akira, S. Toll-like receptors in innate immunity. *Int. Immunol.* **17**, 1–14 (2005).

3. Huysamen, C. & Brown, G.D. The fungal pattern recognition receptor, Dectin-1, and the associated cluster of C-type lectin-like receptors. *FEMS Microbiol. Lett.* **290**, 121–128 (2009).
4. Yoneyama, M. & Fujita, T. RIG-I family RNA helicases: cytoplasmic sensor for antiviral innate immunity. *Cytokine Growth Factor Rev.* **18**, 545–551 (2007).
5. Martinon, F., Mayor, A. & Tschopp, J. The inflammasomes: guardians of the body. *Annu. Rev. Immunol.* **27**, 229–265 (2009).
6. Takaoka, A. *et al.* DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature* **448**, 501–505 (2007).
7. Chiu, Y.H., Macmillan, J.B. & Chen, Z.J. RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. *Cell* **138**, 576–591 (2009).
8. Ablasser, A. *et al.* RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III–transcribed RNA intermediate. *Nat. Immunol.* **10**, 1065–1072 (2009).
9. Roberts, T.L. *et al.* HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. *Science* **323**, 1057–1060 (2009).
10. Hornung, V. *et al.* AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* **458**, 514–518 (2009).
11. 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* (2009).
12. Burckstummer, T. *et al.* An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. *Nat. Immunol.* **10**, 266–272 (2009).
13. Dinarello, C.A. Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* **27**, 519–550 (2009).
14. Dinarello, C.A., Novick, D., Rubinstein, M. & Lonnemann, G. Interleukin 18 and interleukin 18 binding protein: possible role in immunosuppression of chronic renal failure. *Blood Purif.* **21**, 258–270 (2003).
15. Boyden, E.D. & Dietrich, W.F. Nalp1b controls mouse macrophage susceptibility to anthrax lethal toxin. *Nat. Genet.* **38**, 240–244 (2006).
16. Latz, E. The inflammasomes: mechanisms of activation and function. *Curr. Opin. Immunol.* **22**, 28–33 (2010).
17. Miao, E.A. *et al.* Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. *Proc. Natl. Acad. Sci. USA* **107**, 3076–3080 (2010).
18. Ludlow, L.E., Johnstone, R.W. & Clarke, C.J. The HIN-200 family: more than interferon-inducible genes? *Exp. Cell Res.* **308**, 1–17 (2005).
19. Fernandes-Alnemri, T. *et al.* The pyroptosome: a supramolecular assembly of ASC dimers mediating inflammatory cell death via caspase-1 activation. *Cell Death Differ.* **14**, 1590–1604 (2007).
20. Poeck, H. *et al.* Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1β production. *Nat. Immunol.* **11**, 63–69 (2010).
21. Landolfo, S., Gariglio, M., Gribaudo, G. & Lembo, D. The Ifi 200 genes: an emerging family of IFN-inducible genes. *Biochimie* **80**, 721–728 (1998).
22. Cole, L.E. *et al.* Macrophage proinflammatory response to *Francisella tularensis* live vaccine strain requires coordination of multiple signaling pathways. *J. Immunol.* **180**, 6885–6891 (2008).
23. Henry, T., Brotcke, A., Weiss, D.S., Thompson, L.J. & Monack, D.M. Type I interferon signaling is required for activation of the inflammasome during Francisella infection. *J. Exp. Med.* **204**, 987–994 (2007).
24. Elkins, K.L., Cowley, S.C. & Bosio, C.M. Innate and adaptive immunity to *Francisella*. *Ann. NY Acad. Sci.* **1105**, 284–324 (2007).
25. 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).
26. Cole, L.E. *et al.* Toll-like receptor 2-mediated signaling requirements for *Francisella tularensis* live vaccine strain infection of murine macrophages. *Infect. Immun.* **75**, 4127–4137 (2007).
27. Lamkanfi, M. & Dixit, V.M. Inflammasomes: guardians of cytosolic sanctity. *Immunol. Rev.* **227**, 95–105 (2009).
28. Kanneganti, T.D. *et al.* Pannexin-1-mediated recognition of bacterial molecules activates the cryopyrin inflammasome independent of Toll-like receptor signaling. *Immunity* **26**, 433–443 (2007).
29. Li, H., Nookala, S., Bina, X.R., Bina, J.E. & Re, F. Innate immune response to *Francisella tularensis* is mediated by TLR2 and caspase-1 activation. *J. Leukoc. Biol.* **80**, 766–773 (2006).
30. Mariathasan, S. *et al.* Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* **440**, 228–232 (2006).
31. Meixenberger, K. *et al. Listeria monocytogenes*-infected human peripheral blood mononuclear cells produce IL-1β, depending on listeriolysin O and NLRP3. *J. Immunol.* **184**, 922–930 (2010).
32. Franchi, L., Kanneganti, T.D., Dubyak, G.R. & Nunez, G. Differential requirement of P2X7 receptor and intracellular K^+ for caspase-1 activation induced by intracellular and extracellular bacteria. *J. Biol. Chem.* **282**, 18810–18818 (2007).
33. Warren, S.E., Mao, D.P., Rodriguez, A.E., Miao, E.A. & Aderem, A. Multiple Nod-like receptors activate caspase 1 during *Listeria monocytogenes* infection. *J. Immunol.* **180**, 7558–7564 (2008).
34. Dinarello, C.A. Biologic basis for interleukin-1 in disease. *Blood* **87**, 2095–2147 (1996).
35. Pien, G.C. & Biron, C.A. Compartmental differences in NK cell responsiveness to IL-12 during lymphocytic choriomeningitis virus infection. *J. Immunol.* **164**, 994–1001 (2000).
36. Daniels, K.A. *et al.* Murine cytomegalovirus is regulated by a discrete subset of natural killer cells reactive with monoclonal antibody to Ly49H. *J. Exp. Med.* **194**, 29–44 (2001).
37. Hornung, V. & Latz, E. Intracellular DNA recognition. *Nat. Rev. Immunol.* **10**, 123–130 (2010).
38. Ishikawa, H., Ma, Z. & Barber, G.N. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature* **461**, 788–792 (2009).
39. Fitzgerald, K.A. *et al.* IKKε and TBK1 are essential components of the IRF3 signaling pathway. *Nat. Immunol.* **4**, 491–496 (2003).
40. Ichinohe, T., Lee, H.K., Ogura, Y., Flavell, R. & Iwasaki, A. Inflammasome recognition of influenza virus is essential for adaptive immune responses. *J. Exp. Med.* **206**, 79–87 (2009).
41. Labow, M. *et al.* Absence of IL-1 signaling and reduced inflammatory response in IL-1 type I receptor-deficient mice. *J. Immunol.* **159**, 2452–2461 (1997).
42. Alcami, A. & Smith, G.L. A soluble receptor for interleukin-1 beta encoded by vaccinia virus: a novel mechanism of virus modulation of the host response to infection. *Cell* **71**, 153–167 (1992).
43. Smith, G.L. & Chan, Y.S. Two vaccinia virus proteins structurally related to the interleukin-1 receptor and the immunoglobulin superfamily. *J. Gen. Virol.* **72**, 511–518 (1991).
44. Johnston, J.B. *et al.* A poxvirus-encoded pyrin domain protein interacts with ASC-1 to inhibit host inflammatory and apoptotic responses to infection. *Immunity* **23**, 587–598 (2005).
45. Henry, T. & Monack, D.M. Activation of the inflammasome upon *Francisella tularensis* infection: interplay of innate immune pathways and virulence factors. *Cell. Microbiol.* **9**, 2543–2551 (2007).
46. Scalzo, A.A. *et al.* Genetic mapping of Cmv1 in the region of mouse chromosome 6 encoding the NK gene complex-associated loci Ly49 and musNKR-P1. *Genomics* **27**, 435–441 (1995).
47. McVicar, D.W. *et al.* Aberrant DAP12 signaling in the 129 strain of mice: implications for the analysis of gene-targeted mice. *J. Immunol.* **169**, 1721–1728 (2002).
48. Brown, M.G. *et al.* Vital involvement of a natural killer cell activation receptor in resistance to viral infection. *Science* **292**, 934–937 (2001).
49. Lee, S.H. *et al.* Susceptibility to mouse cytomegalovirus is associated with deletion of an activating natural killer cell receptor of the C-type lectin superfamily. *Nat. Genet.* **28**, 42–45 (2001).

ONLINE METHODS

Reagents. ATP, LPS, nigericin and poly(dA:dT) were from Sigma-Aldrich. Anthrax lethal toxin was from List Biologicals (5 μg/ml of PA and 5 μg/ml of LF). Vaccinia virus (Western Reserve strain) was from G. Barton; mCMV (Smith strain) was from R. Welsh; and HSV-1 was from D. Knipe. Sendai virus (Cantrell strain) was from Charles River Laboratories. *F. tularensis* LVS (29684; American Type Culture Collection) was prepared as described[50]. *S. typhimurium* (SL1344 lab strain) was from M. O'Riordan, and *L. monocytogenes* (clinical isolate 10403s) was from V. Boyartchuk. Polyclonal antibody to mouse AIM2 was from E. Alnemri.

Plasmid constructs. Full-length human AIM2 was as described[10]. Full-length mouse AIM2 and mouse ASC were from Invivogen. Expression plasmids (based on the vector pCI) encoding human caspase-1 and ASC-hemagglutinin were from Millenium Pharmaceuticals[10].

Mice. Embryoic stem cells with a gene-trap insertion in *Aim2* (International Gene Trap Consortium) were microinjected into C57BL/6 blastocysts. Chimeric offspring were backcrossed to C57BL/6 mice and germline transmission was confirmed by PCR with gene-trap-specific primers. Long PCR used a 5′ exon 5 primer and a 3′ gene-trap vector primer to amplify the gene-trap integration site in *Aim2*; the products amplified were examined by sequencing. This showed that the gene-trap vector inserted into intron 6 of *Aim2*. Because of duplication of part of *Aim2* at the 3′ end of the integration site, heterozygous and homozygous mice could not be distinguished by PCR of genomic DNA. For definitive identification of homozygous mice, reverse-transcription PCR was done with RNA extracted from blood and following primers: F, 5′-CACACTCGACGTGGCAGATAGGAC-3′; R1, 5′-CAGC ACCGTGACAACAAGTGG-3′; R2, 5′-TCGATGCGATCTGCGTTCTTC-3′. F and R1 amplified wild-type *Aim2* transcripts; F and R2 amplified *Aim2* transcripts with the gene-trap insertion. *Asc*$^{-/-}$ mice (Millennium Pharmaceuticals) were backcrossed onto the C57BL/6 background. *Irf3*$^{-/-}$*Irf7*$^{-/-}$ mice were generated at the University of Massachusetts Medical School from *Irf3*$^{-/-}$ and *Irf7*$^{-/-}$ mice obtained from T. Taniguchi. C57BL/6 mice (Jackson Laboratories) were bred at the University of Massachusetts Medical School. All mouse strains were bred and maintained in specific pathogen–free conditions in the animal facilities of the University of Massachusetts Medical School and were carried out in accordance with the guidelines set forth by the University of Massachusetts Medical School Department of Animal Medicine and the Institutional Animal Care and Use Committee.

Cell culture and stimulation. TEMs were isolated from mice 4 d after intraperitoneal injection of sterile 3% (wt/vol) thioglycollate (Remel). BMDMs and BMDCs were generated as described[10,51]. Cells were first primed for 3–4 h with LPS (200 ng/ml) before treatment with various reagents. ATP (5 mM) or nigericin (10 μM) was added 1 h before supernatants were collected. Cells were transfected with poly(dA:dT) DNA through the use of Lipofectamine 2000 at a concentration of 1.5 μg/ml. Virus doses (in MOI) were as follows unless stated otherwise: vaccinia virus, 0.5–5; mCMV, 5 (splenocytes) or 10 (macrophages and DCs); HSV, 10 or 40. For experiments involving *F. tularensis* LVS, *S. typhimurium* or *L. monocytogenes*, cells were cultured in antibiotic-free medium unless indicated otherwise. Macrophages were infected for 4 h with *F. tularensis* LVS (MOI, 50). After being washed twice with PBS, infected cells were incubated for 45 min in medium containing gentamicin (50 μg/ml). Cells were washed twice with PBS and then incubated with serum-free medium. For *L. monocytogenes*, cells were stimulated for 1 h with *L. monocytogenes* at an MOI of 1 or 5 and then medium was replaced with medium containing gentamicin (100 μg/ml). For *S. typhimurium*, at 2 h after infection supernatants were collected and medium containing gentamicin (50 μg/ml) was added. After 1 h, the medium was again replaced with medium containing gentamicin (10 μg/ml) and supernatants were collected after 3h.

ELISA. Cell culture supernatants were assayed by ELISA for IL-1β (BD Biosciences) and IL-18 (R&D Systems). A sandwich ELISA for mouse IFN-β was used as described[52].

Immunoblot analysis. Supernatants were collected and proteins were precipitated by methanol-chloroform extraction, and cell lysates were collected. Immunoblot analysis was done as described[10] with antibody to mouse caspase-1 p10 (sc-514; Santa Cruz Biotechnology), anti–mouse IL-1β (AF-401-NA; R&D Systems), rabbit polyclonal anti–mouse AIM2 (E. Alnemri), anti-Flag (M2; Sigma), anti-viperin (P. Cresswell) or anti-β-actin (AC-15; Sigma).

Quantitative real-time PCR for pro-IL-1β. These assays were done as described[22].

Reporter assays. All reporter-gene assays were done as described[10].

Cell-viability assay. *Aim2*$^{+/+}$ and *Aim2*$^{-/-}$ cells were treated with poly(dA:dT) as described above and viability was assessed at 24 h by calcein staining as described[10].

***In vivo* mCMV infection.** The Smith strain of mCMV was propagated *in vivo* in the salivary glands of BALB/c mice[53]. Infected salivary gland homogenates were diluted in Hank's balanced salt solution and mice were inoculated intraperitoneally with 1×10^6 or 1×10^5 plaque-forming units (**Fig. 7i**), as titrated on monolayers of mouse α-1,3-galactosyltransferase–knockout fibroblasts.

Immunofluorescence staining and intracellular IFN-γ assay. Leukocytes were isolated and stained for intracellular IFN-γ with reagents and protocols from BD Pharmingen. Cells (2×10^6) were cultured for 4 h at 37 °C in medium containing GolgiPlug (0.2 μl per sample) in the presence or absence of PMA (phorbol 12-myristate 13-acetate; 50 ng/ml; Sigma-Aldrich) and ionomycin (500 ng/ml; Sigma-Aldrich). Cells were washed with flow cytometry buffer (1× PBS with 2% (vol/vol) FBS and 0.1% (wt/vol) sodium azide) buffer and then were incubated for 5 min at 4 °C in 100 μl flow cytometry buffer plus 0.5 μl antibody to the receptor FcγRII. After being washed, cells were then incubated for 30 min at 4 °C with combinations of the following monoclonal antibodies: peridinin chlorophyll protein–cyanine 5.5–conjugated anti-NK1.1 (PK136; BD Pharmingen), fluorescein isothiocyanate–conjugated anti-CD3 (145-2C11; BD Pharmingen), phycoerythrin–conjugated anti-NKp46 (29A1.4; eBioscience), Alexa Fluor 700–conjugated anti-CD8a (53-6.7; eBioscience), phycoerythrin-indotricarbocyanine–conjugated anti-CD69 (H1.2F3; BD Pharmingen) or Alexa Fluor 647–conjugated anti-Ly49H (3D10; eBioscience). Samples were washed twice, then were fixed and made permeable for 20 min at 4 °C with 100 μl Cytofix/Cytoperm (BD Pharmingen). After fixation, samples were washed twice with Perm/Wash solution and then were stained for 30 min at 4 °C with Pacific Blue–conjugated rat immunoglobulin G1 monoclonal antibody to mouse IFN-γ (XMG1.2; BD Pharmingen). Samples were then washed twice with Perm/Wash solution and once with flow cytometry buffer, followed by analysis on an LSR II. Data were analyzed with FlowJo software (TreeStar). For these analyses, 100,000–200,000 events were calculated, which ensured a sizable NK cell population for valid analysis of NK subsets.

Plaque assay. Viral loads in the spleen were titrated by plaque assay on monolayers of mouse α-1,3-galactosyltransferase–knockout fibroblasts.

Statistical analysis. Two-way analysis of variance followed by the Bonferroni's post-test was used, unless otherwise stated, with Prism Software. *P* values of less than 0.05 were considered significant.

50. Elkins, K.L., Winegar, R.K., Nacy, C.A. & Fortier, A.H. Introduction of *Francisella tularensis* at skin sites induces resistance to infection and generation of protective immunity. *Microb. Pathog.* **13**, 417–421 (1992).
51. Rathinam, V.A., Hoag, K.A. & Mansfield, L.S. Dendritic cells from C57BL/6 mice undergo activation and induce Th1-effector cell responses against *Campylobacter jejuni*. *Microbes Infect.* **10**, 1316–1324 (2008).
52. Roberts, Z.J. *et al.* The chemotherapeutic agent DMXAA potently and specifically activates the TBK1-IRF-3 signaling axis. *J. Exp. Med.* **204**, 1559–1569 (2007).
53. Bukowski, J.F., Woda, B.A. & Welsh, R.M. Pathogenesis of murine cytomegalovirus infection in natural killer cell-depleted mice. *J. Virol.* **52**, 119–128 (1984).