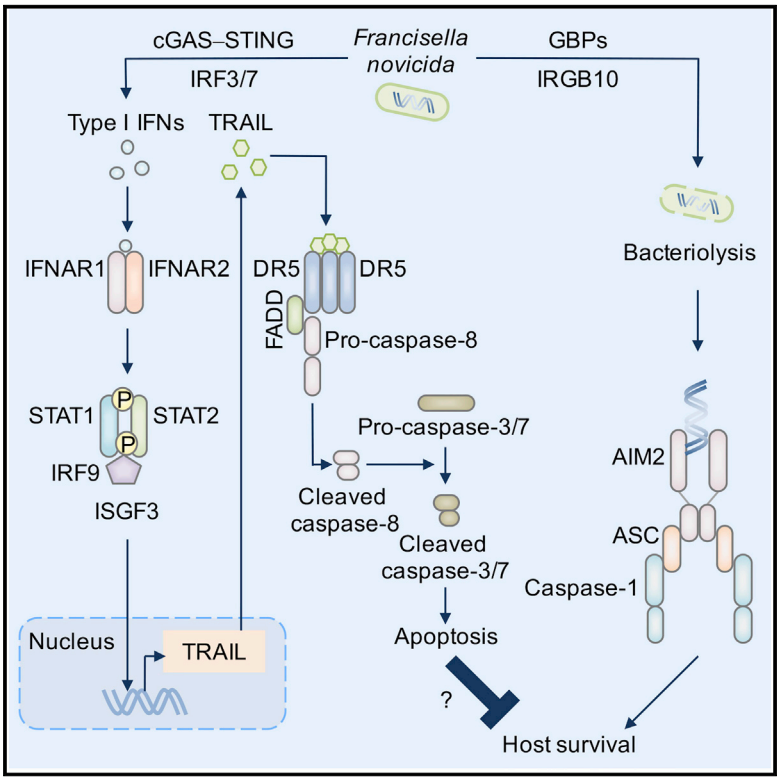
Cell Reports

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

Graphical Abstract



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*

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.

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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 Kanneganti[1,3,*]
[1]Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA
[2]Integrated Biomedical Sciences Program, University of Tennessee Health Science Center, Memphis, TN 38163, USA
[3]Lead Contact
*Correspondence: thirumala-devi.kanneganti@stjude.org
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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; Rathinam 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

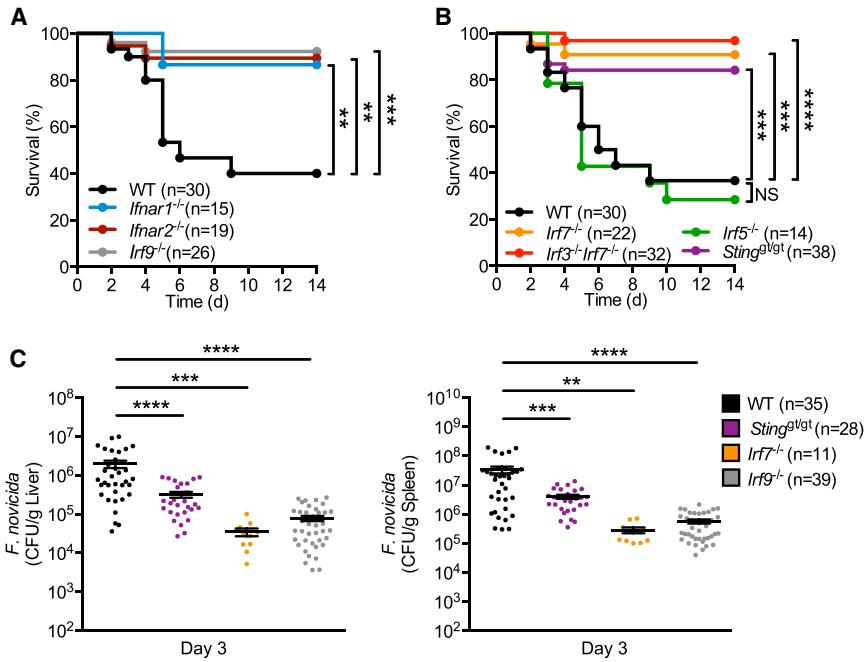

Figure 1. Mice Lacking IFNAR1 or IFNAR2 Are Resistant to *F. novicida*

(A) Survival of WT, *Ifnar1*$^{-/-}$, *Ifnar2*$^{-/-}$, and *Irf9*$^{-/-}$ mice infected with *F. novicida*.

(B) Survival of WT, *Irf7*$^{-/-}$, *Irf3*$^{-/-}$*Irf7*$^{-/-}$, *Irf5*$^{-/-}$ and *Sting*$^{gt/gt}$ mice infected with *F. novicida*.

(C) Bacterial burdens in the liver and spleen of WT, *Sting*$^{gt/gt}$, *Irf7*$^{-/-}$, and *Irf9*$^{-/-}$ mice 3 days after infection with *F. novicida*.

NS, not significant; **p < 0.01, ***p < 0.001, ****p < 0.0001; log-rank test (A and B) or one-way ANOVA with Dunnett's multiple-comparisons test (C). Data are pooled from three (C) or four (A and B) independent experiments (mean and SEM in C).

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 (IRF) 9 (*Irf9*$^{-/-}$), a component of IFN-stimulated gene factor 3 (ISGF3) 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 *Irf7*$^{-/-}$ and *Irf3*$^{-/-}$*Irf7*$^{-/-}$ 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*$^{gt/gt}$) (Figure 1B). Consistent with these results, we found significantly lower bacterial burdens in the liver and spleen of *Sting*$^{gt/gt}$ mice, *Irf7*$^{-/-}$ 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 (*Ifnar2*$^{-/-}$*Aim2*$^{-/-}$). *Ifnar2*$^{-/-}$*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*$^{-/-}$*Aim2*$^{-/-}$ mice with *F. novicida* and monitored their survival. *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice were resistant to the infection, similar to *Ifnar2*$^{-/-}$ 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 *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ and *Ifnar2*$^{-/-}$ 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 *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice. An increased number of splenic CD4^{+} T cells in naive *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice is unlikely to contribute to the susceptibility (Figure S1A), because the bacterial burden in *Ifnar2*$^{-/-}$*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 *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice, confirming AIM2-dependent IL-18 production in the infected mice (Figure 2C). *Ifnar2*$^{-/-}$ 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 *Ifnar2*$^{-/-}$ mice and *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice suggest that the dominance of IFNAR signaling-mediated responses over AIM2 is independent of IL-18.

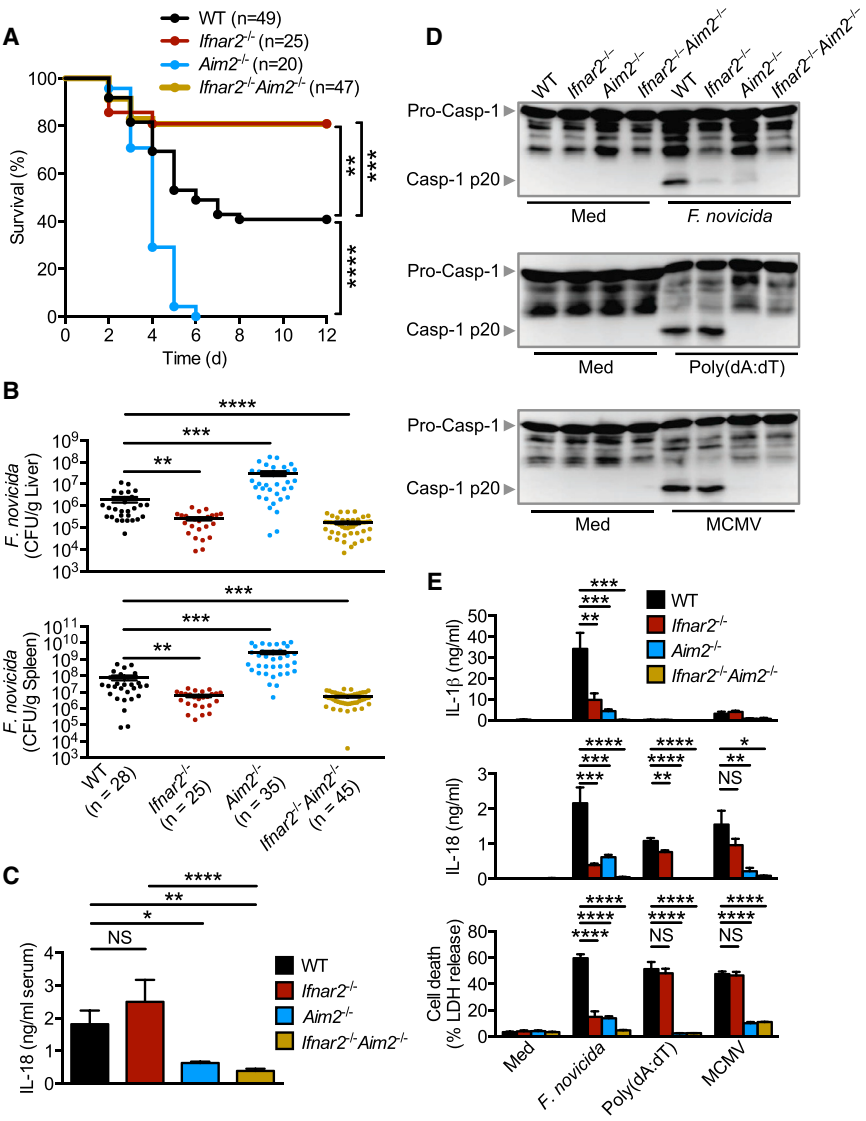

Figure 2. Detrimental Role of Type I IFN Signaling Is Dominant over AIM2 Inflammasome Responses during *F. novicida* Infection

(A) Survival of WT, *Ifnar2*$^{-/-}$, *Aim2*$^{-/-}$, and *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice infected with *F. novicida*.

(B) Bacterial burdens in the liver and spleen of WT, *Ifnar2*$^{-/-}$, *Aim2*$^{-/-}$, and *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice 3 days after *F. novicida* infection.

(C) Concentration of IL-18 in the sera of WT, *Ifnar2*$^{-/-}$, *Aim2*$^{-/-}$, and *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice 1 day after infection.

(D) Immunoblot analysis of pro-caspase-1 (Pro-Casp-1) and the caspase-1 subunit p20 (Casp-1 p20) in unprimed WT or mutant BMDMs left uninfected or untreated (Med, medium alone) or assessed 20 hr after infection with *F. novicida* (MOI, 100; top), 5 hr after transfection with poly(dA:dT) (middle), or 10 hr after infection with mouse cytomegalovirus (MCMV; bottom).

(E) Release of IL-1β (top) or IL-18 (middle) and death (bottom) of unprimed BMDMs after treatment, as in (D).

NS, not significant; *p < 0.05; **p < 0.01, ***p < 0.001, ****p < 0.0001; long-rank test (A) or one-way ANOVA with Dunnett's multiple comparisons test (B, C, and E). Data are pooled from three (B) or four (A and C) independent experiments or are from one experiment representative of two independent experiments (D and E) (mean and SEM in B, C, and E).

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 *Ifnar2*$^{-/-}$ cells after poly(dA:dT) (poly(deoxyadenylic-deoxythymidylic)acid sodium salt) transfection or mouse cytomegalovirus (MCMV) infection (Figures 2D, 2E, and S1D). Consistent with previous reports (Man et al., 2015, 2016), transfection of poly(dA:dT) did not induce IL-1β release in WT BMDMs (Figure 2E), possibly due to an inability of poly(dA:dT) transfection in mediating IL-1β expression. Collectively, these results demonstrate that unlike in macrophages, AIM2 inflammasome activation *in vivo* is retained even in the absence of type I IFN signaling.

Inflammatory Cytokine Production Is Intact in the Absence of Type I IFN Signaling

Previous studies showed that type I IFNs suppress production of pro-inflammatory cytokines to prevent inflammation-associated damage and lethality during *S. pneumonia* infection (Castiglia et al., 2016). To identify whether the levels of pro-inflammatory cytokines are differentially regulated by type I IFN signaling during infection by *F. novicida*, we analyzed a range of cytokines and chemokines 1 day after *F. novicida* infection, when WT, *Ifnar2*$^{-/-}$, and *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice had a similar bacterial burden (Figure 3A). Levels of the pro-inflammatory cytokines GM-CSF, tumor necrosis factor (TNF), IL-6, MIP1-α, IL-17, IL-12, and IFNγ and the anti-inflammatory cytokine IL-10 in the liver were comparable among WT, *Ifnar2*$^{-/-}$, and *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice (Figure 3B). The levels of these proinflammatory cytokines were also similar in the sera of WT, *Ifnar2*$^{-/-}$, and *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice at this time point (Figure 3C). We also measured levels of the cytokines 2 days after infection, when bacterial burdens were similar among the animals (Figure S2A). Whereas levels of TNF, IL-6, MIP1-α, IFNγ, and IL-10 were similar in the liver (Figure S2B), levels of GM-CSF and IL-12 were decreased in both *Ifnar2*$^{-/-}$ and *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice and the level of IL-17 was decreased in *Ifnar2*$^{-/-}$ mice (Figure S2B). We found similar levels of TNF, IL-6, MIP1-α, and IL-10

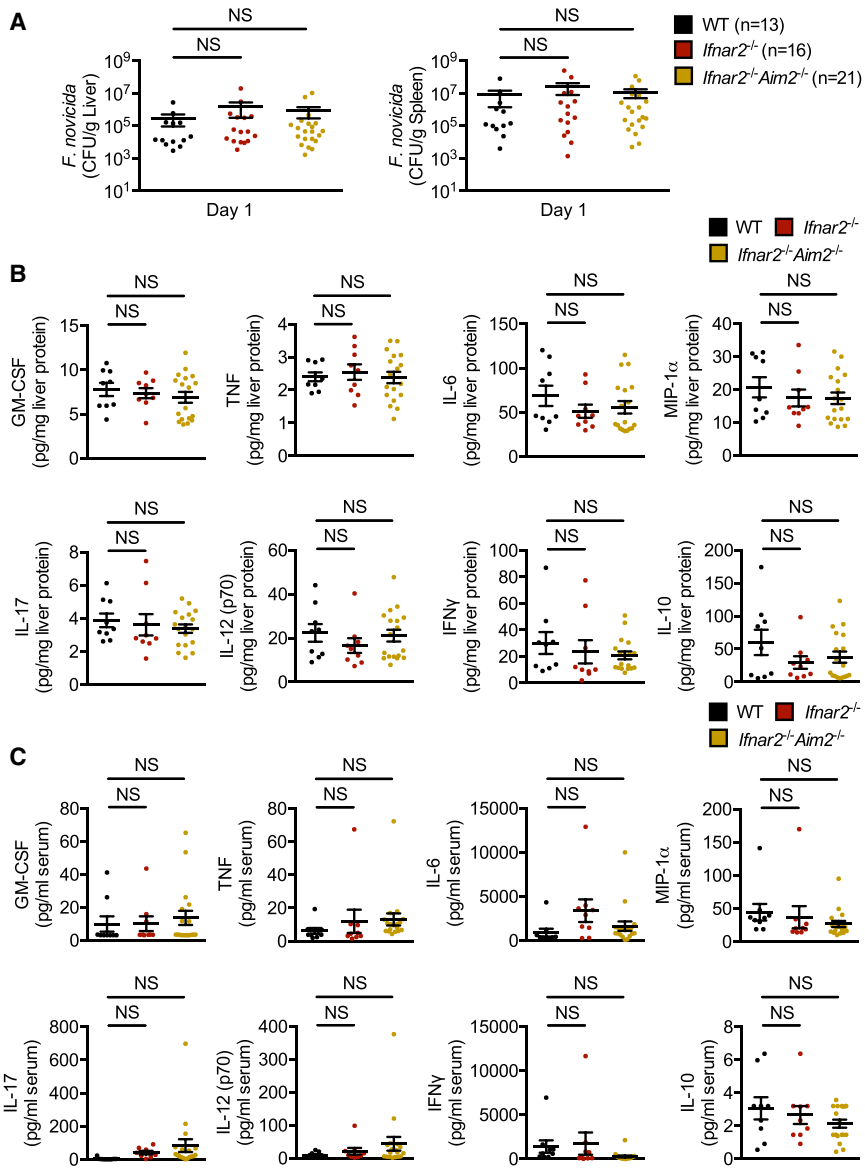

Figure 3. Type I IFN Signaling Does Not Alter Production of a Range of Inflammatory Cytokines during *F. novicida* Infection

(A) Bacterial burdens in the liver and spleen of WT, *Ifnar2*$^{-/-}$, and *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice 1 day after infection with *F. novicida*.

(B and C) Concentrations of GM-CSF, TNF, IL-6, MIP1-α, IL-17, IL-12 (p70), IFNγ, and IL-10 in the liver (B) or serum (C) of WT, *Ifnar2*$^{-/-}$, and *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice 1 day after infection, as in (A).

NS, not significant; one-way ANOVA with Dunnett's multiple-comparisons test. Data are pooled from two (B and C) or three (A) independent experiments (mean and SEM in A–C).

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

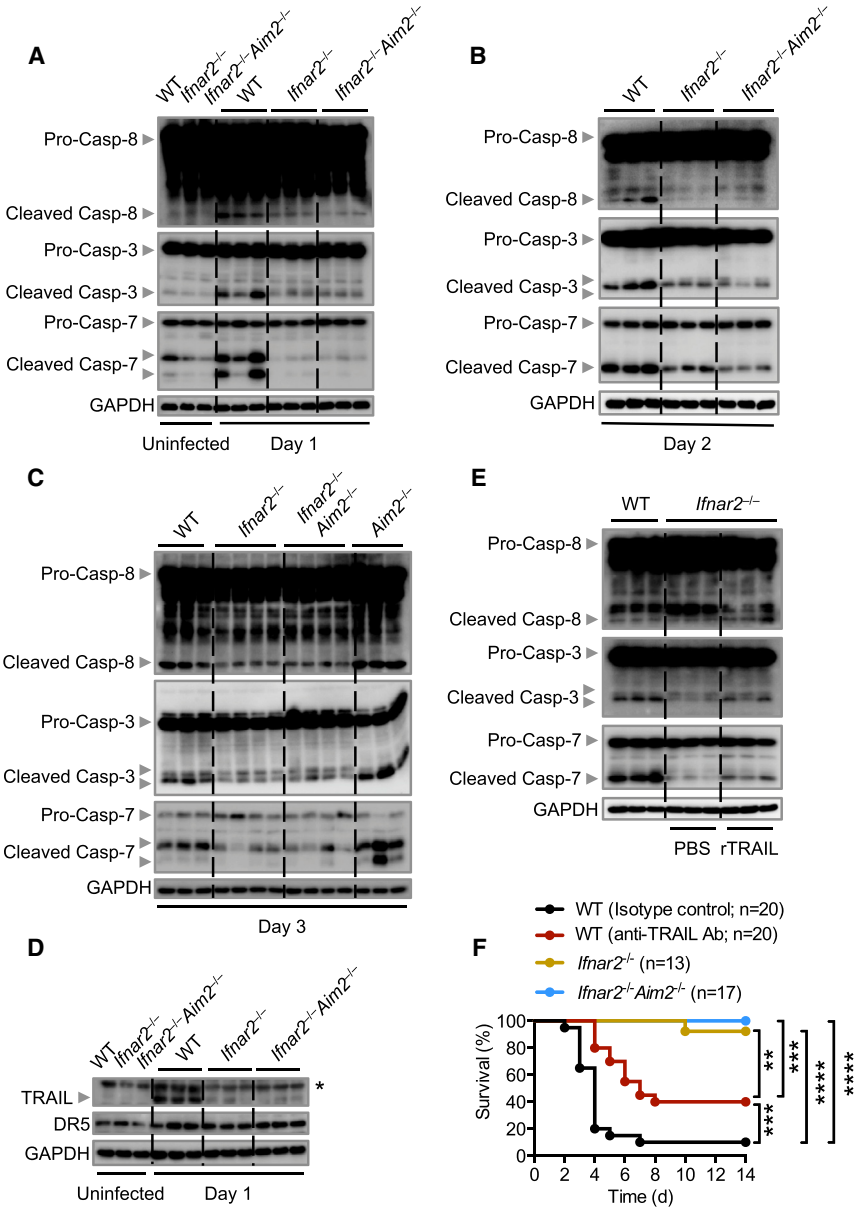

Figure 4. Type I IFN Signaling Induces Apoptotic Cell Death

(A–C) Immunoblot analysis of pro-caspase-8, cleaved caspase-8, pro-caspase-3, cleaved caspase-3, pro-caspase-7, cleaved caspase-7, and GAPDH in liver tissue from uninfected animals or WT, *Ifnar2*$^{-/-}$, and *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice 1 day (A) or 2 days (B) after *F. novicida* infection or from WT, *Ifnar2*$^{-/-}$, *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$, and *Aim2*$^{-/-}$ mice 3 days (C) after *F. novicida* infection.

(D) Immunoblot analysis of TRAIL, DR5, and GAPDH in liver tissues of uninfected animals or WT, *Ifnar2*$^{-/-}$, and *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice 1 day after infection. The asterisk indicates a non-specific band.

(E) Immunoblot analysis of pro-caspase-8, cleaved caspase-8, pro-caspase-3, cleaved caspase-3, pro-caspase-7, cleaved caspase-7, and GAPDH in liver tissue from WT and *Ifnar2*$^{-/-}$ mice treated with PBS or rTRAIL 1 day after *F. novicida* infection.

(F) Survival of WT mice that received TRAIL-neutralizing antibody or isotype control, *Ifnar2*$^{-/-}$ mice, and *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice infected with *F. novicida*.

NS, not significant; **p < 0.01; ***p < 0.001, ****p < 0.0001; long-rank test (F). Data are pooled from three independent experiments (F) or are from one experiment representative of two (B and E) or four (A, C and D) independent experiments.

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 the liver and found that expression of TRAIL was upregulated in the liver of WT mice 1 day after infection; however, the induction was abrogated in *Ifnar2*$^{-/-}$ and *Ifnar2*$^{-/-}$*Aim2*$^{-/-}$ mice (Figures 4D, S4B, and S4C). DR5 was similarly induced between the animals (Figures 4D, S4B, 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. 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 1.5×10^5 or 2.25×10^5 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 the rTRAIL 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.

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AUTHOR CONTRIBUTIONS

Q.Z., S.M.M., and T.-D.K. conceptualized the study; Q.Z., S.M.M., R.K., and R.K.S.M. 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.

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