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Critical role of AIM2 in *Mycobacterium tuberculosis* infection

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Abstract

Absent in melanoma 2 (AIM2) is a sensor of cytosolic DNA that is responsible for activation of the inflammasome and host immune responses to DNA viruses and intracellular bacteria. However, the role of AIM2 in host defenses against *Mycobacterium tuberculosis* is unknown. Here, we show that AIM2-deficient mice were highly susceptible to intratracheal infection with *M. tuberculosis* and that this was associated with defective IL-1β and IL-18 production together with impaired T_h1 responses. Macrophages from AIM2-deficient mice infected with *M. tuberculosis* showed severely impaired secretion of IL-1β and IL-18 as well as activation of the inflammasome, determined by caspase-1 cleavage. Genomic DNA extracted from *M. tuberculosis* (Mtb DNA) induced caspase-1 activation and IL-1β/IL-18 secretion in an AIM2-dependent manner. Mtb DNA, which was present in the cytosol, co-localized with AIM2. Taken together, these findings demonstrate that AIM2 plays an important role in *M. tuberculosis* infection through the recognition of Mtb DNA.

Keywords: host defense, inflammasome, macrophages

Introduction

Tuberculosis is caused by *Mycobacterium tuberculosis* and is a serious disease worldwide causing about 2 million deaths each year. The risk of disease is increased by the emergence of acquired immune deficiency syndrome and multidrug-resistant mycobacteria (1). *Mycobacterium tuberculosis* mainly invades and parasitizes macrophages by inhibiting phagosome maturation into phagolysosomes. Macrophages have several recognition systems to defend against mycobacterial invasion. Toll-like receptors (TLRs) recognize mycobacterial components such as glycolipids and CpG motif DNA (2–4). Several recent findings have also indicated that pattern recognition receptors other than TLRs, such as C-type lectin receptors and NOD-like receptors (NLRs), are implicated in the innate recognition of mycobacterial components (5–10). TLRs and C-type lectin receptors are membrane-bound molecules recognizing mycobacterial components in the extracellular compartments, whereas NLRs are present in the cytosol. Thus, several pattern recognition receptors showing distinct subcellular expression patterns recognize structurally and functionally different components of mycobacteria, contributing to protection by evoking an immune response.

Among the NLR family of proteins, NLR pyrin domain containing 3 (NLRP3) is known to activate the inflammasome, a multi-protein platform leading to the processing of the IL-1 family of cytokines (11–14). NLRP3 inflammasome, which is composed of NLRP3, adaptor protein Apoptosis-associated speck-like protein containing a CARD (ASC) and caspase-1, is activated by mycobacteria (15–18). Following activation, inactive caspase-1 is processed by autocleavage via ASC and is converted into the active form of caspase-1 containing

10 kDa/20 kDa subunits. The active form of caspase-1 then cleaves pro-IL-1β and pro-IL-18 into mature forms of IL-1β and IL-18, respectively. The IL-1 family of cytokines, including IL-1β and IL-18, possess potent pro-inflammatory activities (19–21) and are responsible for the host defense against mycobacteria (22–29). However, several studies suggest that NLRP3 or caspase-1, which mediates the processing of the IL-1 family of cytokines, is not essential for the induction of protective immunity to *M. tuberculosis in vivo* (25, 26, 30–32). Thus, the signaling pathway leading to IL-1β/IL-18 production in mycobacterial infection remains controversial.

Recent studies identified that AIM2 (absent in melanoma 2), which possesses HIN-200 and pyrin domains, recognizes cytosolic DNA leading to activation of the inflammasome and secretion of IL-1β and IL-18 (33–36). Several studies have demonstrated that AIM2 is mandatory for the host defense against DNA viruses (Vaccinia virus and mouse cytomegalovirus) and intracellular bacteria (*Francisella tularensis* and *Listeria monocytogenes*) (37–41). However, the role of AIM2-dependent inflammasome activation in mycobacterial infection remains unknown.

In this study, we analyzed the role of AIM2 in mycobacterial infection. AIM2-deficient mice were highly sensitive to *M. tuberculosis* infection. AIM2-deficient macrophages showed impaired activation of the inflammasome and defective production of IL-1β and IL-18 after *M. tuberculosis* infection. Genomic DNA from *M. tuberculosis* was present within the cytosol after infection and induced activation of the inflammasome in an AIM2-dependent manner. These findings demonstrate the critical role of AIM2 in *M. tuberculosis* infection.

Methods

Mice and bacteria

The *Aim2* gene was isolated from genomic DNA extracted from embryonic stem cells (V6.5) by PCR using Elongase Enzyme Mix (Invitrogen). The targeting vector was constructed by replacing a 2.5-kb fragment encoding the exons of *Aim2* with a neomycin-resistance gene cassette and a herpes simplex virus thymidine kinase gene driven by the PGK promoter for negative selection. After transfection of the targeting vector into embryonic stem cells, colonies resistant to both G418 and ganciclovir were selected and screened by PCR and Southern blot. Homologous recombinants were microinjected into blastocysts of C57BL/6 female mice, and heterozygous F1 progenies were intercrossed to obtain AIM2-deficient mice. AIM2-deficient mice and their wild-type littermates from these intercrosses were used and all animal experiments were conducted with the approval of the Animal Research Committee of the Graduate School of Medicine at Osaka University.

Mycobacterium tuberculosis strain H37Rv (ATCC358121) was grown in Middlebrook 7H9-ADC medium for 2 weeks and stored at −80°C until use.

In vivo infection of mice

Mice were intratracheally infected with *M. tuberculosis* H37Rv (1×10^6 CFU per mouse). At 4 weeks after infection, homogenates of the lungs and livers were plated onto 7H10-OADC agar. For histological analysis, the lungs were fixed with 4% PFA, embedded in paraffin, cut into sections, and stained with hematoxylin and eosin or by the Ziehl–Neelsen method.

Harvest of BALF, blood and cells from tissues

Bronchoalveolar lavage fluid (BALF) was collected from uninfected and infected mice by washing the lung airways with phosphate buffered saline (PBS) at 3 weeks post-infection, and blood was collected from the hearts of uninfected and infected mice. $CD4^+$ T cells (4×10^5 cells) were isolated from the spleens at 3 weeks after infection, and then were stimulated with PPD (2 μg ml^{-1}; Japan BCG Laboratory) in the presence of APC (4×10^5 cells) for 48 h.

Mycobacterium tuberculosis genomic DNA extraction

Mycobacterium tuberculosis (1×10^9 CFU) was homogenized with glass beads (0.1 mm; ASONE) and proteins were removed using Phenol/Chloroform/Isoamyl alcohol and Chloroform/Isoamyl alcohol. Genomic DNA was precipitated using isopropanol.

Cell culture and stimulation

Mice were intraperitoneally injected with 4% thioglycollate (Sigma) and 3 days later macrophages were isolated from the peritoneal cavity. Cells were stimulated with LPS (200 ng ml^{-1}) for 3 h and then transfected with poly(dA:dT) (Sigma) or *M. tuberculosis* DNA using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen). Peritoneal macrophages were infected with *M. tuberculosis* (MOI of 3) for 6 h. Cells were washed three times with PBS and then incubated for 24 h.

ELISA

The concentration of IFN-γ, IL-1β or IL-12p40 in culture supernatants was measured by ELISA according to the manufacturer's instructions (R&D Systems). The ELISA kit for IL-18 was purchased from Medical & Biological Laboratories.

Immuno-precipitation and immuno-blot analysis

Supernatants were precleared with protein G–Sepharose (GE Healthcare), incubated with anti-caspase-1 p10 rabbit antibody (2 μg; Santa Cruz Biotechnology). Cell pellets were lysed in lysis buffer (1% Nonidet P-40, 150 mM NaCl and 50 mM Tris-HCl, pH 7.5) and together with the immuno-precipitants, separated on SDS-PAGE and transferred to PVDF membranes (Millipore). The membranes were incubated with anti-caspase-1 p10 antibody (1:200) and anti-β-actin antibody (1:500; Sigma). Bound antibody was detected with SuperSignal West Dura Extended Duration Substrate (Thermo).

Immuno-fluorescence analysis

Mycobacterium tuberculosis genomic DNA was labeled with Hoechst 33342 (100 ng ml^{-1}; Invitrogen) by incubation for 24 h. Hoechst-labeled *M. tuberculosis* was washed five times with PBS before use. RAW264.7 cells were infected with Hoechst-labeled *M. tuberculosis* (MOI of 3) for 6 h, washed three times with PBS and then incubated for 24 h. Cells were fixed with 4% PFA and permeabilized with 0.4% saponin. The cells were incubated with rabbit anti-Rab7 antibody (1:200; Santa Cruz Biotechnology) or rabbit anti-AIM2 antibody (1:200; Santa Cruz Biotechnology) or rat anti-LAMP1 antibody (1:200; BD Biosciences) for 1 h at room temperature. Cells

were then incubated with Alexa 488 anti-rabbit IgG antibody (Invitrogen) or Alexa 594 anti-rat IgG antibody (Invitrogen) for 40 min at room temperature. The immuno-stained cells were mounted with ProLong Gold antifade reagent (Invitrogen) on glass slides and analyzed using a fluorescence microscope (FV1000-D IX81; Olympus).

Statistical analysis

Differences between control and experimental groups were evaluated using Student's *t* test. Values of $P < 0.05$ were considered to indicate statistical significance.

Results

Aim2$^{-/-}$ mice are highly susceptible to Mycobacterium tuberculosis infection

To assess the *in vivo* role of AIM2, we generated AIM2-deficient mice by gene targeting (Supplementary Figure 1A is available at *International Immunology* Online), which was confirmed by Southern and northern blot analyses (Supplementary Figure 1B and C is available at *International Immunology* Online). *Aim2$^{-/-}$* mice were born at normal Mendelian ratios, developed normally and showed no apparent abnormalities when housed in our specific pathogen-free facility. Several previous studies demonstrated that AIM2 recognizes cytosolic DNA, leading to caspase-1 activation and subsequent processing of the IL-1 family of cytokines, such as IL-1β and IL-18 (33–36, 39). Therefore, we first analyzed the response to synthetic B-form double-stranded DNA [poly(dA:dT)] in *Aim2$^{-/-}$* macrophages. Peritoneal macrophages were collected from wild-type and *Aim2$^{-/-}$* mice and then transfected with poly(dA:dT) into the cytosol after priming with LPS. In immuno-blot analysis, a 10 kDa active form of caspase-1 (p10) was detected in wild-type macrophages stimulated with poly(dA:dT). In contrast, the cleaved p10 form of caspase-1 was not detected in *Aim2$^{-/-}$* macrophages (Supplementary Figure 2A is available at *International Immunology* Online). In addition, poly(dA:dT)-induced secretion of IL-1β and IL-18 into the culture supernatants was markedly decreased in *Aim2$^{-/-}$* macrophages, although ATP-induced secretion of IL-1β was normally observed (Supplementary Figure 2B is available at *International Immunology* Online). Thus, in accordance with previous reports, *Aim2$^{-/-}$* mice showed defective DNA-induced activation of the inflammasome.

We examined the involvement of AIM2 in mycobacterial infection using these *Aim2$^{-/-}$* mice. Wild-type and *Aim2$^{-/-}$* mice were intratracheally infected with the virulent H37Rv strain of *M. tuberculosis* and monitored for their survival (Fig. 1A). All *M. tuberculosis*-infected wild-type mice survived to at least 8 weeks post-infection. In contrast, all *Aim2$^{-/-}$* mice died within 7 weeks of infection with *M. tuberculosis*. We also assessed bacterial burdens in the lungs and livers at 4 weeks post-infection (Fig. 1B). Colony-forming unit titers of *M. tuberculosis* in lungs and livers were higher in *Aim2$^{-/-}$* mice than in wild-type mice. We next performed histological analysis of the lungs of mice at 4 weeks post-infection. Gross appearances of the lungs of wild-type and *Aim2$^{-/-}$* mice were markedly different, and many granulomatous changes were evident in *M. tuberculosis*-infected *Aim2$^{-/-}$* mice (Fig. 2A). Hematoxylin and eosin staining

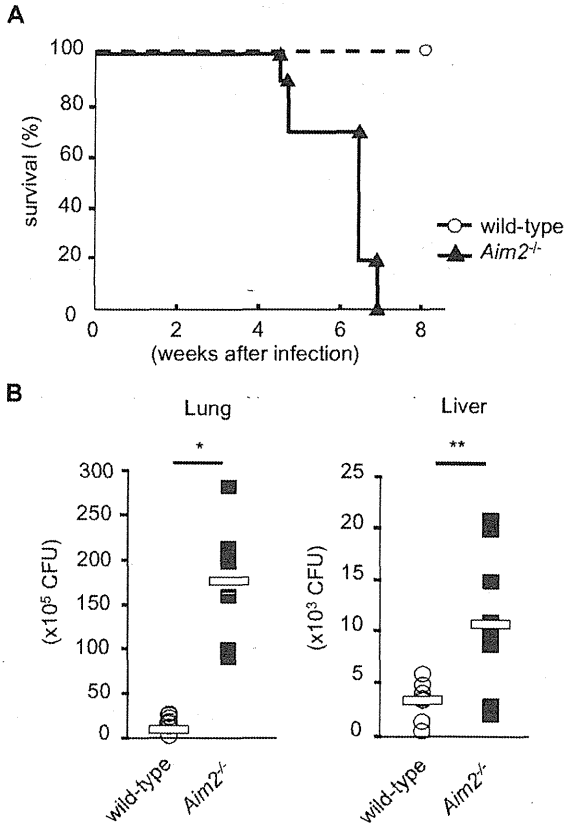

Fig. 1. *Aim2$^{-/-}$* mice are highly sensitive to infection with *M. tuberculosis*. (A) Wild-type ($n = 11$) and *Aim2$^{-/-}$* ($n = 10$) mice were intratracheally infected with *M. tuberculosis* and monitored for their survival. (B) Wild-type ($n = 7$) and *Aim2$^{-/-}$* ($n = 8$) mice were intratracheally infected with *M. tuberculosis*. At 4 weeks after infection, homogenates of the lungs and livers were plated onto 7H10-OADC agar and the CFU titers were counted. Symbols represent individual mice, and bars represent the mean CFU numbers. *, $P < 0.001$; **, $P < 0.01$.

of lung sections from infected *Aim2$^{-/-}$* mice demonstrated infiltration of many inflammatory cells (Fig. 2B). The number of *M. tuberculosis* in the lungs was measured by staining acid-fast bacilli using the Ziehl–Neelsen method (Fig. 2C). In the lungs of *Aim2$^{-/-}$* mice, the number of red-stained *M. tuberculosis* was markedly increased compared with those of wild-type mice. Taken together, these findings demonstrate that *Aim2$^{-/-}$* mice are highly susceptible to intratracheal infection with the virulent H37Rv strain of *M. tuberculosis*.

AIM2 mediates IL-1β/IL-18 production and T_h1 responses in Mycobacterium tuberculosis infection

Recent studies demonstrated that IL-1β is produced from *M. tuberculosis*-infected monocytes and alveolar macrophages mediating the host defense to mycobacteria (22–26, 42). Therefore, we first analyzed the levels of IL-1β in BALF from *M. tuberculosis*-infected mice (Fig. 3A). At 3 weeks after *M. tuberculosis* infection, IL-1β was abundantly detected in BALF from wild-type mice. In contrast, the concentration of IL-1β was profoundly decreased in BALF from *Aim2$^{-/-}$* mice. In addition to IL-1β, IL-18 has also been shown to be important for host resistance to mycobacterial infection (27–29). Therefore, we next

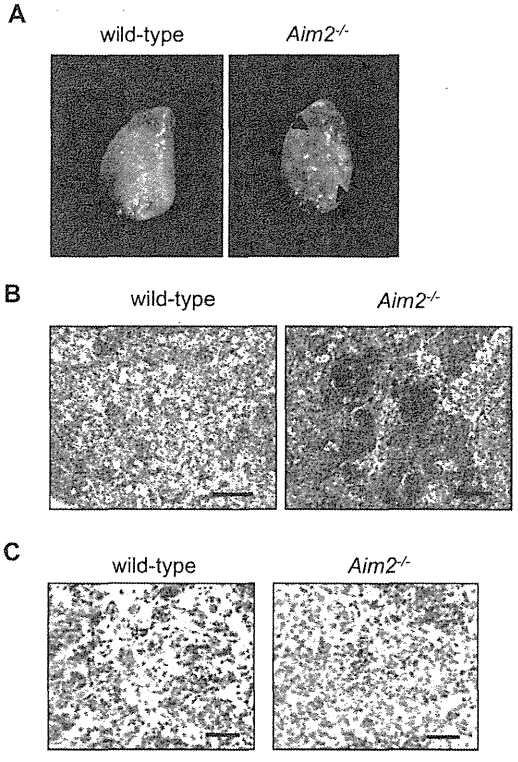

Fig. 2. High susceptibility of *Aim2*$^{-/-}$ mice to infection with *M. tuberculosis*. (A) Lung tissues from wild-type and *Aim2*$^{-/-}$ mice at 4 weeks post-intratracheal infection with *M. tuberculosis*. Arrows are shown to granulomatous lesion. (B) Lung tissue sections were stained with hematoxylin and eosin. Scale bars represent 100 μm. (C) Lung tissue sections were stained by the Ziehl–Neelsen method. Scale bars represent 40 μm.

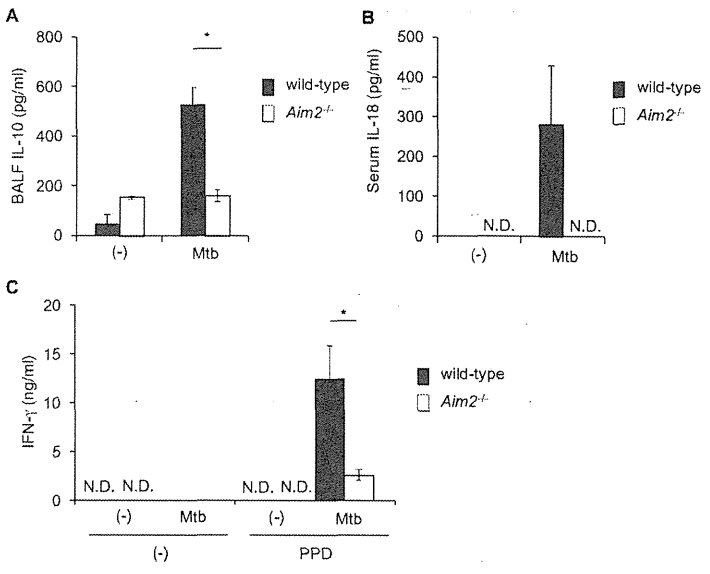

Fig. 3. Impaired production of IL-1β and IL-18 in *M. tuberculosis*-infected *Aim2*$^{-/-}$ mice. (A) BALF was collected from uninfected and infected mice at 3 weeks post-mycobacterial infection. BALF concentration of IL-1β was measured by ELISA. Data are presented as means ± SD of triplicate determinants and are one representative of two independent experiments. *, $P < 0.01$. (B) At 3 weeks after mycobacterial infection, sera were collected from uninfected and infected mice. Concentration of IL-18 was measured by ELISA. Data are presented as means ± SD of triplicate determinants and are one representative of two independent experiments. N.D., not detected. (C) CD4$^+$ T cells were isolated from the spleens of *M. tuberculosis*-uninfected and infected mice. The cells were co-cultured with APC for 48 h in the presence of PPD. The levels of IFN-γ in the cell supernatants were measured by ELISA. Data are presented as means ± SD of triplicate determinants and are one representative of two independent experiments. *, $P < 0.05$. N.D., not detected.

measured the serum levels of IL-18 in *M. tuberculosis*-infected mice (Fig. 3B). Serum concentration of IL-18 was increased in wild-type mice at 3 weeks post-infection. In contrast, IL-18 was not detected in sera from *M. tuberculosis*-infected *Aim2*$^{-/-}$ mice. We also assessed antigen-specific T_h1 responses following infection. CD4$^+$ T cells were isolated from the spleens of wild-type and *Aim2*$^{-/-}$ mice at 4 weeks post-infection and stimulated with a mycobacterial-specific antigen [purified protein derivative (PPD) of *Mycobacterium bovis*] in the presence of antigen-presenting cells (Fig. 3C). PPD stimulation induced marked production of IFN-γ in *M. tuberculosis*-infected wild-type mice. Antigen-specific production of IFN-γ was severely reduced in CD4$^+$ T cells derived from *M. tuberculosis*-infected *Aim2*$^{-/-}$ mice. These results indicate that the absence of AIM2 results in impaired production of IL-1β and IL-18 as well as T_h1 responses after *M. tuberculosis* infection.

AIM2 mediates Mycobacterium tuberculosis-induced inflammasome activation

We assessed the activation of caspase-1 to determine how AIM2-dependent immune responses develop following *M. tuberculosis* infection (Fig. 4A). Cleaved p10 form of caspase-1 was detected in *M. tuberculosis*-infected macrophages of wild-type mice. In contrast, cleavage of caspase-1 was severely reduced in *M. tuberculosis*-infected *Aim2*$^{-/-}$ macrophages.

We also analyzed cytokine production in *M. tuberculosis*-infected macrophages. Peritoneal macrophages from wild-type and *Aim2*$^{-/-}$ mice were infected with *M. tuberculosis* and the levels of cytokines in culture supernatants were measured by ELISA (Fig. 4B). *M. tuberculosis*-induced production of IL-12p40 or IFN-γ was comparable between wild-type and *Aim2*$^{-/-}$ macrophages. However, the production of IL-1β and IL-18 was severely reduced in *Aim2*$^{-/-}$ macrophages. Similar mRNA expression levels of *Il1b* and *Il18* in *M. tuberculosis*-infected wild-type and *Aim2*$^{-/-}$ macrophages were detected using real-time quantitative RT-PCR (Supplementary Figure 3 is available at *International Immunology* Online), indicating that AIM2 controls the production of IL-1β and IL-18 at the post-transcriptional level. Induction of *Ifnb*, encoding IFN-β, was enhanced in *M. tuberculosis*-infected *Aim2*$^{-/-}$ macrophages, confirming previous results (37). Thus, *M. tuberculosis*-infected *Aim2*$^{-/-}$ macrophages show defective caspase-1 activation leading to selective impairment in IL-1β and IL-18 secretion.

Mycobacterium tuberculosis genomic DNA activates AIM2 inflammasome

AIM2 has been shown to recognize cytosolic DNA (33–41). Therefore, we tested whether genomic DNA purified from

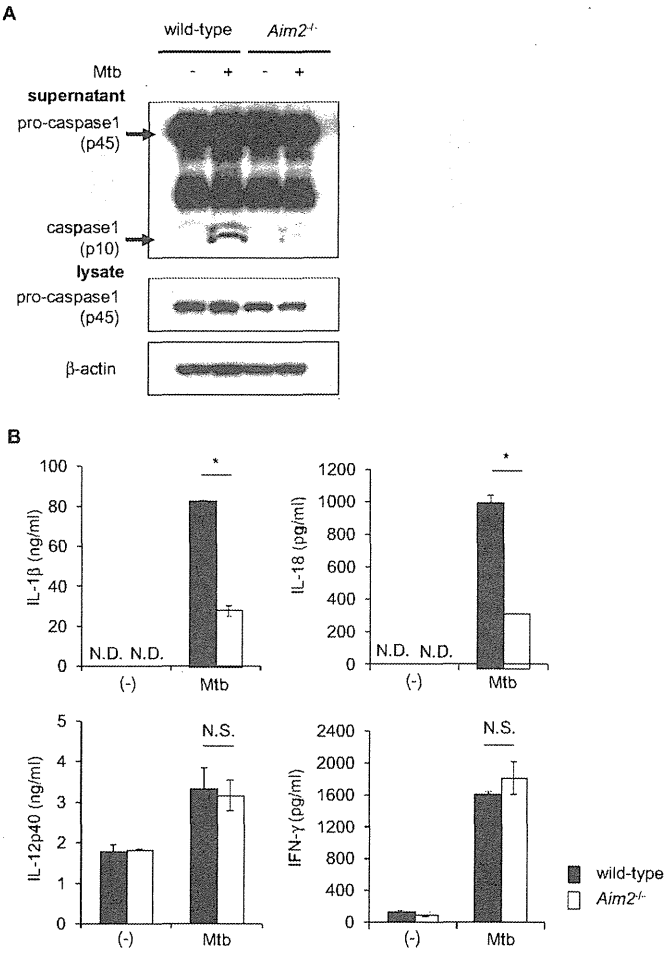

Fig. 4. AIM2-dependent inflammasome activation in *M. tuberculosis* infection. (A) *M. tuberculosis*-infected peritoneal macrophage culture supernatants were immuno-precipitated and the cell pellets were lysed. Caspase-1 specific bands were detected by western blotting and β-actin was used as a control for the cell lysate. One representative of three independent experiments is shown. (B) Thioglycollate-induced peritoneal macrophages were infected with *M. tuberculosis* (MOI of 3). The levels of the indicated cytokines in the culture supernatants were measured by ELISA. Data are presented as means ± SD of triplicate determinants and are one representative of three independent experiments. *, $P < 0.01$. N.S., not significant; N.D., not detected.

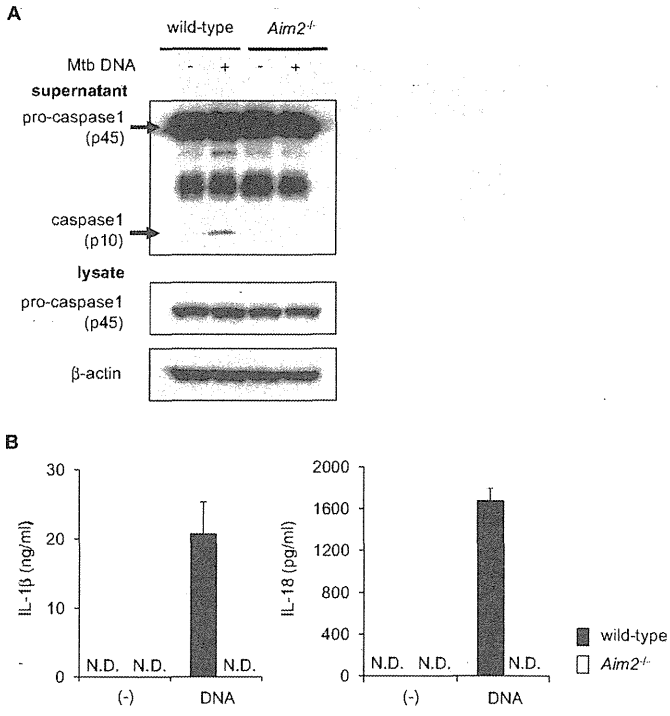

Fig. 5. AIM2 inflammasome is activated by *M. tuberculosis* genomic DNA. (A) Peritoneal macrophages were stimulated with LPS and transfected with Mtb DNA using Lipofectamine 2000. The culture supernatants were immuno-precipitated and the cells were lysed. Caspase-1 specific bands were detected by western blotting. β-actin was used as a control for the cell lysate. One representative of three independent experiments is shown. (B) Thioglycollate-elicited peritoneal macrophages were stimulated with LPS and transfected with Mtb DNA using Lipofectamine 2000. The production of IL-1β and IL-18 in the culture supernatants was measured by ELISA. Data are presented as means ± SD of triplicate determinants and are one representative of three independent experiments. N.D., not detected.

M. tuberculosis (Mtb DNA) activates caspase-1. Mtb DNA was transfected into the cytoplasm of peritoneal macrophages primed with LPS and analyzed for caspase-1 activation (Fig. 5A). The cleaved p10 form of caspase-1 was detected in LPS/Mtb DNA-stimulated wild-type macrophages. In contrast, the active form of caspase-1 was not induced in *Aim2*$^{-/-}$ macrophages stimulated with LPS/Mtb DNA. We also analyzed the secretion of IL-1β and IL-18 (Fig. 5B). Wild-type peritoneal macrophages stimulated with LPS/Mtb DNA secreted substantial amounts of IL-1β and IL-18. In contrast, the production of IL-1β and IL-18 was profoundly reduced in *Aim2*$^{-/-}$ macrophages. These findings indicate that AIM2 mediates Mtb DNA-dependent induction of caspase-1 activation and subsequent IL-1β/IL-18 secretion.

Mycobacterium tuberculosis genomic DNA is co-localized with cytosolic AIM2

We next determined the cellular compartment where Mtb DNA is recognized by AIM2. Recent studies demonstrated that virulent *M. tuberculosis* escapes from phagosomes into the cytosol (43). *Mycobacterium tuberculosis* was incubated with Hoechst 33342 to label genomic DNA and then infected into RAW264.7 macrophages. Some Mtb DNA was not co-localized with Rab7, which is recruited to the phagosomal membrane (Fig. 6A). Although LAMP1 is enriched in the phagolysosomal compartment, some Mtb DNA was not co-localized with it (Fig. 6B, upper panels). In contrast, genomic DNA from non-virulent *M. bovis* bacillus Calmette-Guerin (BCG) was fully merged with phagosome markers, Rab7 and LAMP1 (Supplementary Figure 4 is available at *International Immunology* Online). These findings indicate that Mtb DNA is present in the cytosol. We further visualized cytosolic AIM2 (Fig. 6B, lower panels). Mtb DNA, which was not present within the phagosome, co-localized with AIM2. We also analyzed co-localization of AIM2 and Mtb DNA in peritoneal macrophages (Supplementary Figure 5 is available at *International Immunology* Online). As was the case in RAW264.7 macrophages, Mtb DNA was merged with

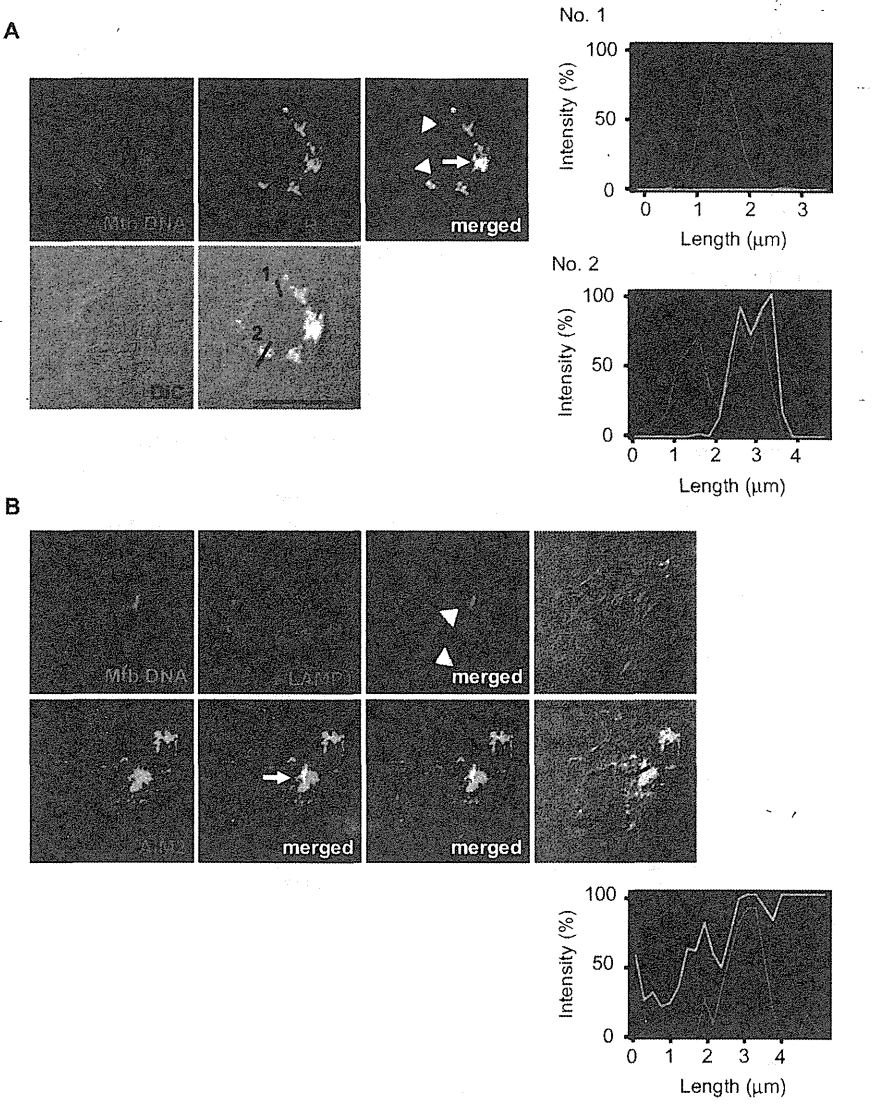

Fig. 6. Co-localization of cytosolic *M. tuberculosis* genomic DNA with AIM2. (A) RAW264.7 macrophages were infected with Hoechst-labeled *M. tuberculosis* (red) and stained with antibody to Rab7 (green). Scale bar represents 20 μm. Arrow heads indicate localization of the red signal alone. Arrow indicates co-localization of the red and green signals. Fluorescence intensities of the red and green signals were quantified along selected lines 1 or 2 (no. 1, upper panel; no. 2, lower panel). (B) RAW264.7 macrophages were infected with Hoechst-labeled *M. tuberculosis* (red) and stained with antibody to LAMP1 (blue, upper panels) or AIM2 (green, lower panels). Scale bar represents 20 μm. Arrow heads indicate localization of the red signal alone. Arrow indicates co-localization of the red and green signals. Fluorescence intensities of the blue, red and green signals were quantified along a selected line.

AIM2, but not with LAMP1. These findings demonstrate that the recognition of Mtb DNA by AIM2 occurs within the cytosolic compartment.

Discussion

In this study, we analyzed the role of AIM2 in the host defense against *M. tuberculosis* using AIM2-deficient mice. *Aim2*$^{-/-}$ mice were highly susceptible to mycobacterial infection compared with wild-type mice and showed severely reduced production of IL-1β and IL-18. IL-1β plays an important role in anti-mycobacterial host defense responses (22–26), and IL-18 is responsible for resistance to *M. tuberculosis* infection via induction of IFN-γ-mediated T_h1 responses (27–29). Therefore, defective production of IL-1 family cytokines, such as IL-1β and IL-18, might cause a high sensitivity to *M. tuberculosis* infection in *Aim2*$^{-/-}$ mice.

Mycobacterium tuberculosis has been shown to activate the inflammasome via NLRP3 (15–18). However, several studies showed that mice deficient in NLRP3 are not highly sensitive to *M. tuberculosis* infection (26, 30–32). This study clearly shows that *Aim2*$^{-/-}$ mice are highly sensitive to infection with *M. tuberculosis* with defective activation of caspase-1, identifying AIM2 as the important molecule for activation of the inflammasome in *M. tuberculosis* infection. However, mice deficient in ASC or caspase-1,

critical components of the inflammasome, do not show dramatically severe phenotypes as compared with those of IL-1β-deficient mice in *M. tuberculosis* infection (25, 26). In this regard, AIM2 might also activate a signaling pathway, leading to inflammasome-independent production of the IL-1 family of cytokines. *Mycobacterium tuberculosis*-infected *Aim2*$^{-/-}$ macrophages expressed high amounts of IFN-β, confirming a previous study that showed that poly(dA:dT) induces increased amounts of IFN-β in *Aim2*$^{-/-}$ splenocytes and macrophages (37). Thus, AIM2 might be involved in the signaling pathway responsible for suppression of IFN-β. IFN-β is induced by *M. tuberculosis* infection and then suppresses the production of IL-1β in macrophages and dendritic cells (44,45). Indeed, the type I IFNs have been shown to contribute to impaired host resistance to *M. tuberculosis* in mice (26, 46–48). Thus, it is possible that AIM2 activates two signaling pathways, one mediating the inflammasome-dependent processing of the IL-1 family of cytokines and a second that mediates activation of unknown pathways that sustain the production of IL-1β by suppressing type I IFNs.

The cleaved p10 form of caspase-1 was not detected, and production of IL-1β and IL-18 was almost completely abrogated in Mtb DNA-stimulated *Aim2*$^{-/-}$ macrophages. In contrast, IL-1β and IL-18 were moderately produced and the cleaved form of caspase-1 was also slightly detected in *M. tuberculosis*-infected *Aim2*$^{-/-}$ peritoneal macrophages. This might be due to NLRP3-dependent recognition of *M. tuberculosis* (15–18). An AIM2-independent and inflammasome-independent mechanism for IL-1β release might also be operating in mycobacterial infection. Matrix metalloproteinase-9 (MMP-9, also known as gelatinase B) is robustly induced in mycobacteria-infected macrophages, causing inactive IL-1β to be processed into a biologically active form (49, 50). Thus, MMP-9 might be involved in the inflammasome-independent processing of the IL-1 family of cytokines during *M. tuberculosis* infection.

We showed that Mtb DNA co-localized with AIM2 in the cytosol, but the localization of mycobacteria within macrophages is still under debate (51). Virulent *M. tuberculosis* resides within phagosomes by inhibiting their maturation into phagolysosomes (52–54). But there are several reports supporting cytosolic escape of virulent mycobacteria (43, 51, 55–57). *Mycobacterium marinum* can escape from phagosomes into the cytosol by actin-based motility (55), an activity which depends on the region of difference 1 [RD1 (57)]. *Mycobacterium tuberculosis* and *Mycobacterium leprae* can translocate from phagosomes to the cytosol of myeloid cells in an RD1-dependent manner and following cell death (43). This might be compatible with our results for *M. tuberculosis*-induced activation of the AIM2 inflammasome, which also requires induction of pyroptosis, a form of cell death (33–39). Our data suggest that *M. bovis* BCG, lacking the RD1 locus, fails to escape from phagosomes into the cytosol. In addition, *M. bovis* BCG does not induce IL-1β secretion from macrophages (32). Thus, AIM2 recognizes *M. tuberculosis* upon translocation into the cytosol. It will be interesting to determine how Mtb DNA becomes exposed and is recognized by AIM2 in the cytosol.

We have identified a novel recognition mechanism in mycobacterial infection through the cytosolic DNA sensor AIM2. Several pattern recognition receptors, such as TLRs and C-type lectin receptors mediate gene expression in mycobacterial infection. AIM2 is co-operatively involved in the immune response to mycobacterial infection with these innate immune mycobacterial sensors.

Supplementary data

Supplementary data are available at *International Immunology* Online.

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