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Type I Interferon Signaling Regulates Activation of the Absent in Melanoma 2 Inflammasome during *Streptococcus pneumoniae* Infection

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***Streptococcus pneumoniae*, a Gram-positive bacterial pathogen, causes pneumonia, meningitis, and septicemia. Innate immune responses are critical for the control and pathology of pneumococcal infections. It has been demonstrated that *S. pneumoniae* induces the production of type I interferons (IFNs) by host cells and that type I IFNs regulate resistance and chemokine responses to *S. pneumoniae* infection in an autocrine/paracrine manner. In this study, we examined the effects of type I IFNs on macrophage proinflammatory cytokine production in response to *S. pneumoniae*. The production of interleukin-18 (IL-18), but not other cytokines tested, was significantly decreased by the absence or blockade of the IFN-α/β receptor, suggesting that type I IFN signaling is necessary for IL-18 production. Type I IFN signaling was also required for *S. pneumoniae*-induced activation of caspase-1, a cysteine protease that plays a central role in maturation and secretion of IL-18. Earlier studies proposed that the AIM2 and NLRP3 inflammasomes mediate caspase-1 activation in response to *S. pneumoniae*. From our results, the AIM2 inflammasome rather than the NLRP3 inflammasome seemed to require type I IFN signaling for its optimal activation. Consistently, AIM2, but not NLRP3, was upregulated in *S. pneumoniae*-infected macrophages in a manner dependent on the IFN-α/β receptor. Furthermore, type I IFN signaling was found to contribute to IL-18 production in pneumococcal pneumonia *in vivo*. Taken together, these results suggest that type I IFNs regulate *S. pneumoniae*-induced activation of the AIM2 inflammasome by upregulating AIM2 expression. This study revealed a novel role for type I IFNs in innate responses to *S. pneumoniae*.**

The Gram-positive bacterial pathogen *Streptococcus pneumoniae* is a leading cause of pneumonia, meningitis, and septicemia and is responsible for significant mortality and morbidity worldwide (1). Due to the severe disease burden and mortality, the incidence of drug-resistant *S. pneumoniae*, and an increase in the number of immunocompromised patients, it is increasingly important to understand the pathogenic processes of pneumococcal diseases in order to develop novel therapeutic modalities and effective vaccines. The polysaccharide capsule and pneumolysin (PLY) are critical virulence factors of *S. pneumoniae* (1–3). PLY is a member of cholesterol-dependent cytolysins and forms ring- or arc-shaped pores on cholesterol-containing membranes. As PLY lacks a signal sequence for secretion, it has been hypothesized that the cytolysin is released during the breakdown of the pneumococcal cell wall. PLY plays multiple roles in the pathogenicity of *S. pneumoniae*, including disruption of tissue barriers, inhibition of ciliary beating on epithelial cells and bactericidal activity of neutrophils, and possible subversion of complement-mediated opsonization (3, 4). It has also been demonstrated that PLY is involved in the activation of innate immune responses to *S. pneumoniae*, which generate inflammation and/or contribute to host defense (3, 5–7).

Pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs) and Nod-like receptors (NLRs), play a central role in innate immunity (8, 9). PRRs sense specific pathogen-derived molecular structures and, in turn, provide signals that activate innate defense mechanisms. Pneumococcal components and products are sensed by several PRRs, leading to the induction of inflammatory mediators, some of which contribute to host defense against *S. pneumoniae*. TLR2, TLR9, and TLR4 have been reported to participate in the production of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), in response to *S. pneumoniae* and pneumococcal uptake (6, 10–12). NLR family, pyrin domain containing 3 (NLRP3) and absent in melanoma 2 (AIM2) are cytoplasmic PRRs, which sense various stimuli and cytosolic DNA, respectively (13–15). After stimulation with specific agonists, NLRP3 and AIM2 form inflammasomes which activate caspase-1, which, in turn, processes pro-IL-1β and pro-IL-18 into their bioactive forms. Recent studies have suggested that the AIM2 and NLRP3 inflammasomes mediate *S. pneumoniae*-induced activation of caspase-1 and secretion of bioactive IL-1β and IL-18 (16–19). Moreover, *S. pneumoniae* induces the production of type I interferons (IFNs) through stimulator of interferon genes (STING), an adaptor of cytosolic DNA sensors (16, 20). Thus, *S. pneumoniae* appears to be recognized by at least one intracellular DNA sensor. Several groups have proposed that PLY mediates the delivery of pneumococcal DNA into the cytoplasm of host cells by forming pores in the cell membrane (11, 16, 17, 20). Especially during pneumococcal infection of macrophages, PLY is thought to dis-

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rupt the membrane of *S. pneumoniae*-containing phagosomes, in which engulfed bacteria are autolyzed or killed, thereby mediating the release of pneumococcal components, including DNA, into the cytoplasm (11, 16, 17). Strikingly, the induction of bioactive IL-1β and IL-18 and type I IFNs by *S. pneumoniae* critically depends on phagocytosis of bacteria and the expression of intact PLY (7, 16, 17). A previous study showed that recognition of *S. pneumoniae* by NOD2, a cytosolic sensor for muramyl dipeptide, was dependent on bacterial uptake and PLY (21, 22). This also suggests that PLY mediates cytoplasmic delivery of pneumococcal components.

Type I IFNs are produced during viral infections and are responsible for the first line of defense against viruses (23). Type I IFNs signal through a common receptor, the IFN-α/β receptor (IFNAR), which is a heterodimer of IFNAR1 and IFNAR2, resulting in the activation of the Janus kinase/signal transducer and activator of transcription pathway; this leads to the expression of IFN-inducible genes, some of which have antiviral effects. For the past decade, a number of studies have suggested that the production of type I IFNs is induced not only by viruses but also by bacterial pathogens (24). In bacterial infections, the role of type I IFNs varies with the type of pathogen; they can be both beneficial and detrimental to the host. Type I IFNs appear to contribute to, have no effect on, or impair host defense against *S. pneumoniae*, probably depending on the experimental design (20, 25–27). In pneumococcal intranasal or intratracheal infection models, genetic ablation of IFNAR1 resulted in increased nasopharyngeal colonization with *S. pneumoniae* in one study (20) but did not influence the clearance of bacteria from the lung and upper respiratory tract in other studies (26, 27). In the latter studies, IFNAR1 deficiency facilitated pneumococcal clearance in influenza virus-*S. pneumoniae* coinfection models (26, 27). In addition, a recent study showed that the transition of *S. pneumoniae* from the lung into the bloodstream was enhanced in the absence of IFNAR1 (25).

It has also been shown that type I IFNs positively or negatively regulate the production of chemokines, such as C-C motif chemokine 5 (CCL5; also known as RANTES), C-X-C motif chemokine 1 (CXCL1; also known as KC), CXCL2 (also known as Mip-2), and CCL2 (also known as MCP-1), in response to *S. pneumoniae* or a combination of influenza virus and *S. pneumoniae* in an autocrine and/or paracrine manner (16, 26, 27). This may, in part, account for the complicated effects of type I IFNs on host defense against pneumococcal infections. In addition to chemokines, proinflammatory cytokines are involved in host defense against *S. pneumoniae* (28–33), and hence, examining the effects of type I IFNs on cytokine production would be helpful to understand the exact role of type I IFNs in pneumococcal diseases. In this study, we found that type I IFN signaling is required for the production of IL-18 upon infection of macrophages with *S. pneumoniae*. Caspase-1 activation in response to *S. pneumoniae* was also dependent on IFNAR1. The AIM2 inflammasome rather than the NLRP3 inflammasome seemed to require type I IFN signaling for its optimal activation, which is most likely because of upregulation of AIM2 that is downstream of type I IFN signaling. Furthermore, type I IFN signaling was found to contribute to IL-18 production in pneumococcal pneumonia *in vivo*. Taken together, the results revealed a novel role for type I IFNs in innate responses to *S. pneumoniae*.

MATERIALS AND METHODS

Mice. Female C57BL/6 mice were purchased from Japan SLC (Hamamatsu, Japan). IFNAR1$^{-/-}$ mice were kindly provided by Michel Aguet (Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland). NLRP3$^{-/-}$ mice were generated by Jürg Tschopp (University of Lausanne). Mice were maintained under specific-pathogen-free conditions and used at 8 to 10 weeks of age. All of the experimental procedures performed on mice were approved by the Animal Ethics and Research Committee of Kyoto University Graduate School of Medicine, Kyoto, Japan.

Bacterial strain. *Streptococcus pneumoniae* D39 (serotype 2) was purchased from the National Collection of Type Cultures (NCTC 7466; Central Public Health Laboratory, London, United Kingdom). Pneumococci were grown overnight on blood agar plates at 37°C and 5% CO_2. Colonies were inoculated into Todd-Hewitt broth (BD Biosciences, San Jose, CA) supplemented with 0.5% yeast extract, grown until midlogarithmic phase (optical density at 600 nm [OD_{600}] = 0.5), and centrifuged at 6,000 × *g* for 15 min. The bacterial pellet was suspended in phosphate-buffered saline (PBS) and stocked at −80°C. The concentration was determined by colony counting on blood agar plates.

Preparation of macrophages and *in vitro* infection with *S. pneumoniae*. Mice were injected intraperitoneally (i.p.) with 3% thioglycolate broth (Eiken Chemical, Tokyo, Japan), and peritoneal exudate cells (PECs) were collected 3 days later. After being washed with RPMI 1640 medium (Nacalai Tesque, Kyoto, Japan), PECs were suspended with RPMI 1640 supplemented with 10% fetal calf serum (FCS) and incubated in 48-well microplates at a density of 2.5×10^5 cells/well at 37°C plus 5% CO_2, and nonadherent cells were removed after 2 h. More than 95% of the adherent PECs were F4/80$^+$ macrophages as determined by flow cytometry. An immortalized AIM2-deficient macrophage cell line was kindly gifted from E. S. Alnemri (34). Macrophages were infected with *S. pneumoniae* at a multiplicity of infection (MOI) of 10 for 8 h, then 100 μg/ml of gentamicin (Wako Pure Chemical Industries, Osaka, Japan) was added to the cultures, and the cultures were incubated for an additional 16 h. Anti-mouse IFNAR1 blocking MAb (Biolegend, San Diego, CA) and purified mouse IgG1 (Biolegend) were added to the cultures at the same time as *S. pneumoniae*. Ultrapure *Escherichia coli* O111:B4 lipopolysaccharide (LPS) and nigericin were purchased from Invivogen (San Diego, CA) and Sigma-Aldrich (St. Louis, MO), respectively.

ELISA. Levels of cytokines in culture supernatants were determined by two-site sandwich enzyme-linked immunosorbent assay (ELISA). ELISA kits for mouse IL-1β, IL-6, TNF-α, and IL-12p40 were purchased from eBioscience (San Diego, CA). A mouse IL-18 ELISA kit was purchased from Medical & Biological Laboratories (Nagoya, Japan). A mouse IL-1α ELISA kit was purchased from BD Biosciences.

Western blot analysis. PECs were cultured on 12-well plates at a density of 5×10^5 cells/well in RPMI 1640 plus FCS at 37°C for 2 h. Then nonadherent cells were washed out, and the medium was replaced with Opti-MEM (Life Technologies, Carlsbad, CA). Adherent cells were infected with *S. pneumoniae* at an MOI of 10. Supernatants were collected 24 h after infection, and the cells were lysed with radioimmunoprecipitation assay buffer (Nacalai Tesque). The supernatants were concentrated 20-fold using 20% (wt/vol) trichloroacetate (Nacalai Tesque). The precipitates and cell lysates were subjected to SDS-PAGE and subsequently transferred to polyvinylidene difluoride membranes. The membranes were immunoblotted with anti-caspase-1 Ab (Santa Cruz Biotechnology, Santa Cruz, CA), anti-IL-1β Ab (R&D Systems, Minneapolis, MN), anti-IL-18 MAb (Medical & Biological Laboratories), or anti-β-actin MAb (Sigma-Aldrich).

Quantification of *S. pneumoniae* organisms associating with and phagocytosed by macrophages. The number of *S. pneumoniae* organisms associating with or phagocytosed by macrophages was determined as previously described (17). Adherent macrophages were infected with *S. pneumoniae* at an MOI of 10 for 4, 6, or 8 h. To enumerate *S. pneumoniae* organisms associating with macrophages, cells were washed with chilled

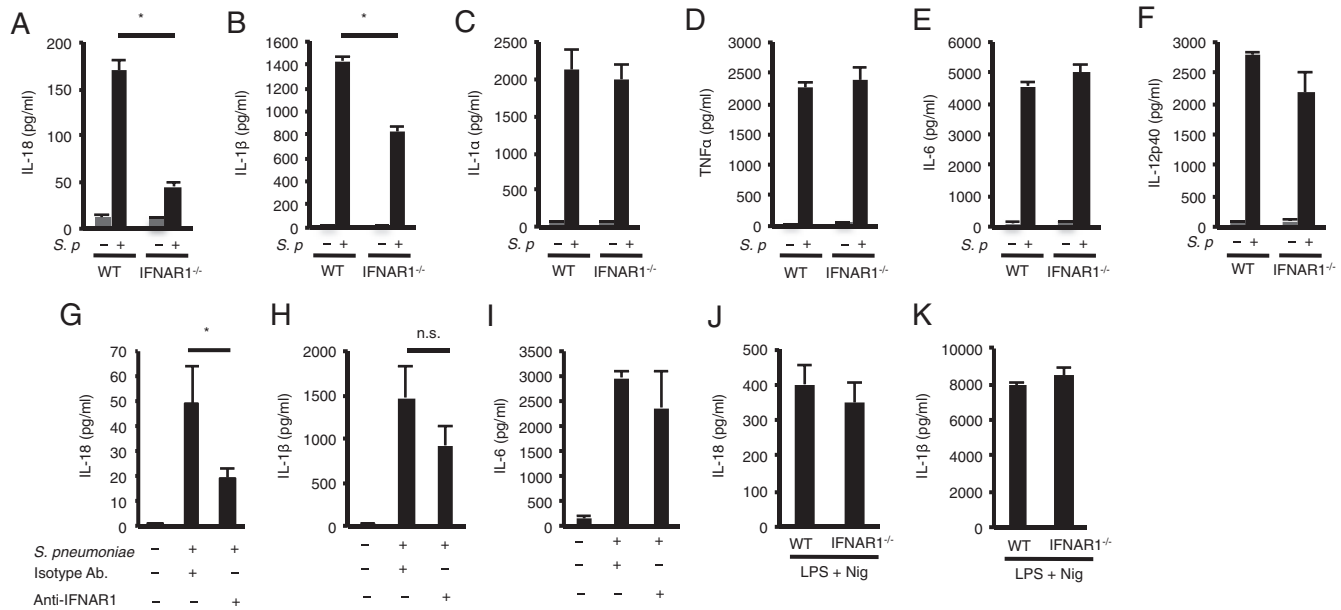

FIG 1 Type I IFN signaling is required for IL-18 production in response to *S. pneumoniae*. (A to F) Adherent PECs from C57BL/6 (WT) and IFNAR1$^{-/-}$ mice were left uninfected or infected with *S. pneumoniae* D39 at an MOI of 10. Gentamicin (final concentration of 100 μg/ml) was added to the cultures 8 h after infection, and culture supernatants were collected after an additional 16 h of incubation (24 h after infection). Levels of IL-18 (A), IL-1β (B), IL-1α (C), TNF-α (D), IL-6 (E), and IL-12p40 (F) in the culture supernatants were determined by ELISA. (G to I) Anti-IFNAR1 blocking MAb (10 μg/well) or isotype control Ab (10 μg/well) was added to the cultures at the same time as *S. pneumoniae*. Levels of IL-18 (G), IL-1β (H), and IL-6 (I) were determined by ELISA. (J and K) Adherent PECs were stimulated with LPS (10 ng/ml) for 3 h and sequentially with nigericin (5 μM). After 21 h, culture supernatants were collected to assess the levels of IL-18 (J) and IL-1β (K) by ELISA. All of the experiments were repeated at least two times, with similar results. Data are presented as the means and standard deviations of triplicate assays. Statistical significance was determined by one-way ANOVA followed by Bonferroni's test. *, $P < 0.05$; n.s., not significant.

PBS three times to remove nonassociating bacteria and lysed in PBS containing 0.1% Triton X-100. The cell lysates were diluted with PBS and plated on blood agar plates. CFU were counted after overnight incubation. To enumerate the phagocytosed bacteria, macrophages were infected with *S. pneumoniae* as described above and additionally cultured for 30 min in the presence of 100 μg/ml of gentamicin. The cells were then washed, and the bacteria were counted. To monitor the survival of *S. pneumoniae* inside macrophages, the numbers of intracellular bacteria were determined every 0.5 h from 1 h to 3 h after gentamicin addition.

Quantitative RT-PCR analysis. Total cellular RNA of adherent PECs was extracted by using NucleoSpin RNA Cleanup (MACHEREY-NAGEL, Düren, Germany), and quantitative real-time RT-PCR was carried out using the ABI Prism 7000 (Life Technologies) and Express Two-Step SYBR GreenER (Life Technologies), according to the manufacturer's instructions. Primers for quantitative real-time RT-PCR were as follows: for β-actin, 5′-GCC CTG AGG CTC TTT TCC AG-3′ (forward) and 5′-TGC CAC AGG ATT CCA TAC CC-3′ (reverse); for AIM2, 5′-TTG TAT CTA GGC TGA TCC TGG GAC-3′ (forward) and 5′-ACC TGC ACT TTG AAT CAG GTG GTC-3′ (reverse); for NLRP3, 5′-AGA GCC TAC AGT TGG GTG AAA TG-3′ (forward) and 5′-CCA CGC CTA CCA GGA AAT CTC-3′ (reverse); and for IL-6, 5′-TTC CAT CCA GTT GCC TTC TTG-3′ (forward) and 5′-GAA GGC CGT GGT TGT CACC-3′ (reverse).

Intranasal infection of mice. Mice were anesthetized with pentobarbital (Nacalai Tesque) and inoculated with 5×10^7 bacteria in 30 μl of PBS. Bronchoalveolar lavage fluid (BALF) was collected 12 h after *S. pneumoniae* infection. BALF samples from noninfected mice were collected as a negative control. The collected BALF samples were first centrifuged at $200 \times g$ for 3 min at 4°C, and the supernatants were transferred to new tubes for cytokine detection. The pellets were washed and the total cell number was determined. A total of 10^6 cells from each sample were incubated with anti-mouse CD16/32 MAb (Biolegend) for 30 min at 4°C. Then the cells were stained with phycoerythrin (PE)-conjugated anti-mouse F4/80 MAb (Biolegend) and fluorescein isothiocyanate (FITC)-conjugated anti-Gr-1 MAb (Biolegend) for 30 min at 4°C and analyzed on a FACSCalibur flow cytometer (BD Biosciences). The proportions of F4/80$^+$ macrophages and F4/80$^-$ Gr-1$^+$ neutrophils were calculated.

Statistical analysis. For comparisons between two groups, Student's *t* test was used. Multigroup comparisons of mean values were made according to analysis of variance (ANOVA) and the Bonferroni *post hoc* test. Statistical significance was determined as a *P* value of <0.05.

RESULTS

Type I IFN signaling is required for IL-18 production in response to *S. pneumoniae*. To determine whether type I IFNs regulate the production of proinflammatory cytokines, thioglycolate-elicited macrophages from wild-type (WT) or IFNAR1-deficient mice were infected with *S. pneumoniae* for 24 h, and cytokine levels in the culture supernatants were determined. In the absence of IFNAR1, *S. pneumoniae*-induced production of IL-18 was markedly reduced, while that of IL-1β was marginally reduced (Fig. 1A and B). The production of IL-1α, TNF-α, IL-6, and IL-12p40 was not affected by IFNAR1 deficiency (Fig. 1C to F). Blockade of IFNAR1 using an anti-IFNAR1 MAb resulted in a significant reduction in the production of IL-18 but not of IL-1β and IL-6 (Fig. 1G to I). Therefore, it appears that type I IFN signaling is involved in the production of IL-18 during *S. pneumoniae* infection. WT and IFNAR1-deficient macrophages produced comparable levels of IL-18 and IL-1β after stimulation with LPS plus nigericin, which activates the NLRP3 inflammasome, suggesting that secretion of these cytokines can occur without type I IFN signaling in this situation (Fig. 1J and K).

Type I IFN signaling contributes to *S. pneumoniae*-induced caspase-1 activation. IL-18 and IL-1β require cleavage by caspase-1 to be secreted in their active forms. We examined the

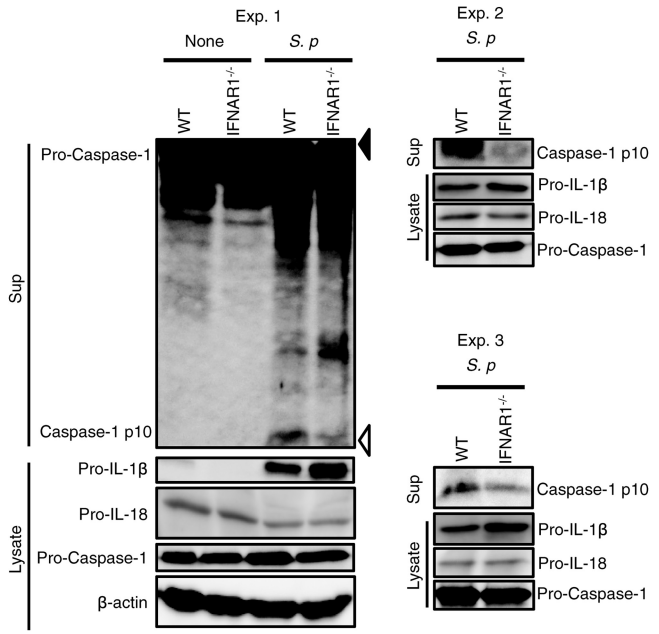

FIG 2 Type I IFN signaling contributes to *S. pneumoniae* (*S. p*)-induced caspase-1 activation. Adherent PECs from WT and IFNAR1$^{-/-}$ mice were left uninfected or infected with *S. pneumoniae* D39 at an MOI of 10 for 24 h as described for Fig. 1, and culture supernatants and cell lysates were then collected. The culture supernatants were concentrated by TCAtrichloroacetic acid precipitation. The precipitates and cell lysates were subjected to Western blot analysis using antibodies specific for caspase-1, IL-1β, IL-18, or β-actin. Filled and open triangles indicate molecular mass markers of 47 kDa and 9 kDa, respectively. All of the experiments were repeated three times, with similar results.

role of type I IFNs in caspase-1 activation in macrophages infected with *S. pneumoniae*. Infection of macrophages with this bacterium induced the secretion of the p10 fragment of active caspase-1, which was significantly reduced in the absence of IFNAR1 (Fig. 2). Thus, it is suggested that type I IFN signaling contributes to induction of caspase-1 activation by *S. pneumoniae*. Consistent with previous observations that type I IFNs suppress the expression of pro-IL-1β (35), the protein level of pro-IL-1β was higher in IFNAR1-deficient macrophages than in WT macrophages (Fig. 2). On the other hand, protein levels of pro-IL-18 were comparable between WT and IFNAR1-deficient macrophages. This may explain why *S. pneumoniae*-induced IL-1β production was only modestly affected by deficiency of IFNAR1, despite a significant reduction in IL-18 production and caspase-1 activation.

Type I IFN signaling is dispensable for uptake and killing of *S. pneumoniae* by macrophages. We have previously shown that the production of IL-18 and the activation of caspase-1 in response to *S. pneumoniae* depend on phagocytosis of pneumococci by host macrophages (17). To know whether type I IFNs are involved in pneumococcal uptake and killing, WT and IFNAR1-deficient macrophages were tested for the ability to ingest and kill *S. pneumoniae*. IFNAR1-deficient macrophages showed normal uptake and killing of *S. pneumoniae* (Fig. 3), suggesting that IFNAR1 deficiency does not affect phagocytosis and killing of this pathogen by macrophages.

Type I IFN signaling is required for induction of AIM2-mediated IL-18 production by *S. pneumoniae*. *S. pneumoniae* induces the production of IL-18 and activation of caspase-1 through the AIM2 and NLRP3 inflammasomes (16–19). Thus, we examined which kind of inflammasome requires type I IFN signaling for optimal activation upon *S. pneumoniae* infection. IFNAR1 blockade significantly reduced the production of IL-18, but not of IL-1β, by NLRP3-deficient macrophages infected with *S. pneumoniae* (Fig. 4). This indicates that NLRP3-independent production of IL-18 requires type I IFN signaling. On the other hand, AIM2-deficient macrophages hardly secreted IL-18 and IL-1β after infection with *S. pneumoniae* (Fig. 4), and thus, it was difficult to test the effect of inhibition of type I IFN signaling on IL-18 production in the absence of AIM2. AIM2-deficient macrophages could secrete a large amount of IL-1β in response to LPS plus nigericin used as a positive control (data not shown). Based on these results, we concluded that upon *S. pneumoniae* infection, AIM2 rather than NLRP3 mediates the production of IL-18 in a manner dependent on type I IFN signaling.

Type I IFN signaling is required for upregulation of AIM2 in *S. pneumoniae* infection. It has been demonstrated that stimulation of macrophages with TLR ligands or proinflammatory cytokines, such as TNF-α, leads to upregulation of NLRP3 through the activation of NF-κB, which is critical for NLRP3 inflammasome activation (36, 37). On the other hand, AIM2 is an IFN-inducible protein (13). Thus, we determined the expression levels of NLRP3 and AIM2 in *S. pneumoniae*-infected macrophages. The expression of AIM2, NLRP3, and IL-6 was strongly increased following infection with *S. pneumoniae* (Fig. 5). In IFNAR1-deficient mac-

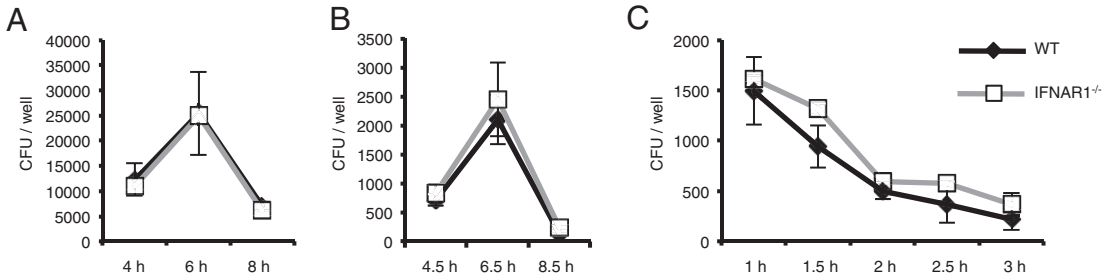

FIG 3 Type I IFN signaling is dispensable for uptake and killing of *S. pneumoniae* by macrophages. Adherent PECs were infected with *S. pneumoniae* D39 at an MOI of 10. (A) Bacteria associating with macrophages were counted at 4, 6, and 8 h after infection as described in Materials and Methods. (B) Phagocytosed bacteria were counted. Non-cell-associated bacteria were removed by washing at 4, 6, and 8 h after infection, and macrophages were cultured for an additional 30 min in the presence of 100 μg/ml of gentamicin. The bacteria in cell lysates were then counted. (C) The viability of phagocytosed bacteria was determined. Gentamicin was added to the cultures 4 h after infection, and the cells were additionally incubated for the indicated times. The bacteria in cell lysates were then counted. All of the experiments were repeated two times, with similar results. Data are presented as the means and standard deviations of triplicate assays.

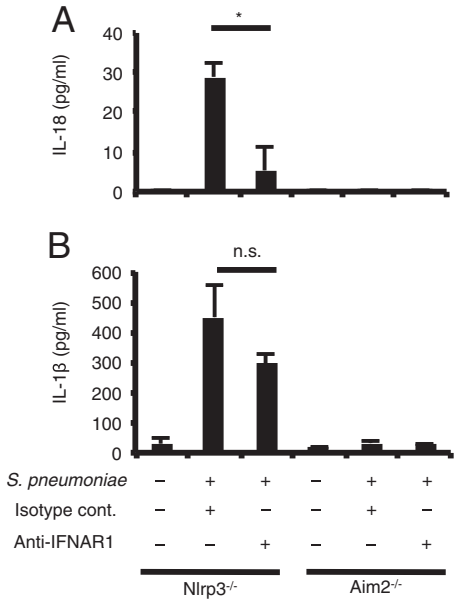

FIG 4 Type I IFN signaling promotes AIM2-mediated IL-18 induction by *S. pneumoniae*. Adherent PECs from NLRP3$^{-/-}$ mice and immortalized AIM2$^{-/-}$ macrophages were left uninfected or infected with *S. pneumoniae* D39 at an MOI of 10. Gentamicin (final concentration 100 μg/ml) was added to cultures 8 h after infection, and culture supernatants were collected after an additional 16 h of incubation (24 h after infection). Anti-IFNAR1 blocking MAb (10 μg/ml) or isotype control Ab (10 μg/ml) was added to the cultures at the same time as *S. pneumoniae*. Levels of IL-18 (A) and IL-1β (B) were determined by ELISA. All of the experiments were repeated two times, with similar results. Data are presented as the means and standard deviations of triplicate assays. Statistical significance was determined by one-way ANOVA followed by Bonferroni's test. *, $P < 0.05$; n.s., not significant.

rophages, the increase in AIM2 expression was impaired (Fig. 5A), whereas expression levels of NLRP3 and IL-6 were almost comparable to those in WT macrophages (Fig. 5B and C). Therefore, type I IFN signaling appears to be required for upregulation of AIM2 during *S. pneumoniae* infection.

Type I IFN signaling facilitates IL-18 production in pneumococcal pneumonia *in vivo*. Finally, we examined the involvement of type I IFN signaling in cytokine production in pneumococcal pneumonia *in vivo*. Levels of IL-18, IL-1β, and IL-6 in BALF were significantly elevated after intranasal infection with *S. pneumoniae* (Fig. 6A to C). Although the concentrations of IL-1β and IL-6 were not reduced by the absence of IFNAR1 (Fig. 6B and C), IL-18 levels were significantly lower in IFNAR1-deficient mice than in WT mice (Fig. 6A). There was no significant difference between WT and IFNAR1-deficient mice in the number of monocytes/macrophages and neutrophils, which are possible sources of IL-18, in BALF after pneumococcal infection (Fig. 6D and E). In conclusion, consistent with the above *in vitro* observations, type I IFN signaling appears to be important for IL-18 production in *S. pneumoniae* infection *in vivo*.

DISCUSSION

Type I IFNs are well-known antiviral mediators (23). They also have anti-inflammatory effects and are widely used to control autoimmune diseases. In addition, type I IFNs have recently been suggested to have functions in bacterial infections (24). In earlier studies, type I IFN signaling had positive or negative effects on host resistance and chemokine responses to *S. pneumoniae* (16, 20, 25–27). In our present study, we found that type I IFN signaling contributes to the activation of caspase-1 and production of IL-18 in response to *S. pneumoniae*. Considering the importance of this inflammatory response in the control and pathology of pneumococcal infections, our findings would be helpful to better understand host-pathogen interactions in pneumococcal diseases.

Type I IFN signaling has been reported to contribute to AIM2 inflammasome activation induced by *Francisella tularensis*, *Listeria monocytogenes*, or nonvirulent mycobacteria (38–41). We previously showed that *L. monocytogenes* induced the upregulation of AIM2 in an IFNAR1-dependent manner (42). One study suggested that upon *F. tularensis* infection, type I IFN signaling leads to upregulation of AIM2, thereby enhancing the activation of the AIM2 inflammasome (40), whereas another study proposed type I IFN signaling enhances *F. tularensis*-induced AIM2 inflammasome activation by promoting the killing of engulfed bacteria to generate the cytosolic DNA rather than by upregulating AIM2 expression (38). We observed that the lack of IFNAR1 led to an impaired upregulation of AIM2, but not to defective uptake and killing of pneumococci, in *S. pneumoniae*-infected macrophages. Judging from the results, it is most likely that type I IFNs enhance the activation of the AIM2 inflammasome through upregulation of AIM2 during *S. pneumoniae* infection.

A previous study has suggested that type I IFNs suppress the expression of pro-IL-1β (35). In agreement with this, the protein

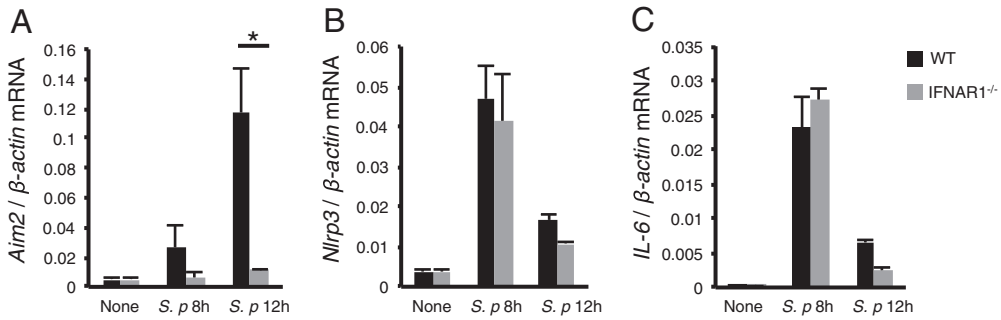

FIG 5 Type I IFN signaling is required for upregulation of AIM2 during *S. pneumoniae* infection. Adherent PECs from WT and IFNAR1$^{-/-}$ mice were infected with *S. pneumoniae* D39 at an MOI of 10 for 8 h or 12 h, and the expression of *Aim2* (A), *Nlrp3* (B), and *Il6* (C) was analyzed by real-time RT-PCR. The target mRNA concentrations were normalized to β-actin mRNA concentrations. All of the experiments were repeated three times, with similar results. Data are presented as the means and standard deviations of triplicate assays. Tests for statistical significance were performed by using the Student *t* test. *, $P < 0.05$.

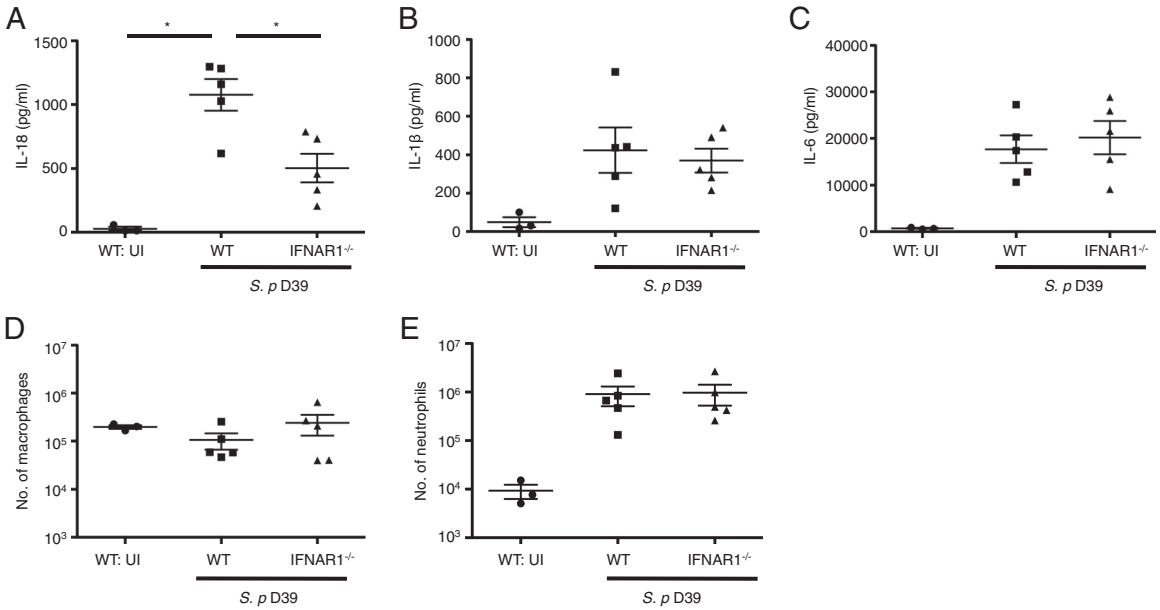

FIG 6 Type I IFN signaling facilitates IL-18 production in pneumococcal pneumonia *in vivo*. WT and IFNAR1$^{-/-}$ mice were left uninfected or intranasally challenged with *S. pneumoniae* D39, BALF was collected from the mice 12 h after infection, and the levels of IL-18 (A), IL-1β (B), and IL-6 (C) were measured by ELISA. The numbers of macrophages (D) and neutrophils (E) in each BALF sample were determined by cell counting and flow cytometric analysis. Tests for statistical significance were performed by using one-way ANOVA followed by Bonferroni's test. *, $P < 0.05$.

level of pro-IL-1β was higher in IFNAR1-deficient macrophages than in WT macrophages after *S. pneumoniae* infection. One may speculate that an increase in the amount of pro-IL-1β leads to an increase in its cleaved form when in the presence of active caspase-1. However, IL-1β production in response to *S. pneumoniae* was not enhanced but marginally reduced in the absence of IFNAR1, which is probably a reflection of diminished caspase-1 activation under this condition. The elevation of IL-1β in BALF after *S. pneumoniae* infection was not affected by deficiency of IFNAR1, despite a significant reduction in BALF IL-18 levels in the absence of IFNAR1. This might be due to an increase in pro-IL-1β expression in the absence of IFNAR1. Another possibility is that IL-1β might be produced by an inflammasome-independent mechanism, which does not require type I IFN signaling, in pneumococcal pneumonia. Indeed, in our recent experiments, BALF IL-18 levels, but not BALF IL-1β levels, were elevated in a caspase-1-dependent manner after intranasal infection with *S. pneumoniae* (R. Fang and K. Tsuchiya, unpublished data). Taken together, our results show that type I IFN signaling appears to differentially regulate the production of IL-18 and IL-1β upon *S. pneumoniae* infection.

IL-18 and IL-1β have been reported to be protective against pneumococcal pneumonia (28, 29, 32, 33, 43). In earlier studies, IL-18 was shown to promote the clearance of *S. pneumoniae* in a nasopharyngeal colonization model and pneumonia models (29, 43). Thus, the type I IFN enhancement of AIM2-mediated IL-18 production may play a protective role in host defense against pneumococcal respiratory tract infections. Consistently, both IFNAR and IL-18 have been shown to contribute to the clearance of *S. pneumoniae* from the nasopharynx in colonization models (20, 43). However, several studies have suggested that in some situations, type I IFN signaling impairs host defense against pneumococcal pneumonia. Importantly, type I IFN signaling not only regulates the AIM2 inflammasome but also has effects on other innate immune responses to *S. pneumoniae*, including the inhibition of the production of chemokines involved in optimal protection (26, 27). It is therefore conceivable that type I IFNs may exacerbate pneumococcal pneumonia through affecting chemokine production and/or other mechanisms despite their role in enhancing AIM2 inflammasome activation. Alternatively, enhanced activation of the AIM2 inflammasome in the lung may be detrimental to the host, for example, because of increased cell death due to pyroptosis, among other reasons (14, 15). In contrast to pneumonia models, deficiency or blockade of IL-18 ameliorated pathology in pneumococcal meningitis models, with or without reduced bacterial outgrowth in the brain (44, 45), suggesting that IL-18 plays a host-detrimental role in *S. pneumoniae* meningitis. IL-18 is an IFN-γ-inducing cytokine. It was proposed that IL-18-stimulated IFN-γ production by NK cells contributes to pathogenesis in pneumococcal meningitis (46). Accordingly, IL-18 is a key cytokine when considering pathology of and host defense against *S. pneumoniae* infections, and thus, type I IFN signaling may influence the outcome of pneumococcal diseases by enhancing inflammasome activation and IL-18 production.

In this study, we found that type I IFN signaling regulates caspase-1 activation and IL-18 production during *S. pneumoniae* infection. Our results suggest a novel role for type I IFNs in an inflammasome-mediated response to *S. pneumoniae*, providing an insight into the cytokine network that is involved in the control and pathology of pneumococcal infections.

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