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AIM2 inflammasome is dispensable for the host defense against *Pseudomonas aeruginosa* infection

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Abstract

Respiratory tract infection with *Pseudomonas aeruginosa* is a major cause of hospital-acquired pneumonia in immune-compromised individuals. Lung infection with *P. aeruginosa* is often associated with production of various inflammatory cytokines including IL-1β. Production of IL-1β requires proteolytic cleavage by a multiprotein complex termed inflammasome. AIM2 inflammasome recognizes foreign cytosolic double stranded DNA. A role of AIM2 in *P. aeruginosa* infection has not been reported previously. In this study, we found that *P. aeruginosa* infection induced degradation of AIM2 protein in macrophages and induction of AIM2 mRNA expression in macrophages and in the lung of mice. Interestingly, *P. aeruginosa* infection induced a similar level of IL-1β, IL-6 and TNF production in wild-type and AIM2-deficient mice. Similarly, no significant differences in bacterial clearance, neutrophil infiltration and NF-κB activation were observed between wild-type and AIM2-deficient mice following *P. aeruginosa* lung infection. Our data suggest that AIM2 inflammasome is dispensable for the host defense against *P. aeruginosa* infection.

Key words: *Pseudomonas aeruginosa*, AIM2 inflammasome, respiratory infection, macrophage.

Introduction

Pseudomonas aeruginosa, an opportunistic Gram-negative bacterium, is an important cause of infection among immunocompromised individuals (1). *P. aeruginosa* chronically infects cystic fibrosis (CF) patients, leading to declined pulmonary function and increased morbidity and mortality (2). *P. aeruginosa* infection in the airway triggers excessive production of various cytokines and chemokines including IL-1β (3). The levels of IL-1β are significantly increased in the bronchoalveolar lavage fluid and sputum of cystic fibrosis patients, and in mouse lung following *P. aeruginosa* infection (4, 5).

Production of mature form of IL-1β requires proteolytic cleavage of its inactive precursor (6). This proteolytic cleavage is tightly controlled by caspase-1, a cysteine protease in cytosol. Activation of caspase-1 is regulated by multiprotein complexes termed inflammasomes, which consist of members of nucleotide binding and oligomerization domain (NOD)-like receptors (NLR) family or pyrin and HIN domain-containing protein (PYHIN) family member absent in melanoma-2 (AIM2) (6-8). NLRs and AIM2 are cytoplasmic pattern recognition receptors (PRRs) that detect microbial molecules and endogenous danger signals as intracellular sensors. Once activated they rapidly assemble into inflammasomes and recruit procaspase-1 for cleavage and activation (9). The NLR family CARD domain–containing protein 4 (NLRC4) inflammasome has been shown contributing to IL-1β production in response to *P. aeruginosa* infection (5, 10-12). However, cells from animals with homozygous deletion of NLR family members NLRC4, or NLRP3 produced similar levels of IL-1β in response to *P. aeruginosa* pilin stimulation as the wild-type cells did (13). Thus, additional members of inflammasome may also contribute to *P. aeruginosa*-mediated IL-1β production.

AIM2 is a cytosolic double-stranded DNA (dsDNA) sensor essential for innate immune responses against DNA viruses and bacteria (14-20). It is composed of an N-terminal pyrin domain (PYD) associated with adaptor protein ASC for caspase-1 recruitment and a C-terminal haematopoietic interferon-inducible nuclear protein 200 (HIN200) domain, which is responsible for binding to cytosolic dsDNA (6). Upon binding to dsDNA, AIM2 releases from an auto-inhibited state and assembles into a large inflammasome complex with ASC and procaspase-1 (21). However, the role of AIM2 inflammasome in the innate immune responses during *P. aeruginosa* infection remains undefined. Given the ability of *P. aeruginosa* to reside and replicate within host cells (22), and the important role of AIM2 inflammasome in the activation of IL-1β during host defense against various intracellular bacteria (14-20), it is possible that the AIM2 inflammasome is involved in mediation of inflammatory responses during *P. aeruginosa* lung infection.

In this study, we found that *P. aeruginosa* infection mediates degradation of AIM2 protein and stimulation of AIM2 mRNA expression, suggesting a potential involvement of AIM2 in *P. aeruginosa* infection. Surprisingly, AIM2-deficient mice showed no defect in bacterial clearance, neutrophil infiltration, IL-1β production and transcription factor NF-κB activation in the lung following *P. aeruginosa* infection. These findings suggest that there may be functional redundancy in inflam-

masome-mediated caspase-1 activation and IL-1β production.

Materials and methods

Ethic statement

Animal protocols for this study were approved by the University Committee on Laboratory Animals, Dalhousie University (permit number: 13-115), in accordance with the guidelines of the Canadian Council on Animal Care. Animals were housed in specific pathogen free facilities, and anesthetized with ketamine to minimize suffering during relevant procedures.

Animals

AIM2-deficient ($AIM2^{-/-}$) mice on the C57BL/6 background were purchased from Jackson Laboratory**.** C57BL/6 mice were purchased from Charles River Laboratories and used as wild-type controls. $AIM2^{-/-}$ mice were age- and sex- matched with C57BL/6 mice.

Antibodies

Abs to AIM2 (13095) was purchased from Cell Signaling Technology (Beverly, MA). Abs to actin (sc1616) and all HRP-linked secondary Abs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Abs to caspase-1 (p20) (AG-20B-0042) was purchased from AdipoGen (San Diego, CA).

Bacterial preparation and macrophage activation

P. aeruginosa strain 8821, a gift from Dr. A. Chakrabarty (University of Illinois, Chicago, IL, USA), was a mucoid strain isolated from a cystic fibrosis patient (23). P. aeruginosa was cultured as described previously (24). Briefly, suspension culture was grown until reaching the early stationary phase. Bacteria were washed in phosphate buffer solution (PBS) and resuspended in saline for *in vivo* studies or PBS for *in vitro* macrophage activation experiments.

Bone marrow-derived macrophages (BMMs) were used for *in vitro* study. Bone marrow cells were flushed from C57BL/6 or $AIM2^{-/-}$ mice femurs and tibias and cultured in complete medium (DMEM medium containing 10% FBS, 1% Penicillin/Strep and M-CSF from supernatant of L929). Cells were cultured for 7 days until they are mature, and the medium was replaced with antibiotic-free DMEM medium with 10% FBS. Cells were infected with *P. aeruginosa* strain 8821 at the multiplicity of infection (MOI) of 10 or 20 bacteria per macrophage for various time points. After incubation, cell-free supernatants were collected for measuring cytokines by ELISA. Cell pellets were lysed for Western blotting or real-time quantitative PCR.

Cytokine production

The concentrations of IL-1β, IL-6 and TNF in the lungs, bronchoalveolar lavage fluid (BALF) and cell-free supernatants were determined by ELISA as described previously using Ab pairs from R&D System (Minneapolis, MN), and developed with an amplification system (Invitrogen) (25).

Western blotting

Cells were lysed in RIPA buffer supplemented with a cocktail of protease and phosphatase inhibitors. Cells lysates (36 – 42 μg) were subjected to electrophoresis in 12% SDS-polyacrylamide gels. Gels were transferred to polyvinylidene difluoride (PVDF) membrane, blocked with 5% non-fat milk powder, blotted with primary and secondary antibodies, and detected by an ECL detection system (Western Lightning Plus-ECL; PerkinElmer). For detection of caspase-1 p20 in cell supernatants, proteins were precipitated by the addition of an equal volume of methanol and 0.25 volumes of chloroform as described previously (26).

Real-time quantitative PCR

1-2 X 10^6 cells or 100 mg of mouse lung tissues were collected and processed in TRIzol (Invitrogen). The total mRNA was purified using RNeasy kit (Qiagen) and cDNA was synthesized using a reverse transcription system (Clontech). AIM2 primer sequences, Forward 5'- AGCTGAAAACTGCTCTGCTGC -3' and Reverse 5'- AGCACCGTGACAACAAGTGG- 3', were designed by Primer-BLAST (NCBI). According to manufacturer's instruction, RT-PCR arrays were conducted in triplicate and AIM2 mRNA was quantified using syber green method on a sequence detection system (ABI Prism 7000; Applied Biosystems). Hypoxanthine-guanine phosphoribosyltransferase (HPRT) was used as an endogenous reference. Data were analyzed using relative standard curve method according to the manufacturer's protocol.

Lung infection with P. aeruginosa, collection of lung and BALF

Mice were intranasally infected with 1 X 10^7 CFU of P. aeruginosa strain 8821 for 4 h or 1 X 10^9 CFU for 24 h. After 4 h or 24 h infection, BALF was obtained by lavaging the lung with 3 X 1 ml of PBS containing soybean trypsin inhibitor (100 μg/ml). The lung tissues were obtained for detection of cytokines, myeloperoxidase (MPO), bacterial CFU counting and histology.

Lung tissues were homogenized at maximum speed for 10s in 50 mM HEPES buffer (4 μl/mg lung) containing soybean trypsin inhibitor (100 μg/ml). For bacterial CFU counting, 10 μl of homogenate was plated on an agar dish and incubated for 24 h at 37°C. For cytokines and MPO assays, the homogenate was centrifuged at 4°C for 30 min at 18000 X g. The supernatant was stored at -80°C for later cytokine analysis. The pellets were resuspended and homogenized in 0.5% cetyltrimethylammonium chloride (CTAC) (4 μl/mg lung) and centrifuged again at 18000 X g for 30 min. The clear extracts were collected for MPO assay.

BALF (10 μl) was plated on an agar dish and incubated for 24 h at 37°C for CFU counting. For detection of cytokines and MPO assay, BALF was centrifuged at 480 X g for 5 min at 4°C. The supernatants were used for cytokine analysis. The cell pellets were resuspended in 1 ml of NH_4Cl buffer to lyse erythrocytes and centrifuged. The supernatants were discarded. The pellets were resuspended in 0.5% CTAC (250 μl/mouse) and centrifuged again. The clear extracts were collected for MPO assay.

MPO assay

The MPO assay was used to determine the infiltra-

tion of neutrophils into the lungs of the mice as described previously (27). Briefly, samples in duplicate (75 µl) were mixed with equal volumes of the substrate (3,3′,5,5′-tetramethyl-benzidine dihydrochloride, 3 mM; Resorcinol, 120 µM; and H_2O_2, 2.2 mM) for 2 minutes. The reaction was stopped by adding 150 µl of 2 M H_2SO_4. The optical density was measured at 450 nm.

EMSA analysis

A consensus double-stranded NF-κB oligonucleotide (5′-AGTTGAGGGGACTTTCCCAGGC-3′; Promega) was used for EMSA. Nuclear protein extracts were obtained using a nuclear extract kit (Active Motif, Carlsbad, CA), according to the manufacturer's protocol. EMSA performed as previously described (28). Briefly, 10 µg of extracted nuclear protein was added into 10 µl of binding buffer (Promega E358A) with 1 µg of poly(deoxyinosinic-deoxycytidylic acid) (GE healthcare) and incubated for 15 min at room temperature. The double-stranded NF-κB oligonucleotide was ^{32}P-labeled and added into each reaction mixture, which was incubated for 30 min at room temperature and separated by electrophoresis on a 6% polyacrylamide gel in 0.5 X Tris–boric acid–EDTA buffer. Gels were vacuum dried and subjected to autoradiography.

Histology

Mice lungs were fixed in 10% formalin overnight then in 100% ethanol for paraffin embedding and sectioning. Slides were deparaffinized with CitriSolv (Fisher Scientific), and rehydrated through decreasing concentrations of ethanol. Slides were stained with Harris H&E to illustrate lung histology.

Statistics

Data are presented as the mean ± SEM of the indicated number of experiments. Statistical significance was determined by assessing means with ANOVA and the Tukey-Kramer multiple comparison tests, or by using an unpaired t test. Differences were considered significant at $p < 0.05$.

Results

P. aeruginosa infection induces AIM2 protein degradation and stimulates AIM2 mRNA expression

The early host responses to pathogen in airway are mainly mediated by local resident cells. Macrophages have been identified to play an important role in the first line of defense against *P. aeruginosa* in respiratory tract (29, 30). To determine the AIM2 gene expression in macrophages during *P. aeruginosa* infection, macrophages cultured from murine bone marrow were infected with *P. aeruginosa* stain 8821 at a MOI of 1:10 for various times. We first examined the AIM2 mRNA expression in macrophages at 2, 4 or 6 h time points by real-time quantitative PCR. The AIM2 mRNA expression level was found to be increased and reached its highest level around 4 h after *P. aeruginosa* infection (Fig 1A). Similarly, the AIM2 mRNA expression was enhanced in the lung *in vivo* after *P. aeruginosa* lung infection for 4 h (Fig. 1B).

We next assessed the AIM2 protein level in macrophages. Mouse bone marrow derived macrophages were treated with *P. aeruginosa* (MOI of 1: 10) for 1, 2, 4 or 6 h. Cell lysates were subjected to Western blot analysis for AIM2. Surprisingly, the level of AIM2 protein was significantly reduced over the infection time, and it was mostly degraded at 6 h (Fig. 1C and 1D).

AIM2 deficiency does not impair IL-1β production in macrophages following P. aeruginosa infection in vitro

To examine whether AIM2 plays a role in IL-1β production during *P. aeruginosa* infection, AIM2-deficient and wild-type macrophages were treated with *P. aeruginosa* at a MOI of 1:10 for various times up to 24 h. Cell free supernatants were collected for detection of IL-1β production by ELISA. The level of IL-1β started to increase at 3 h. There is no significant difference of IL-1β production between wild-type and AIM2-deficient cells (Fig. 2A). Moreover, we tested additional two cytokines, TNF (Fig. 2B) and IL-6 (Fig. 2C), which are two inflammatory cytokines independent of the inflammasome-mediated activation pathway. Similarly, no significant difference of TNF and IL-6 production was found between wild-type and AIM2-deficient macrophages.

AIM2 deficiency does not impair caspase-1 activation

Assembly of inflammasome complexes leads to acti-

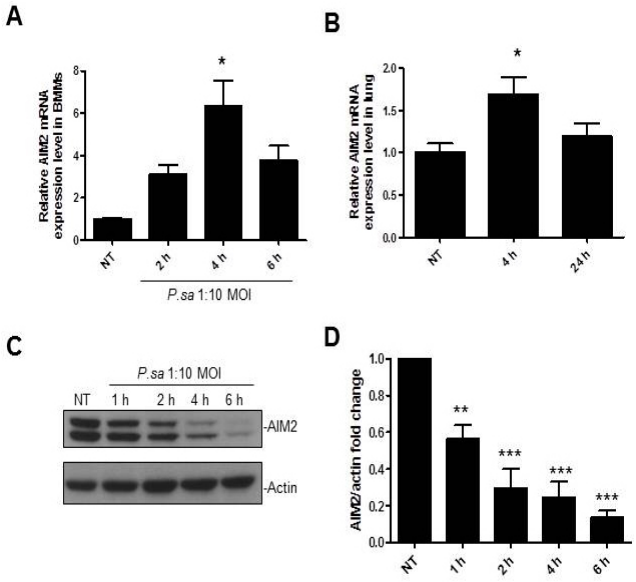

Figure 1. AIM2 mRNA expression is induced, but the AIM2 protein is reduced following *P. aeruginosa* infection. (A) Bone marrow-derived macrophages (BMMs) were infected with *P. aeruginosa* strain 8821 (*P.sa*) at a MOI of 1:10 for 2, 4, 6 h, or left untreated (NT). Total RNA isolated from these cells was reverse transcribed to cDNA and subjected to real-time quantitative PCR for AIM2. The AIM2 gene expression was normalized to endogenous control HPRT (n = 3 ± SEM, *p<0.05). (B) Wild-type (+/+) mice were infected intranasally with 1 X 10^7 (4 h) or 1 X 10^9 (24 h) CFU/mouse *P. aeruginosa* strain 8821 or equivalent volume of saline (NT). AIM2 gene expression in lung tissues was analyzed using real-time quantitative PCR and normalized to HPRT (n=3 ± SEM. *p<0.05). (C) Wild type (+/+) BMMs were infected with *P. aeruginosa* strain 8821 at a MOI of 1:10 for 1, 2, 4, 6 h, or left untreated (NT). Cell lysates were analyzed by Western blotting for AIM2 as well as actin loading control. (D) Scanning densitometry was performed for detecting AIM2 expression and data are expressed as fold change normalized to actin (n = 4 ± SEM, **p<0.01, ***p<0.0001).

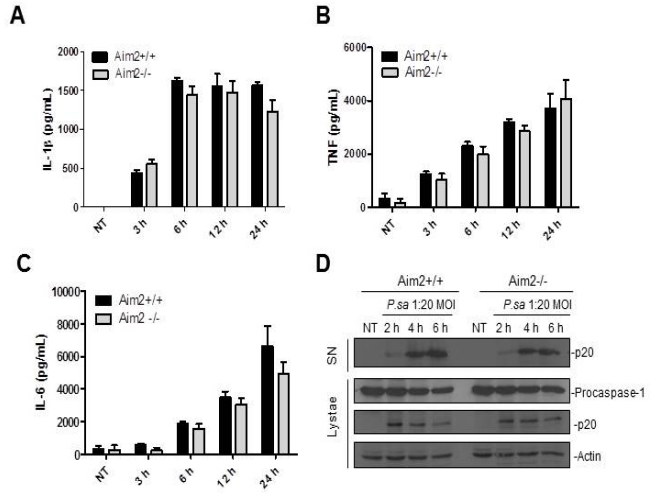

Figure 2. AIM2 deficiency does not significantly impair cytokine production or caspase-1 activation following *P. aeruginosa* infection *in vitro*. Wild type (+/+) and Aim2-deficient (-/-) mouse bone marrow-derived macrophages (BMMs) were infected with *P. aeruginosa* strain 8821 at a MOI of 1:10 for 3, 6, 12 or 24 h , or left untreated (NT). Cell-free supernatants were collected and assessed for IL-1β (A), TNF (B) and IL-6 (C) production by ELISA (n=3 independent experiments). (D) Lysates and cell supernatants were collected from Wild type (+/+) and Aim2-deficient (-/-) BMMs after treatment with *P. aeruginosa* strain 8821 (*P.sa*) at a MOI of 1:20 for 2, 4 or 6 h, or left untreated (NT). Procaspase-1, p20 and actin loading control in the cell lysates and secreted p20 in the cell-free supernatants (SN) were detected by Western blotting. (one representative of 2 independent experiments).

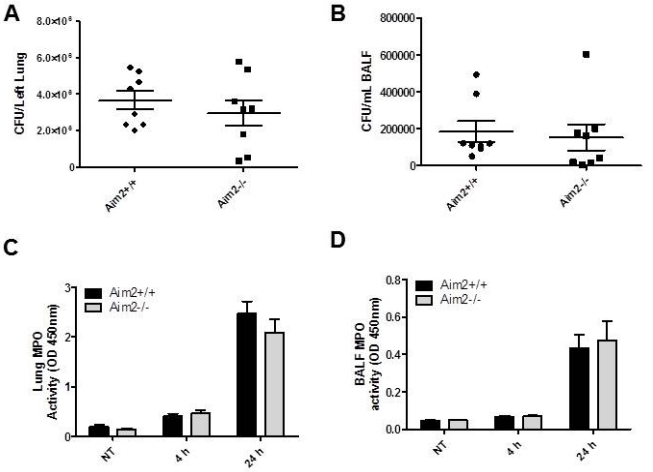

Figure 3. AIM2 deficiency has no effect on bacterial clearance and neutrophil infiltration in lung following *P. aeruginosa* infection. Wild type (+/+) and Aim2-deficient (-/-) mice were intranasally infected with of *P. aeruginosa* strain 8821 (1 X 10^7 CFU/mouse for 4 h or 1 X 10^9 CFU/mouse for 24 h) or treated with saline as a control (NT). Bacterial burden was examined by colony counting for the mice infected with *P. aeruginosa* for 24 h (lung tissues: A and BALF: B). MPO activity was measured in the lung (C) and BALF (D) lysates (n = 8-11 independent experiments).

vation of caspase-1 by cleavage of procaspase-1 into two subunits, p20 and p10 (31). To examine whether AIM2 inflammasome is required for *P. aeruginosa*-induced caspase-1 activation *in vitro*, we set out to detect the activated form of caspase-1 p20 in both wild-type and AIM2-deficient cell lysates and supernatants, as well as its inactive form procaspase-1, by Western blotting. Wild-type and AIM2-deficient macrophages were infected with *P. aeruginosa* stain 8821 at a MOI of 1:20 for 2, 4 or 6 h. The activated caspase-1 p20 was detected in both wild type and AIM2-deficient cell lysates and supernatants in response to *P. aeruginosa* infection. The active caspase-1 subunits have been previously shown to be released into extracellular environment (32-34). The p20 cleavage in cell lysates was found to be induced at 2 h and then decreased up to 6 h, which is in agreement of the increase of p20 in culture supernatants along the infection time (Fig. 2D). This suggests that caspase-1 activation is not impaired in AIM2-deficient macrophages upon *P. aeruginosa* infection.

AIM2-deficiency does not impair the clearance of P. aeruginosa and neutrophil infiltration in vivo

To determine whether AIM2 inflammasome plays a role in the clearance of *P. aeruginosa* and neutrophil infiltration in the airways, wild-type and AIM2-deficient mice were infected intranasally with 1 X 10^7 or 1 X 10^9 CFU/mouse for 4 h or 24 h. Lungs and BALF were collected for detection of bacterial burden by CFU counting (24 h) and neutrophil infiltration by MPO assay (4 and 24 h). The bacterial burden in the lungs and BALF of AIM2-deficient mice were not significantly different to that in wild-type mice at 24 h (Figure 3A and 3B).

Upon pathogen infection, neutrophils are recruited into the infected site (35, 36). We further characterized the neutrophil infiltration into the respiratory tract by measuring the activity of the neutrophil specific MPO. The MPO activity was not significantly impaired in the lungs and BALF of AIM2-deficient mice compared to that in wild type mice at 4 h and 24 h (Figure 3C and 3D). Moreover, lung histology showed similar level of inflammatory cell infiltration in the lung between AIM2-deficient and wild-type mice (Fig. 4). These results suggest that AIM2 deficiency has no effect on bacterial clearance and neutrophil recruitment in the airways following *P. aeruginosa* lung infection *in vivo*.

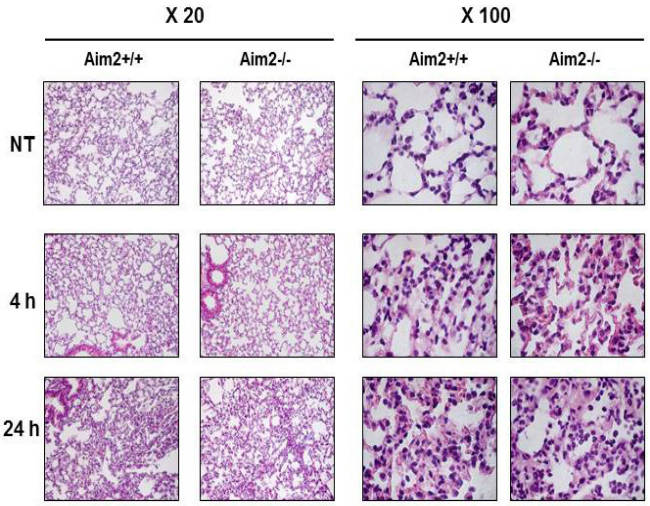

Figure 4. AIM2 deficiency has little effect on inflammatory cell infiltration into the airways following *P. aeruginosa* lung infection. Wild type (+/+) and Aim2-deficient (Aim2-/-) mice were intranasally infected with *P. aeruginosa* strain 8821 (1 X 10^7 CFU/mouse for 4 h or 1 X 10^9 CFU/mouse for 24 h) or treated with saline as a control (NT). The upper lobe of the left lung was collected for H&E staining (original magnification X 20 or X 100). Pictures are representative of 6 mice from each group.

AIM2 deficiency does not impair IL-1β production and NF-κB activation in response to P. aeruginosa lung infection in vivo

Synthesis of pro-IL-1β is primarily regulated by the transcription factor NF-κB (37). To determine a role for AIM2 inflammasome in cytokine production following *P. aeruginosa* lung infection *in vivo*, the lung and BALF supernatants were collected for determination of IL-1β, TNF and IL-6 by ELISA after 4 or 24 h infection. IL-1β (Fig. 5A and 5B), TNF (Fig. 5C and 5D) and IL-6 (Fig. 5E and 5F) in lungs and BALF were induced as early as 4 h and significantly increased at 24 h. However, no statistically significant difference was found in these cytokines between wild-type and AIM2-deficient mice. Consistent with this finding, *P. aeruginosa*-induced activation of NF-κB in the lungs of AIM2-deficient mice was not impaired compared to that in wild-type mice as measured by EMSA (Fig. 6A and B). These findings suggest that AIM2 deficiency has no significant effect on transcription factor NF-κB activation and its downstream cytokine production following *P. aeruginosa* lung infection.

Discussion

P. aeruginosa infection impairs lung function which often is associated with excessive production of cytokines and chemokines including IL-1β in immunocompromised individuals and CF patients (1, 2). Previous studies have revealed that host immune system employs the two types of pattern recognition receptors, the TLRs and NLRs, to recognize the invading *P. aeruginosa* (5, 10-12, 38). We and others have demonstrated the involvement of several cell membrane-associated TLRs including TLR2, 4, 5, 6 in the recognition of the extracellular *P. aeruginosa* (24, 38-40). The NLRs are cytoplasmic receptors that are responsible for detecting

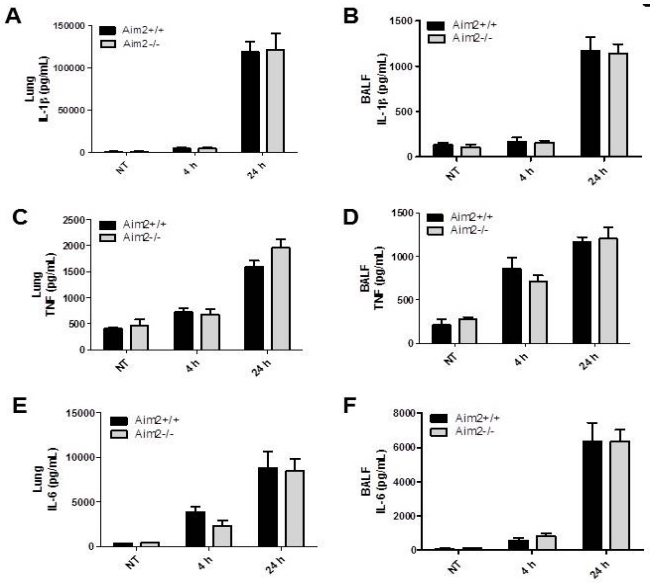

Figure 5. AIM2 deficiency does not significantly impair cytokine production following *P. aeruginosa* lung infection *in vivo*. Wild type (+/+) and AIM2-deficient (-/-) mice were infected intranasally with 1 X 10^7 (4 h) or 1 X 10^9 (24 h) CFU/mouse *P. aeruginosa* strain 8821 or equivalent volume of saline (NT). After 4 or 24 h, lung tissues and BALF were collected for the determination of IL-1β (A, B), TNF (C, D) and IL-6 (E, F) by ELISA (n = 8-11 independent experiments).

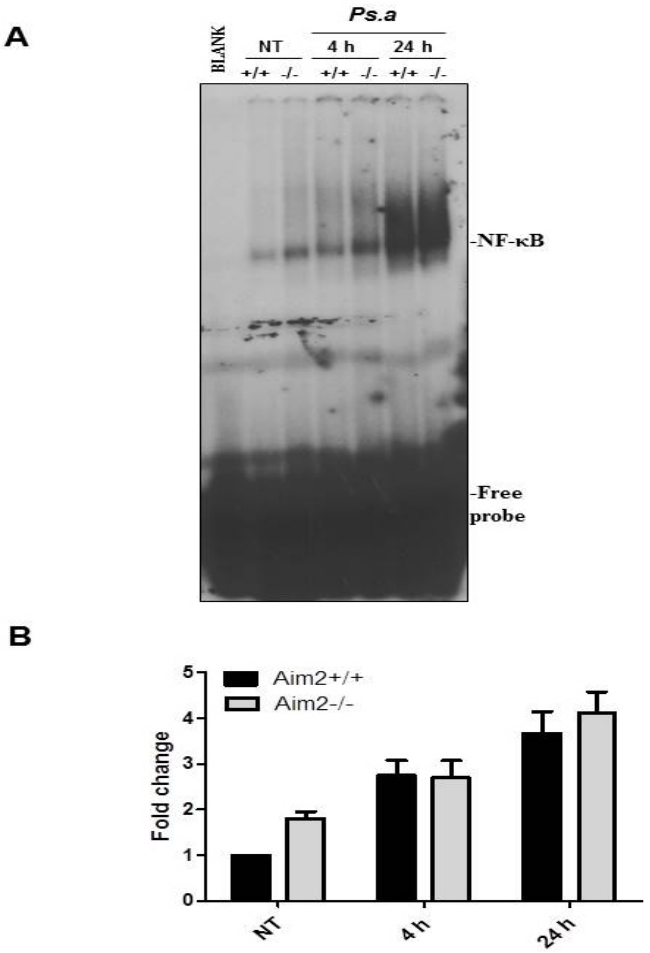

Figure 6. Aim2-deficiency does not significantly affect NF-κB activation *in vivo* following *P. aeruginosa* infection. Wild type (+/+) and Aim2-deficient (-/-) mice were infected intranasally with 1 X 10^7 (4 h) or 1 X 10^9 (24 h) CFU/mouse *P. aeruginosa* strain 8821 or equivalent volume of saline (NT). Nuclear proteins were extracted from long tissues and subjected to EMSA by incubation with ^{32}P-labeled NF-κB DNA probes (A). Scan densitometry was performed for analysis (B), and data are expressed as fold change versus wild-type untreated lung (n = 6 independent experiments).

the *P. aeruginosa* inside cells (9, 38). Activation of the TLRs or NLRs triggers downstream signaling pathways leading to the production of cytokines and chemokines. Specifically, the expression of inactive pro-form of the proinflammatory cytokine IL-1β is regulated by TLR pathways, and the pro-IL-1β were subsequently cleaved by inflammasome, which consists of activated NLRs (41, 42). NLRC4 inflammsome has been found to recognize *P. aeruginosa* and mediates IL-1β production (10). However, a recent report showed that *P. aeruginosa* pillin was able to trigger the inflammasome-mediated immune responses in a NLRC4-independent manner (13), suggesting that additional mechanisms may be involved in the recognition of *P. aeruginosa*. In this study, we examined the role of AIM2 inflammasome in host response to *P. aeruginosa* infection *in vitro* and *in vivo*. Unexpectedly, our data suggest that AIM2 is not required for *P. aeruginosa*-mediated IL-1β production.

AIM2 mRNA expression was increased following *P. aeruginosa* infection. In contrast, the AIM2 protein level was reduced in macrophages, suggesting the involvement of AIM2 in *P. aeruginosa* infection. However, how and why AIM2 is degraded remains to be determined. It has been well recognized that inflammasome activity

needs to be tightly controlled by host immune system to avoid overproduction of cytokines and excessive pyroptotic cell death (43, 44). To achieve this, host regulatory mechanisms down-regulate the inflammasome activity by inhibition or degradation of inflammasome (45-51). A host regulatory process termed autophagy may be involved in AIM2 degradation following *P .aeruginosa* infection. Autophagy is an evolutionarily conserved process that degrades and recycles cellular organelles and long-lived proteins in eukaryotic cells (52, 53). A study has shown that induction of AIM2 inflammasome triggers autophagosome formation and increased autophagy activity leading to destruction of AIM2 through ubiquitination (51). We recently showed that autophagy is induced by *P .aeruginosa* infection (54). It is possible that autophagy accompanies inflammasome activation to temper inflammation by eliminating active inflammasomes.

AIM2 inflammasome senses the dsDNA released from microbes in host cytosol (6). AIM2 has been found to be activated in dendritic cells and macrophages upon sensing several intracellular pathogens, including cytosolic bacterial pathogen *Francisella tularensis*, *Listeria monocytogenes*, *Porphyromonas gingivalis*, *Mycobacterium bovis* and human papillomaviruses (HPV) (16-20). AIM2 deficiency results in impaired IL-1β production and caspase-1 activation following infection by these pathogens (16-20). Given the ability of *P. aeruginosa* to reside and replicate within host cell, we examined whether AIM2 inflammasome was activated by *P. aeruginosa*. No impairment of IL-1β production and caspase-1 activation was found in AIM2-deficient macrophages. Similarly, the level of IL-1β secretion in the lungs and BALF of AIM2-deficient mice was not significantly impaired *in vivo* as well. Thus, *P. aeruginosa* possess unique property to induce IL-1β production and caspase-1 activation which is independent of AIM2.

The synthesis of pro-IL-1β is regulated by transcription factor NF-κB through the TLRs signaling pathway (37, 38, 41). Once the TLRs are activated by *P. aeruginosa*, a MyD88-dependent downstream signaling is triggered, leading to activation of NF-κB. The liberated NF-κB then moves into nucleus and activates NF-κB–mediated proinflammatory gene expression (37). No difference of NF-κB activation was found between AIM2-deficient and wild-type mice following *P. aeruginosa*, suggesting that TLR-NF-κB pathway was not affected by AIM2 deficiency. Previous studies have shown that inflammasome deficiency displays enhanced bacterial burden and attenuated neutrophil infiltration in airways in response to respiratory pathogen infection (10, 11). However, our results show that the bacterial clearance and neutrophil infiltration in the airways were not significantly impaired in AIM2-deficienct mice compared to wild-type mice. Accordingly, our findings from both *in vitro* and *in vivo* studies suggest that AIM2 inflammsome is functionally redundant in the inflammasome-mediated caspase-1 activation and IL-1β production during *P. aeruginosa* infection.

Author Contributions

Conceived and designed the experiments: ZP and TJL. Performed the experiments: ZP. Analyzed the data: ZP, GS, RDJ and TJL. Wrote the paper: ZP, GS, and TJL.

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