Detrimental Type I Interferon Signaling Dominates Protective AIM2 Inflammasome Responses during Francisella novicida Infection

Authors
Qifan Zhu, Si Ming Man, Rajendra Karki, R.K. Subbarao Malireddi, Thirumala-Devi Kanneganti

Correspondence
thirumala-devi.kanneganti@stjude.org

In Brief
Zhu et al. show that, although type I IFN signaling is required for activating AIM2 inflammasome in response to Francisella novicida in macrophages, these components have strikingly opposing effects in vivo. Deleterious type I IFN signaling dominates protective AIM2 inflammasome responses by inducing apoptotic cell death.

Highlights
- Signaling by IFNAR1 or IFNAR2 is detrimental to mice infected with F. novicida
- Type I IFN-mediated susceptibility to F. novicida depends on STING, IRF7, and IRF9
- Pathogenic type I IFN signaling overrides protective AIM2 inflammasome responses
- Type I IFNs activate apoptotic caspases to drive detrimental cell death in vivo

Zhu et al., 2018, Cell Reports 22, 3168–3174
March 20, 2018 © 2018 The Authors.
https://doi.org/10.1016/j.celrep.2018.02.096
Detrimental Type I Interferon Signaling Dominates Protective AIM2 Inflammasome Responses during *Francisella novicida* Infection

Qifan Zhu,1,2 Si Ming Man,1 Rajendra Karki,1 R.K. Subbarao Malireddi,1 and Thirumala-Devi Kanneganti1,3,*

1Department of Immunology, St. Jude Children’s Research Hospital, Memphis, TN 38105, USA
2Integrated Biomedical Sciences Program, University of Tennessee Health Science Center, Memphis, TN 38163, USA
3Lead Contact
*Correspondence: thirumala-devi.kanneganti@stjude.org
https://doi.org/10.1016/j.celrep.2018.02.096

**SUMMARY**

Interferons (IFNs) and inflammasomes are essential mediators of anti-microbial immunity. Type I IFN signaling drives activation of the AIM2 inflammasome in macrophages; however, the relative contribution of IFNs and inflammasome responses in host defense is less understood. We report intact AIM2 inflammasome responses in mice lacking type I IFN signaling during infection with *F. novicida*. Lack of type I IFN signaling conferred protection to *F. novicida* infection in contrast to the increased susceptibility in AIM2-deficient mice. Mice lacking both AIM2 and IFNAR2 were protected against the infection. The detrimental effects of type I IFN signaling were due to its ability to induce activation of apoptotic caspases and cell death. These results demonstrate the contrasting effects of type I IFN signaling and AIM2 during *F. novicida* infection *in vivo* and indicate a dominant role for type I IFNs in mediating detrimental responses despite the protective AIM2 inflammasome responses.

**INTRODUCTION**

Innate immune sensors mediate the recognition of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) and are central regulators of host defense against microbial infections. Upon activation, immune sensors induce the production of inflammatory cytokines and interferons. Certain innate immune cytoplasmic sensors have a unique ability to form a caspase-1-activating multi-protein complex termed the inflammasome. These sensors include members of the nucleotide-binding domain, leucine-rich repeat containing protein (NLR) family, absent in melanoma 2 (AIM2), and pyrin (Man et al., 2017). Of these, AIM2 binds directly to double-stranded DNA (dsDNA) and assembles the inflammasome to mediate the release of interleukin (IL)-1β and IL-18 and the induction of pyroptosis. AIM2 orchestrates protective inflammasome-dependent responses against microbial infection *in vivo*, including infection by the Gram-negative bacterial pathogen *Francisella novicida* (Man et al., 2017).

Components of the type I interferon (IFN) signaling pathway are essential for the activation of AIM2 inflammasome during infection by *F. novicida* and *Listeria monocytogenes* (Cole et al., 2008; Fernandes-Alnemri et al., 2010; Henry et al., 2007; Jones et al., 2010; Man et al., 2015; Meunier et al., 2015; Rathnam et al., 2010). During *F. novicida* infection, the DNA sensor cGAS and its adaptor STING mediate the production of type I IFNs, which in turn drive expression of effector proteins of cell-autonomous immunity, leading to bacteriolysis and release of bacterial DNA for recognition by AIM2 (Man et al., 2017).

Type I IFN signaling during bacterial infection *in vivo* can be beneficial or detrimental depending on the pathogen (Malireddi and Kanneganti, 2013). Whereas type I IFN signaling is protective in mice infected with *Streptococcus pneumoniae* or *Streptococcus pyogenes*, it is detrimental in the case of *F. novicida* or *L. monocytogenes* (McNab et al., 2015). The requirement for type I IFN signaling to activate the AIM2 inflammasome in macrophages is counterintuitive to the observation that type I IFN signaling is detrimental during *in vivo* infection with *F. novicida*, whereas the AIM2 inflammasome is protective. How the interplay between the opposing effects of these signaling pathways determines the outcome during *F. novicida* infection has remained unclear. Here, we showed that the detrimental effects of type I IFN signaling override protective AIM2 inflammasome responses.

**RESULTS AND DISCUSSION**

**IFNAR Signaling Enhances Susceptibility to Infection by *F. novicida***

A study has demonstrated non-redundant functions for type I IFN receptor subunits IFNAR1 and IFNAR2 independently of each other (de Weerd et al., 2013). To directly compare the effects of IFNAR1 and IFNAR2 during *F. novicida* infection, we infected wild-type (WT) mice and mice lacking IFNAR1 (Ifnar1<−/−>) or IFNAR2 (Ifnar2<−/−>) with *F. novicida* and monitored their survival. Whereas 60% of the infected WT mice died within 14 days, only 13% of the *Ifnar1<−/−>* mice and 11% of the *Ifnar2<−/−>* mice succumbed to infection (Figure 1A), suggesting that signaling by both IFNAR1 and IFNAR2 contributes to increased susceptibility to infection by *F. novicida*. The resistance to
F. novicida infection in Ifnar1<−/− mice is consistent with the observations of a previous study (Henry et al., 2010). Furthermore, only 8% of mice lacking the transcription factor IFN-regulatory factor 3 (IRF3) transducing type I IFN signaling, succumbed to infection by F. novicida over 14 days, essentially phenocopying Ifnar1<−/− and Ifnar2<−/− mice (Figure 1A). IRFs are central components regulating type I IFN production in response to diverse pathogens. We found that Ifr7<−/− and Ifr3<−/−/Ifr7<−/− mice were resistant to the infection (Figure 1B). However, mice lacking IRF5 (Irf5<−/−), an IRF not critical for IFN production, were as susceptible as WT mice (Figure 1B). We also observed increased resistance in mice lacking STING (Sting<−/−>gt) (Figure 1B). Consistent with these results, we found significantly lower bacterial burdens in the liver and spleen of Sting<−/−gt mice, Ifr7<−/− mice, and Irf9<−/− mice 3 days after infection compared with WT mice (Figure 1C). Altogether, these results suggest that the effector responses mediated by type I IFN signaling enhance susceptibility to F. novicida infection in vivo.

The Detrimental Effects of Type I IFN Signaling Dominate the Protective Effects of AIM2 during F. novicida Infection In Vivo

To directly assess the relative contribution of IFNs and AIM2 in host defense during F. novicida infection, we compared mice lacking components of the type I IFN signaling pathway, such as IFNAR2, with mice lacking AIM2. Whereas Ifnar2<−/− mice were resistant to infection by F. novicida, with only 19% of the mice dying, all Aim2<−/− mice succumbed to infection within 6 days (Figure 2A). The disparate roles of type I IFN signaling and AIM2 during F. novicida infection in vivo suggest a more complex functional relationship between these two pathways.

To formally investigate the cumulative effects of type I IFN signaling and AIM2 during F. novicida infection, we bred Ifnar2<−/− mice and Aim2<−/− mice to generate mice lacking both IFNAR2 and AIM2 (Irf7<−/−>Aim2<−/−>). Irf7<−/−Aim2<−/− mice had similar numbers of neutrophils, macrophages, dendritic cells, CD8+ T cells, and B cells in the spleen; T cells in the thymus; and neutrophils, monocytes, and B cells in the bone marrow compared with WT mice (Figures S1A–S1C). We infected Ifnar2<−/− mice and monitored their survival. Irf7<−/−Aim2<−/− mice were resistant to the infection, similar to Irf7<−/− mice, with only 19% of the mice dying of infection over 12 days compared with 100% in Aim2<−/− mice (Figure 2A). Consistent with the reduction in mortality, both Irf7<−/−Aim2<−/− and Irf7<−/− mice had significantly lower bacterial burdens in the liver and spleen 3 days after infection compared with WT mice, while Aim2<−/− mice were more susceptible to infection and had a greater bacterial burden (Figure 2B). These results demonstrate that type I IFN signaling is dominant over AIM2 inflammasome during F. novicida infection in vivo. Because both IFNAR1 and IFNAR2 are required for mediating detrimental type I IFN signaling, it is likely that Ifnar1<−/−Aim2<−/− mice are also resistant to the infection, phenocopying Irf7<−/−Aim2<−/− mice. An increased number of splenic CD4+ T cells in naive Irf7<−/−Aim2<−/− mice is unlikely to contribute to the susceptibility (Figure S1A), because the bacterial burden in Irf7<−/−Aim2<−/− mice was significantly lower than in WT mice 3 days after infection when T cell responses barely occurred.

We next investigated whether type I IFN signaling was required for AIM2 inflammasome-induced IL-18 production in vivo during F. novicida infection. Reduced levels of IL-18 were observed in the sera of Aim2<−/− and Irf7<−/−Aim2<−/− mice, confirming AIM2-dependent IL-18 production in the infected mice (Figure 2C). Irf7<−/− mice retained the ability to produce IL-18 after the infection, indicating inflammasome activation independent of type I IFNs in vivo (Figure 2C). The comparable levels of resistance to F. novicida infection in Irf7<−/− mice and Irf7<−/−Aim2<−/− mice suggest that the dominance of IFNAR signaling-mediated responses over AIM2 is independent of IL-18.
As demonstrated previously (Man et al., 2015), Ifnar2−/− bone marrow-derived macrophages (BMDMs) had an impaired ability to activate caspase-1, trigger release of IL-1β and IL-18, and induce cell death in response to F. novicida compared to the WT BMDMs (Figures 2D, 2E, and S1D). Aim2−/− or Ifnar2−/− Aim2−/− BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDMs infected with F. novicida also failed to activate inflammasome responses, confirming specificity of F. novicida infection to the AIM2 inflammasome (Figures 2D, 2E, and S1D). Inflammasome responses occurred normally in F. novicida BMDM...
but increased levels IL-17 and IL-12 in the sera of Ifnar2^{−/−} and Ifnar2^{−/−}/Aim2^{−/−} mice compared with WT animals (Figure S2C), which is consistent with a previous study (Henry et al., 2010), suggesting that circulating IL-17 and IL-12 may also contribute to host defense at this time point. The level of IFNγ was reduced in Ifnar2^{−/−}/Aim2^{−/−} mice, but not Ifnar2^{−/−} mice (Figure S2C). This could be due to a lack of IL-18-induced IFNγ production in Ifnar2^{−/−}/Aim2^{−/−} mice (Figure S2C).

**Type I IFN Signaling Controls Activation of Apoptotic Caspases and Cell Death**

Type I IFN signaling has been shown to exert its detrimental effects to the host via induction of cell death during *L. monocytogenes* and *Brucella abortus* infection (McNab et al., 2015). To determine whether type I IFN signaling promotes excessive cell death during *F. novicida* infection, we measured levels of apoptotic caspase-8, caspase-3, and caspase-7 in liver tissues of WT, Ifnar2^{−/−}, and Ifnar2^{−/−}/Aim2^{−/−} mice. In line with our hypothesis, the levels of cleaved caspase-8, caspase-3, and caspase-7 were lower in Ifnar2^{−/−} and Ifnar2^{−/−}/Aim2^{−/−} mice than in WT mice 1 and 2 days after infection (Figures 4A, 4B, S3A, and S3B). These results suggest that induction of apoptosis is impaired in the absence of type I IFN signaling. Levels of cleaved caspase-3 and caspase-7 were similar between WT and Aim2^{−/−} mice (Figures S3D and S3E). In addition, Ifnar2^{−/−} and Ifnar2^{−/−}/Aim2^{−/−} mice showed decreased cleavage of caspase-8, caspase-3, and caspase-7 on day 3 post-infection (Figures 4C and S3C), whereas Aim2^{−/−} mice had higher levels of cleaved caspase-8, caspase-3, and caspase-7, possibly due to excessive liver damage caused by uncontrolled bacteria replication (Figures 4C and S3C). TUNEL staining also indicated reduced cell death in Ifnar2^{−/−} and Ifnar2^{−/−}/Aim2^{−/−} mice and enhanced cell death in Aim2^{−/−} mice after the infection (Figure S4A). We further tested role of AIM2 in mediating apoptosis in vitro during *F. novicida* infection by measuring cleavage of apoptotic caspases. Cleavage of caspase-8 and caspase-3 was increased in Aim2^{−/−}/BMDMs compared with WT cells after the infection (Figures S3F and S3G). Previous study has shown that AIM2 inflammasome negatively regulates type I IFNs production (Corrales et al., 2016). It is possible that an increased level of type I IFNs in Aim2^{−/−}/BMDMs induces activation of caspase-8 and caspase-3. Caspase-7 activation was decreased in Aim2^{−/−}/BMDMs (Figures S3F and S3G). Caspase-7 has been reported as a substrate of caspase-1 (Lamkanfi et al., 2008). The reduced cleavage of caspase-7 in Aim2^{−/−}/BMDMs could be due to impaired caspase-1 activation during the infection. Overall, these results suggest that Aim2^{−/−}/BMDMs have increased apoptosis after *F. novicida* infection. However, apoptosis...
occurred similarly in infected Aim2−/− mice initially but further increased at a later stage of the infection compared with WT mice. This could be explained by the cell-type-specific differences in AIM2 function between BMDMs and liver, where most cells are hepatocytes. Similar to the liver tissues, BMDMs from Ifnar2−/− and Ifnar2−/−Aim2−/− mice had reduced levels of cleaved caspase-8, caspase-3, and caspase-7 compared with WT cells following F. novicida infection (Figures S3F and S3G), indicating a potential role for type I IFN signaling in activating apoptotic caspases in vitro. The defective caspase-1 activation in Ifnar2−/− and Ifnar2−/−Aim2−/− BMDMs may lead to impaired caspase-7 cleavage in these cells.

TNF-related apoptosis-inducing ligand (TRAIL) is upregulated by type I IFNs and was shown to mediate type I IFN signaling-induced cell death during viral and bacterial infections (McNab et al., 2015). TRAIL binds to its receptor DR5 and triggers apoptosis by driving caspase-8-mediated caspase-3 and caspase-7 activation. We therefore hypothesize that type I IFN signaling may promote cell death through the TRAIL pathway. We measured expression levels of TRAIL and DR5 in mouse liver tissue from WT and Ifnar2−/− mice treated with PBS or rTRAIL 1 day after F. novicida infection. The asterisk indicates a non-specific band.

The defective caspase-1 activation in Ifnar2−/− and Ifnar2−/−Aim2−/− mice had reduced levels of DR5 expression after the infection, while the level of DR5 was similar (Figures S4D and S4C). The level of TRAIL was slightly reduced in Aim2−/− mice after the infection, while the level of DR5 was similar (Figures S4D and S4E). IFNγ can induce TRAIL expression, in addition to type I IFNs (Park et al., 2004). Infected Aim2−/− mice may exhibit reduced IFNγ production (Pierini et al., 2013), which in turn leads to modest reduction in TRAIL. However, the reduction was not significant enough to reduce apoptosis in Aim2−/− mice, suggesting that other mechanisms such as Fas (CD95/ APO-1) signaling may co-exist to promote marginal cell death. Furthermore, treatment of recombinant TRAIL (rTRAIL) increased cleavage of caspase-8, caspase-3, and caspase-7 in Ifnar2−/− mice compared with PBS-treated Ifnar2−/− mice after the infection (Figures 4E and S4F), indicating that type I IFN signaling induces TRAIL-mediated apoptosis during F. novicida infection.
infection. To confirm that type I IFN signaling exacerbates *F. novicida* infection via the TRAIL pathway, we treated WT mice with TRAIL-neutralizing antibody or isotype control and infected these mice, along with *Ifnar2*−/− and *Ifnar2*−/− *Aim2*−/− mice, with *F. novicida*. A higher dose of *F. novicida* was used in these experiments so that any protective effects rendered by TRAIL neutralization would be more prominent. Although 90% of WT mice that received isotype control died, only 60% of WT mice that received TRAIL-neutralizing antibody succumbed to infection over 14 days (Figure 4F). Neutralization of TRAIL also appeared to decrease bacterial burdens in the liver and spleen 3 days post-infection (Figure S4G), demonstrating a detrimental role for TRAIL pathway during *F. novicida* infection in vivo. Moreover, WT mice that received TRAIL-neutralizing antibody had reduced levels of cleaved caspase-8, caspase-3, and caspase-7 compared with the control animals after the infection (Figures S4H and S4I), confirming that the TRAIL pathway leads to activation of apoptotic caspases during *F. novicida* infection.

In this study, we demonstrate that type I IFN induced apoptosis in the liver following *F. novicida* infection and that the pathogenic effects of type I IFN signaling dominate the protective Aim2 responses. Type I IFN signaling has been shown to enhance susceptibility to *L. monocytogenes* and *B. abortus* infection by promoting cell death in the spleen with an ability to induce Trail expression (Carrero et al., 2004, 2006; de Almeida et al., 2011; O’Connell et al., 2004). Moreover, Trail−/− mice have decreased bacterial burdens in the liver and spleen and reduced splenic cell death after *L. monocytogenes* infection (Zheng et al., 2004). Increased apoptosis during *F. novicida* infection could lead to loss of hepatocytes, which have crucial roles in controlling infection (Zhou et al., 2016). An expansion of IL-17-producing γδ T cells was attributed to the phenotype of *Ifnar1*−/− mice in a previous study (Henry et al., 2010). Because reduced activation of apoptotic caspases in *Ifnar2*−/− and *Ifnar2*−/− *Aim2*−/− mice occurred as early as 1 day post-infection, preceding the elevation of IL-17 and IL-12 in the serum, it is intriguing to hypothesize that certain immune cells such as γδ T cells may exhibit dampened cell death in the absence of type I IFN signaling, leading to an increased production of circulating IL-17 and IL-12. Type I IFN signaling is necessary for activating AIM2 inflammasome in BMDMs infected with *F. novicida* via regulation of IRF1 and IRF1-mediated induction of guanylate binding proteins (GBPs) and IRGB10 (Man et al., 2017). However, in contrast to mice lacking type I IFN signaling, mice lacking IRF1, GBPs, or IRGB10 are susceptible to the infection due to impaired AIM2 inflammasome responses (Man et al., 2015, 2016). Activation of AIM2 inflammasome is IFNAR independent in vivo, so expression of IRF1, GBPs, and IRGB10 might be intact in mice lacking type I IFN signaling because of the redundant induction by other types of IFNs. Consistent with this, priming *Ifnar1*−/− BMDMs with IFNγ bypasses the requirement for type I IFN signaling in the activation of the AIM2 inflammasome induced by *F. novicida* infection (Meunier et al., 2015). Overall, our work highlights the dominant role of type I IFN signaling, which exacerbates *F. novicida* infection in vivo, as opposed to the protective role of the AIM2 inflammasome.

**EXPERIMENTAL PROCEDURES**

**Mice**

WT C57BL/6J mice were purchased from The Jackson Laboratory. Details on mutant mice are provided in Supplemental Experimental Procedures. Male and female mice that were 6–8 weeks old were used in this study. Animal studies were conducted according to protocols approved by the St. Jude Animal Care and Use Committee.

**Bacterial Culture and Animal Infection**

*F. novicida* strain U112 was grown in BBL Trypticase soy broth (TSB) (BD) supplemented with 0.2% L-cysteine overnight at 37°C and then 1:10 subcultured for 4 hr before infection. Mice were infected subcutaneously with 1.5 × 10⁵ or 2.25 × 10⁵ of *F. novicida* in 200 μL PBS. For colony-forming unit (CFU) analysis, harvested liver and spleen were homogenized, plated onto TSB agar with 0.2% L-cysteine, and incubated overnight. For survival analysis following TRAIL neutralization, mice were injected intraperitoneally (i.p.) with 300 μg anti-TRAIL monoclonal antibody (mAb) (N2B2) or immunoglobulin G2a (IgG2a) (RTK2758) isotype control (BioLegend) on days 0, 1, and 2 post-infection. For TRAIL experiment, mice were injected i.p. with 10 μg rTRAIL (PeproTech) or PBS on the same day as infection. Details are provided in Supplemental Experimental Procedures.

**Cytokine Analysis**

Cytokine levels were determined by performing multiplex ELISA (Millipore) or IL-18 ELISA (MBL International) according to the manufacturers’ instructions.

**Statistical Analysis**

GraphPad Prism 6.0 software was used for data analysis. Data are shown as mean ± SEM. Statistical significance was determined by performing t tests (two tailed), one-way ANOVA, or a log-rank test. p < 0.05 was considered statistically significant.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.02.096.

**ACKNOWLEDGMENTS**

We thank Parimal Samir (St. Jude) for technical assistance and members of the Kanneganti laboratory for their comments and suggestions. We thank V.M. Dixit (Genentech) for the mutant mice and the Department of Scientific Editing at St. Jude for editing the manuscript. Work from our laboratory is supported by the NIH (AI101935, AI124346, AR056296, and CA163507 to T.-D.K.), the American Lebanese Syrian Associated Charities, and the R.G. Menzies Early Career Fellowship from the National Health and Medical Research Council of Australia (to S.M.M.).

**AUTHOR CONTRIBUTIONS**

Q.Z., S.M.M., and T.-D.K. conceptualized the study; Q.Z., S.M.M., R.K., and T.-D.K. designed the experiments; Q.Z., S.M.M., R.K., and R.K.S.M. performed the experiments; Q.Z., S.M.M., and R.K. conducted the analysis; and Q.Z., S.M.M., and T.-D.K. wrote the manuscript, with input from all authors. T.-D.K. acquired the funding, provided resources, provided overall supervision, and approved the manuscript.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

Received: June 29, 2017

Revised: January 29, 2018

Accepted: February 23, 2018

Published: March 20, 2018
REFERENCES


