The Journal of Immunology

Cutting Edge: *Mycobacterium tuberculosis* but Not Nonvirulent Mycobacteria Inhibits IFN-β and AIM2 Inflammasome–Dependent IL-1 β Production via Its ESX-1 Secretion System

Swati Shah, Amro Bohsali, Sarah E. Ahlbrand, Lalitha Srinivasan, Vijay A. K. Rathinam, Stefanie N. Vogel, Katherine A. Fitzgerald, Fayyaz S. Sutterwala and Volker Briken

J Immunol 2013; 191:3514-3518; Prepublished online 30 August 2013;
doi: 10.4049/jimmunol.1301331
http://www.jimmunol.org/content/191/7/3514

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Cutting Edge

The Journal of Immunology

Cutting Edge: *Mycobacterium tuberculosis* but Not Nonvirulent Mycobacteria Inhibits IFN-β and AIM2 Inflammasome–Dependent IL-1β Production via Its ESX-1 Secretion System

Swati Shah,* Amro Bohsali,* Sarah E. Ahlbrand,* Lalitha Srinivasan,* Vijay A. K. Rathinam,† Stefanie N. Vogel,‡ Katherine A. Fitzgerald,† Fayyaz S. Sutterwala,§,¶ and Volker Briken*

Mycobacterium tuberculosis extracellular DNA gains access to the host cell cytosol via the ESX-1 secretion system. It is puzzling that this extracellular DNA of _M. tuberculosis_ does not induce activation of the AIM2 inflammasome because AIM2 recognizes cytosolic DNA. In this study, we show that nonvirulent mycobacteria such as _Mycobacterium smegmatis_ induce AIM2 inflammasome activation, which is dependent on their strong induction of IFN-β production. In contrast, _M. tuberculosis_, but not an ESX-1–deficient mutant, inhibits the AIM2 inflammasome activation induced by either _M. smegmatis_ or transfected dsDNA. The inhibition does not involve changes in host cell AIM2 mRNA or protein levels but led to decreased activation of caspase-1. We furthermore demonstrate that _M. tuberculosis_ inhibits IFN-β production and signaling, which was partially responsible for the inhibition of AIM2 activation. In conclusion, we report a novel immune evasion mechanism of _M. tuberculosis_ that involves the ESX-1–dependent, direct or indirect, suppression of the host cell AIM2 inflammasome activation during infection. _The Journal of Immunology_, 2013, 191: 3514–3518.

Interleukin-1β is important for host immune defense against *Mycobacterium tuberculosis*, as several studies demonstrated that IL-1β and IL-1R knockout mice are more susceptible to *M. tuberculosis* infections (1, 2). In macrophages and dendritic cells the production of mature IL-1β is dependent on activation of the inflammasome (3). The nucleotide-binding domain and leucine-rich repeat–containing receptor (NLR) proteins such as NLRP3 and NLRC4 are one family of cytosolic receptors that upon ligand binding mediate inflammasome activation. In the case of *M. tuberculosis* the sole NLR capable of inducing inflammasome activation is NLRP3 (1, 2, 4–7).

The significance of type I IFN signaling for activation of inflammasome responses was first reported for *Francisella*-infected macrophages (8). Nevertheless, during the course of *M. tuberculosis* infections IFN-β has the opposite effect and suppresses activation of the NLRP3 inflammasome, suggesting that induction of IFN-β could correlate with increased virulence (9, 10). Mice deficient in IFN regulatory factor-3, a major signaling component of the type I IFN host cell signaling pathway, are much more resistant to *M. tuberculosis* infections (11). The induction of host cell type I IFN signaling after *M. tuberculosis* infection is dependent on the type VII secretion system (ESX-1)–mediated translocation of *M. tuberculosis* extracellular DNA (eDNA) into the host cell cytosol (11, 12). It is confounding that this cytosolic *M. tuberculosis* DNA is not recognized by the host cell inflammasome component AIM2, which should lead to subsequent inflammasome activation. AIM2 binds to dsDNA of intracellular pathogens such as *Francisella* and *Listeria* (13, 14). There is evidence that transfected *M. tuberculosis* dsDNA can interact with AIM2 and activate the AIM2 inflammasome and that AIM2 is important for host resistance to *M. tuberculosis* infection (15).

Materials and Methods

Cell culture and animals

C57BL/6 wild-type (WT) mice were obtained from The Jackson Laboratory. *Nlrc4*$^{-/-}$, *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$, *Nlrp6*$^{-/-}$, and *Nlrp10*$^{-/-}$ mice were provided by Dr. R. Flavell and Millennium Pharmaceuticals. *Aim2*$^{-/-}$ (16) and *Aim2/Nlrp3*$^{-/-}$ double knockout mice were obtained from Dr. K.A. Fitzgerald. *Ifnar1*$^{-/-}$ mice were from Dr. A. Sher (National Institutes of Health). *IFN-*

*Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742; †Division of Infectious Diseases and Immunology, Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01605; ‡Department of Microbiology and Immunology, University of Maryland, Baltimore, MD 21201; §Inflammation Program, Department of Internal Medicine, University of Iowa, Iowa City, IA 52242; and ¶Veterans Affairs Medical Center, Iowa City, IA 52241

Received for publication May 22, 2013. Accepted for publication August 7, 2013.

The work was supported by National Institutes of Health Grants AI072584 (to S.S., A.B., and V.B.), AI087630 (to F.S.S.), AI083713 (to K.A.F.), AI067497 (to K.A.F.), AI018797 (to S.N.V.), and T32 AI09621 (to S.E.A.), as well as by Veterans Administration Merit Review Grant IBX000167A (to F.S.S.).

Address correspondence and reprint requests to Dr. Volker Briken, Department of Cell Biology and Molecular Genetics, University of Maryland, Microbiology Building 231, Room 2201, College Park, MD 20742. E-mail address: vbriken@umd.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: BMDC, bone marrow–derived dendritic cell; BMDM, bone marrow–derived macrophage; eDNA, extracellular DNA; hpi, hours postinfection; LVS, live vaccine strain; NLR, nucleotide-binding domain and leucine-rich repeat–containing receptor; WT, wild-type.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1301331

$\beta^{-/-}$ mice were provided by Dr. S.N. Vogel. All studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Bacteria

Mycobacterium smegmatis (mc^{2}155), *M. tuberculosis* H37Rv (ATCC 25618), and H37Ra (ATCC 25177) were obtained from Dr. W.R. Jacobs, Jr. (Albert Einstein College of Medicine). *Mycobacterium fortuitum* (ATCC 6841) and *Mycobacterium kansasii* strain Hauduroy (ATCC 12478) were obtained from the American Type Culture Collection. *M. tuberculosis* Δ*esxA* and Δ*exoU* PAK (*Pseudomonas aeruginosa*) were provided by Dr. L. Gao and Dr. V. Lee. *M. smegmatis* Δ*esx1*, Δ*eccCb* mutant, and Δ*eccCb* complemented strains were gifts of Dr. K. Derbyshire (17). *Francisella tularensis* live vaccine strain (LVS) was obtained from Dr. Kevin McIver.

Ex vivo infection

Bacterial infections of bone marrow–derived dendritic cells (BMDCs) and bone marrow–derived macrophages (BMDMs) were performed as described (7, 18). For induction of AIM2 inflammasome, BMDCs were pretreated with 20 ng/ml LPS (InvivoGen) for 1 h and then infected with H37Rv for 4 h. Infected BMDCs were then washed twice with PBS and transfected with 0.5 μg/ml poly(deoxyadenylic-thymidylic) acid (Sigma-Aldrich) using Lipofectamine LTX Plus reagent (Invitrogen) for 2 h. The transfection was performed according to the manufacturers' instructions and the supernatants were harvested 2 h after transfection.

IFN-β neutralization

BMDCs from C57BL/6 mice were treated with anti–IFN-β neutralizing Ab 7F-D3 (5 μg/ml; Abcam) for 1 h and infected with *M. smegmatis* at a multiplicity of infection of 10:1 for 2 h as previously described. Cells were then washed with PBS and incubated for an additional 20 h in DMEM chase media. Supernatants were collected for ELISA.

Cell death assays

The adenylate kinase release assay ToxiLight bioassay (Lonza) was used to quantify necrotic cell death. The assay was performed according to the manufacturer's instructions.

Cytokine measurement and immunoblotting

ELISA was used to measure secreted IL-1β (BD Biosciences) and IFN-β (Legend Max; BioLegend), respectively. For immunoblotting, the cell lysate preparation and Western blotting were performed as described earlier (7). The primary Abs used were anti–IL-1β (R&D Systems) at 0.15 μg/ml in 0.1% BSA, anti–caspase-1 (Santa Cruz Biotechnology) at 1:300, anti-AIM2 (Santa Cruz Biotechnology) at 1:500, and anti-tubulin (Cell Signaling Technology) at 1:1000. The above Abs were diluted in 5% milk with TBST. The secondary Abs used were donkey anti-goat (Jackson ImmunoResearch Laboratories) at 1:25,000, goat anti-rabbit (Jackson ImmunoResearch Laboratories) at 1:50,000, and goat anti-mouse (Jackson ImmunoResearch Laboratories) at 1:50,000 dilutions.

Real-time PCR

BMDCs were harvested 8 h postinfection (hpi) using TRIzol (Invitrogen). Real-time PCR was done using SYBR Green PCR Master Mix (Roche) with *GAPDH* as the housekeeping gene. The primers used were: *Aim2*, 5′-GTCACCAGTTCCTCAGTTGT-3′ and 5′-CACCTCCATTGTCCCTGTTTTAT-3′; *Gapdh*, 5′- ATGGGATTTCCATTGATGACA-3′ and 5′-CCACCCATGGCAAATTCC-3′; *Mx1*, 5′-TGTGCAGGCACTATGAGGAG-3′ and 5′-ACTCTGGTCCCCAATGACAG-3′; and *PKR*, 5′-GCACCGGGTTTTGTATCGA-3′ and 5′-GGAGCACGAAGTACAAGCGC-3′.

Statistical analysis

Statistical analysis was performed on at least three independent experiments using GraphPad Prism 5.0 software and one-way ANOVA with a Tukey posttest unless otherwise noted in the figure legends. Shown are representative results of triplicate values with SD. A p value <0.05 was considered statistically significant.

Results and Discussion

Nonvirulent mycobacteria induce AIM2 inflammasome activation

The nonvirulent mycobacterial species such as *M. smegmatis* induce a potent proinflammatory immune response and host cell apoptosis when compared with more virulent mycobacterial species (18), but the activation of host cell inflammasome mediated by *M. smegmatis* infection has not been analyzed. BMDCs of various mouse strains were infected with *M. smegmatis* and the amount of IL-1β in the supernatant was detected and normalized to IL-1β levels secreted by WT BMDCs (Fig. 1A). There were no differences in cell lysis as determined by adenylate kinase release assay and pro–IL-1β ELISA (not shown). Surprisingly, in NLRP3$^{-/-}$ cells the amount of secreted IL-1β dropped by <50%. This is unexpected because the inflammasome activation in *M. tuberculosis* is completely dependent on the presence of NLRP3 (1, 2, 4, 5). NLRP6, NLRP12, NLRC4, and NLRP10 did not significantly contribute to *M. smegmatis*–induced inflammasome activation (Fig. 1A). Interestingly, however, the deficiency of AIM2 resulted in ∼40% reduction in IL-1β response in BMDCs (Fig.1A) and almost 75% reduction in BMDMs (Fig. 1B). The partial reductions in IL-1β secretion suggested redundancy between AIM2 and NLRP3 pathways. Consistently, the IL-1β production in *Aim2/Nlrp3*$^{-/-}$ cells was further reduced when compared with any of the single deletions; surprisingly, however, *M. smegmatis*–infected *Aim2/Nlrp3*$^{-/-}$ BMDCs were still able to secrete up to 40% of the IL-1β (Fig. 1A). This result suggests recognition of *M. smegmatis* cytosolic components by one or more unidentified NLRs. The *M. tuberculosis*–induced activation of NLRP3 is dependent on the *M. tuberculosis* ESX-1 secretion system (7, 19, 20). The core ESX-1 secretion complex is conserved in *M. smegmatis*, making it a compelling model to study the mechanisms of ESX-1–mediated protein secretion

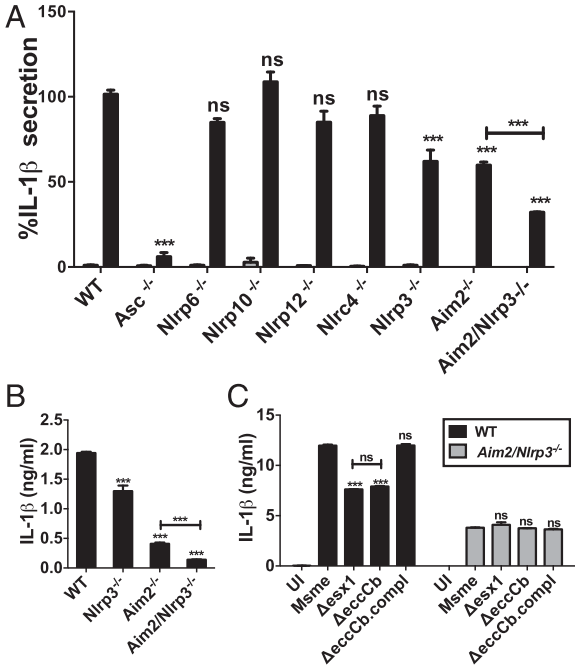

FIGURE 1. *M. smegmatis* activates the AIM2 inflammasome via a partially ESX-1–dependent mechanism. (**A**) BMDCs and (**B**) BMDMs from various indicated knockout mice were either left uninfected (UI, gray bars) or infected with *M. smegmatis* (black bars). The cell supernatants were harvested 16 hpi and analyzed for IL-1β secretion by ELISA. (**C**) BMDCs from WT and *Aim2/Nlrp3*$^{-/-}$ mice were infected with different *M. smegmatis* strains (*M. smegmatis*, Δ*esx1*, Δ*eccCb*, complemented) and the supernatants were analyzed for IL-1β secretion. Data are shown as the means ± SD of triplicate measurements of one representative experiment out of three except for (B) and (C) where the data are from one representative experiment out of two. ***$0.0001 < p < 0.001$. Msme, *M. smegmatis*.

(21, 22). Similar to *M. tuberculosis*, a functional *M. smegmatis* ESX-1 secretion system is required for maximal response of AIM2/NLRP3-dependent secretion of IL-1β because two different *M. smegmatis* mutants with defective ESX-1 show an almost 50% reduction of IL-1β secretion when compared with WT *M. smegmatis* in WT BMDCs, but that difference is abolished in *Aim2/Nlrp3*$^{-/-}$ BMDCs (Fig. 1C).

Next, we wanted to investigate whether other mycobacterial species activate the AIM2 inflammasome. We thus infected WT and AIM2-deficient BMDCs with *M. smegmatis*, *M. fortuitum*, *M. kansasii*, the attenuated *M. tuberculosis* H37Ra, and the virulent *M. tuberculosis* H37Rv. We monitored cell lysis (not shown) and IL-1β secretion for all the infections (Fig. 2A). In general, there was an inverse correlation between virulence of the species and the amount of IL-1β induction, with the least virulent species inducing the most IL-1β in WT BMDCs (Fig. 2A). The infection of AIM2-deficient BMDCs by these mycobacterial species allowed determining the fraction of total IL-1β secretion that was dependent on presence of AIM2. As expected, the *Francisella*-induced inflammasome activation was completely dependent on AIM2, whereas ~40–50% of the *M. smegmatis*, *M. fortuitum*, and *M. kansasii* induction was dependent on AIM2 (Fig. 2A). The attenuated *M. tuberculosis* strain H37Ra induced the lowest amount of AIM2 inflammasome activation, and the virulent *M. tuberculosis* strain H37Rv did not induce any AIM2 activation (Fig. 2A).

Nonvirulent mycobacteria induce IFN-β dependent AIM2 inflammasome activation

The AIM2 inflammasome activation by *Francisella* is stimulated by IFN-β (8). To investigate the potential importance of IFN-β in AIM2 inflammasome activation by nonvirulent mycobacteria, we analyzed the supernatants of BMDCs infected with various mycobacterial species for IFN-β production. Interestingly, the three species (*M. smegmatis*, *M. fortuitum*, and *M. kansasii*) that mediated the strongest AIM2 inflammasome activation also induced a very pronounced IFN-β production of ~800 pg/ml, which was a 20-fold increase over the amount of IFN-β produced by BMDCs after infection with *M. tuberculosis* H37Rv (Fig. 2B). To demonstrate that this IFN-β secretion is important for AIM2 inflammasome activation, we infected BMDCs from WT and *Aim2*$^{-/-}$ mice with *F. tularensis* LVS and *M. smegmatis* in the absence or presence of neutralizing IFN-β Abs and measured the IL-1β secretion after 24 h (Fig. 2C). The addition of neutralizing IFN-β Abs significantly reduced the IL-1β secretion after infection with either *F. tularensis* LVS or *M. smegmatis* (Fig. 2C). Consistently, the production of IL-1β was reduced by similar levels when BMDCs of WT or *Ifnar1*$^{-/-}$ mice were infected (Fig. 2D). The neutralization of IFN-β had no effect on the IL-1β production after *F. tularensis* LVS or *M. smegmatis* infection of *Aim2*$^{-/-}$ BMDCs. These results suggest that IFN-β plays a similar role in the induction of AIM2 inflammasome activation by nonvirulent mycobacterial species, as it does after *Francisella* infections.

M. tuberculosis *inhibits AIM2-dependent IL-1β production*

Finally, we addressed the hypothesis that *M. tuberculosis* can actively inhibit AIM2 inflammasome activation by performing mixed infection experiments. BMDCs from *Nlrp3*$^{-/-}$ mice were infected with *M. tuberculosis* or the *esxA* deletion *M. tuberculosis* mutant (*M. tuberculosis* Δ *esxA*), and either 4 or 8 hpi the supernatants were harvested for analysis of IL-1β secretion (Fig. 3A, 3B). In *M. smegmatis*–infected BMDCs we

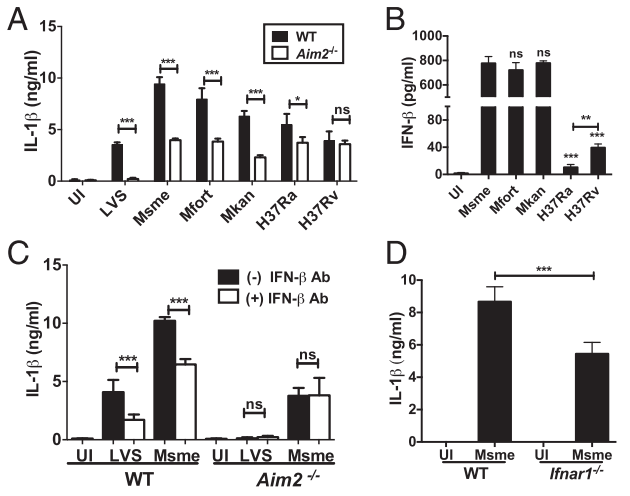

FIGURE 2. Nonvirulent mycobacteria induce IFN-β–dependent AIM2 inflammasome activation. (**A**) WT and *Aim2*$^{-/-}$ BMDCs were infected with *M. smegmatis*, *M. fortuitum*, *M. kansasii*, attenuated *M. tuberculosis* H37Ra, and virulent *M. tuberculosis* H37Rv. *Francisella* LVS infection was used as a positive control. The secreted IL-1β was measured at 16 hpi by ELISA. (**B**) IFN-β ELISA of supernatants from WT BMDCs infected with indicated mycobacterial species. (**C**) IL-1β ELISA of supernatants from WT and *Aim2*$^{-/-}$ *M. smegmatis*–infected BMDCs in the presence or absence of IFN-β neutralizing Abs. LVS was used as a positive control. (**D**) IL-1β ELISA of supernatants from *Ifnar1*$^{-/-}$ or WT BMDCs infected with *M. smegmatis* or left uninfected. Data are shown as the means ± SD of triplicate measurements of one representative experiment out of three. *$0.01 < p < 0.05$, **$0.001 < p < 0.01$, ***$0.0001 < p < 0.001$. Mfort, *M. fortuitum*; Mkan, *M. kansasii*; Msme, *M. smegmatis*; UI, uninfected.

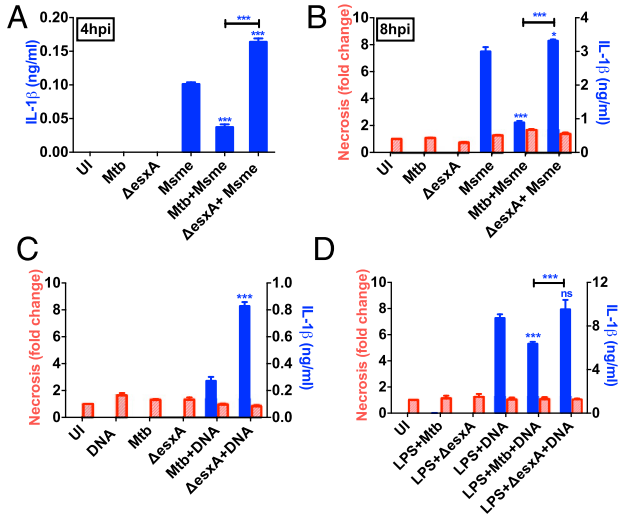

FIGURE 3. *M. tuberculosis* inhibits AIM2-dependent IL-1β production. *Nlrp3*$^{-/-}$ BMDCs were first infected with *M. tuberculosis* or the *M. tuberculosis* Δ *esxA* mutant and then with *M. smegmatis*. Secreted IL-1β (blue, filled bars) was measured by ELISA at (**A**) 4 hpi or (**B**) 8 hpi. Necrotic cell death (red, striped bars) was assayed and is represented as fold change over uninfected. *Nlrp3*$^{-/-}$ BMDCs in the (**C**) absence or (**D**) presence of LPS pretreatment were first infected with *M. tuberculosis* or the *M. tuberculosis* Δ *esxA* mutant and then transfected with poly(deoxyadenylic-thymidylic) acid for 2 h. Both secretion of IL-1β and necrosis were measured as before. Data are shown as the means ± SD of triplicate measurements of one representative experiment out of three. *$0.01 < p < 0.05$, ***$0.0001 < p < 0.001$. Msme, *M. smegmatis*; Mtb, *M. tuberculosis*; UI, uninfected.

detected ~100 pg/ml after 4 hpi and 3000 pg/ml after 8 hpi. Interestingly, when BMDCs, which had been infected with *M. tuberculosis*, were challenged with *M. smegmatis* we observed a 2- to 3-fold reduction in the amount of secreted IL-1β when compared with BMDCs infected only with *M. smegmatis*. The *M. tuberculosis*–mediated inhibition was dependent on a functional ESX-1 secretion system because infection with the *M. tuberculosis Δ esxA* mutant did not inhibit IL-1β secretion after challenge with *M. smegmatis* (Fig. 3A, 3B). The rate of infection of *M. smegmatis* was not affected by the prior infection with *M. tuberculosis* as analyzed via flow cytometry using GFP-labeled *M. smegmatis* (Supplemental Fig. 1A). A similar inhibition by *M. tuberculosis* was also observed for IL-18 induced by *M. smegmatis* infections (Supplemental Fig. 1B). There were no differences in necrosis induction at 8 hpi (Fig. 3B). To confirm the specific inhibition of the AIM2 inflammasome, we transfected the *M. tuberculosis*– or *M. tuberculosis Δ esxA*–infected *Nlrp3*$^{-/-}$ BMDCs with 0.5 μg/ml poly(deoxyadenylic-thymidylic) acid in the absence (Fig. 3C) or presence (Fig. 3D) of LPS pretreatment. Consistent with the previous finding, analysis of IL-1β showed that *M. tuberculosis* does inhibit the activation of the AIM2 inflammasome when compared with *M. tuberculosis Δ esxA*–infected cells. Additionally, we determined that *M. tuberculosis* was not able to inhibit NLRC4 inflammasome activation by *P. aeruginosa* (Supplemental Fig. 2).

Finally, to further support our hypothesis that *M. tuberculosis* mediates inhibition of AIM2 inflammasome activation, we started to investigate the mechanism of this inhibition. First, we determined that there were no significant changes of *Aim2* transcription mediated by *M. tuberculosis* infection (not shown). Consistently, immunoblots of cell lysates from infected BMDCs at 6 hpi showed that there is no difference in AIM2 protein expression (Fig. 4A). Also, the protein expression of pro–IL-1β and procaspase-1 was not affected by *M. tuberculosis*. However, immunoblots of the corresponding supernatants showed that there is decreased secretion of p10 fragment of caspase-1 and the mature IL-1β (p17) fragment in *M. tuberculosis*–infected cells challenged with *M. smegmatis* when compared with *M. smegmatis* or *M. tuberculosis Δ esxA*/*M. smegmatis*–infected cells (Fig. 4A). Interestingly, *M. tuberculosis* infection reduced the amount of *M. smegmatis*–induced IFN-β secretion in an ESX-1–dependent manner (Fig. 4B). Even the addition of high amounts of exogenous IFN-β (400 ng/ml) could only partially overcome the *M. tuberculosis*–mediated inhibition of *M. smegmatis*–induced IL-1β secretion (Fig. 4C). These results demonstrate that *M. tuberculosis* is able to limit IFN-β production in infected host cells, which may explain some of its capacity to inhibit the IFN-β–dependent AIM2 inflammasome activation. To investigate whether *M. tuberculosis* may also inhibit IFN-β signaling, we used IFN-β$^{-/-}$ BMDCs and infected them with *M. tuberculosis* and *M. tuberculosis Δ esxA* followed by treatment with IFN-β, and the transcription of IFN-β–inducible genes *Mx1* and *PKR* was analyzed by quantitative RT-PCR (Fig. 4D, 4E). In both cases *M. tuberculosis* infection reduced the IFN-β–mediated increase in transcription but this inhibition was not dependent on ESX-1, because the *M. tuberculosis Δ esxA* mutant showed a similar reduction. The inhibition of IL-1β production by *M. tuberculosis* has been reported before (23), but the inhibition of IFN-β signaling

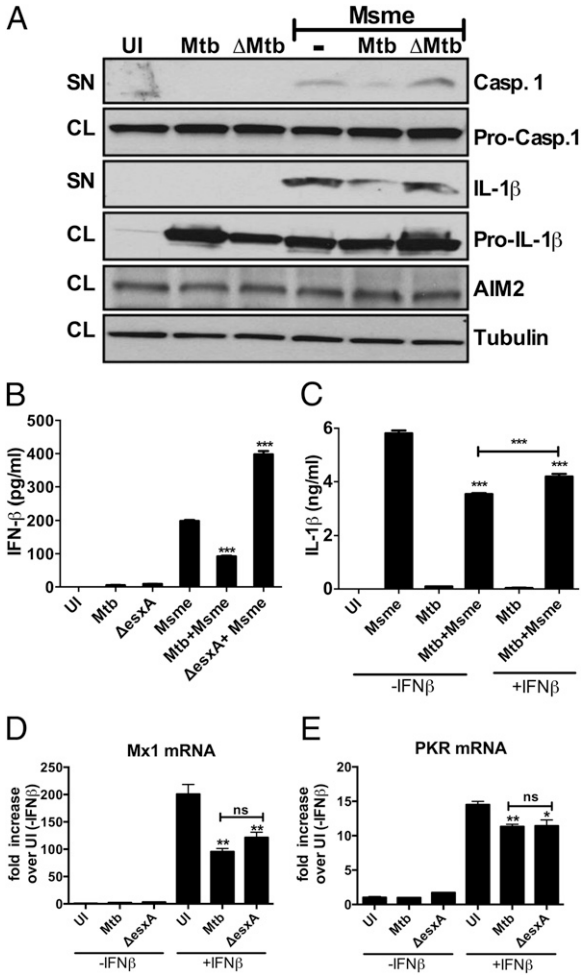

FIGURE 4. Mechanism of AIM2 inflammasome inhibition. *Nlrp3*$^{-/-}$ BMDCs were infected first with *M. tuberculosis* or the *M. tuberculosis Δ esxA* mutant and then with *M. smegmatis*. (**A**) Western blots of supernatants (SN) showing active cleavage fragments of caspase-1 (p10) and IL-1β (p17) and cell lysates (CL) detecting procaspase-1 (p45), pro–IL-1β (p35), tubulin (p55), and AIM2 (p38) protein levels. (**B**) Secreted IFN-β was measured from supernatants collected 8 hpi by ELISA. (**C**) Secreted IL-1β from supernatants of untreated *Nlrp3*$^{-/-}$ BMDCs or those treated with IFN-β (400 ng/ml) and then infected as indicated was measured 8 hpi by ELISA. (**D** and **E**) IFN-β$^{-/-}$ mice were infected with indicated bacteria and treated or not with IFN-β (700 pg/ml) and the mRNA levels of *Mx1* and *PKR* were analyzed after 4 h via quantitative RT-PCR. Data are from one representative experiment out of three. $*0.01 < p < 0.05$, $**0.001 < p < 0.01$, $***0.0001 < p < 0.001$. Msme, *M. smegmatis*; Mtb, *M. tuberculosis*; UI, uninfected.

by *M. tuberculosis* has not been shown previously to our knowledge.

The precise molecular mechanism of the *M. tuberculosis*–mediated AIM2 inflammasome inhibition remains to be elucidated. It seems unlikely that limiting IFN-β production is the only pathway for *M. tuberculosis* to suppress AIM2 inflammasome activation because external addition of IFN-β did not induce IL-1β secretion in *M. tuberculosis*–infected *Nlrp3*$^{-/-}$ BMDCs (Fig. 4C) and only partially restored the *M. tuberculosis*–mediated inhibition of *M. smegmatis*–induced IL-1β. Hence, *M. tuberculosis* may secrete another effector that could inhibit signaling of the IFN-α/β receptor and/or directly modify AIM2 inflammasome activation. Indeed, we provide evidence that *M. tuberculosis* inhibits IFN-β signaling. A detailed analysis of the large number of IFN-β–regulated

genes may reveal a subset whose expression can only be inhibited by *M. tuberculosis* with a functional ESX-1 system. This subset of genes would be the most likely to contain candidates for mediating the AIM2 inflammasome inhibition. Overall, the cosecretion into the host cell cytosol of *M. tuberculosis* eDNA and a putative AIM2 inhibitor and/or IFN-β signaling inhibitor via the ESX-1 system may allow *M. tuberculosis* to take advantage of the type I IFN–mediated inhibition of the NLRP3 inflammasome without the *M. tuberculosis* eDNA inducing activation of the AIM2 inflammasome. The recent finding that *Aim2*$^{-/-}$ mice are very susceptible to *M. tuberculosis* infections supports the potential role of AIM2 inflammasome inhibition for optimal virulence of *M. tuberculosis* (15). Our discovery of a novel immune evasion mechanism engaged by *M. tuberculosis* opens the door for investigations into the identification of the *M. tuberculosis* genes involved in this inhibition and subsequent analysis of their importance for virulence of *M. tuberculosis*.

Acknowledgments

We thank Vickie Knepper-Adrian and Lisa Waggoner for technical assistance.

Disclosures

The authors have no financial conflicts of interest.

References

1. Mayer-Barber, K. D., D. L. Barber, K. Shenderov, S. D. White, M. S. Wilson, A. Cheever, D. Kugler, S. Hieny, P. Caspar, G. Núñez, et al. 2010. Caspase-1 independent IL-1β production is critical for host resistance to *Mycobacterium tuberculosis* and does not require TLR signaling in vivo. *J. Immunol.* 184: 3326–3330.
2. McElvania Tekippe, E., I. C. Allen, P. D. Hulseberg, J. T. Sullivan, J. R. McCann, M. Sandor, M. Braunstein, and J. P. Ting. 2010. Granuloma formation and host defense in chronic *Mycobacterium tuberculosis* infection requires PYCARD/ASC but not NLRP3 or caspase-1. *PLoS ONE* 5: e12320.
3. Schroder, K., and J. Tschopp. 2010. The inflammasomes. *Cell* 140: 821–832.
4. Mishra, B. B., P. Moura-Alves, A. Sonawane, N. Hacohen, G. Griffiths, L. F. Moita, and E. Anes. 2010. *Mycobacterium tuberculosis* protein ESAT-6 is a potent activator of the NLRP3/ASC inflammasome. *Cell. Microbiol.* 12: 1046–1063.
5. Dorhoi, A., G. Nouailles, S. Jörg, K. Hagens, E. Heinemann, L. Pradl, D. Oberbeck-Müller, M. A. Duque-Correa, S. T. Reece, J. Ruland, et al. 2012. Activation of the NLRP3 inflammasome by Mycobacterium tuberculosis is uncoupled from susceptibility to active tuberculosis. *Eur. J. Immunol.* 42: 374–384
6. Wong, K. W., and W. R. Jacobs, Jr. 2011. Critical role for NLRP3 in necrotic death triggered by *Mycobacterium tuberculosis. Cell. Microbiol.* 13: 1371–1384.
7. Abdalla, H., L. Srinivasan, S. Shah, K. D. Mayer-Barber, A. Sher, F. S. Sutterwala, and V. Briken. 2012. *Mycobacterium tuberculosis* infection of dendritic cells leads to partially caspase-1/11-independent IL-1β and IL-18 secretion but not to pyroptosis. *PLoS ONE* 7: e40722.
8. Henry, T., A. Brotcke, D. S. Weiss, L. J. Thompson, and D. M. Monack. 2007. Type I interferon signaling is required for activation of the inflammasome during *Francisella* infection. *J. Exp. Med.* 204: 987–994.
9. Novikov, A., M. Cardone, R. Thompson, K. Shenderov, K. D. Kirschman, K. D. Mayer-Barber, T. G. Myers, R. L. Rabin, G. Trinchieri, A. Sher, and C. G. Feng. 2011. Mycobacterium tuberculosis triggers host type I IFN signaling to regulate IL-1β production in human macrophages. *J. Immunol.* 187: 2540–2547.
10. Mayer-Barber, K. D., B. B. Andrade, D. L. Barber, S. Hieny, C. G. Feng, P. Caspar, S. Oland, S. Gordon, and A. Sher. 2011. Innate and adaptive interferons suppress IL-1α and IL-1β production by distinct pulmonary myeloid subsets during *Mycobacterium tuberculosis* infection. *Immunity* 35: 1023–1034.
11. Manzanillo, P. S., M. U. Shiloh, D. A. Portnoy, and J. S. Cox. 2012. *Mycobacterium tuberculosis* activates the DNA-dependent cytosolic surveillance pathway within macrophages. *Cell Host Microbe* 11: 469–480.
12. Stanley, S. A., J. E. Johndrow, P. Manzanillo, and J. S. Cox. 2007. The type I IFN response to infection with *Mycobacterium tuberculosis* requires ESX-1-mediated secretion and contributes to pathogenesis. *J. Immunol.* 178: 3143–3152.
13. Sauer, J. D., C. E. Witte, J. Zemansky, B. Hanson, P. Lauer, and D. A. Portnoy. 2010. *Listeria monocytogenes* triggers AIM2-mediated pyroptosis upon infrequent bacteriolysis in the macrophage cytosol. *Cell Host Microbe* 7: 412–419.
14. Hornung, V., A. Ablasser, M. Charrel-Dennis, F. Bauernfeind, G. Horvath, D. R. Caffrey, E. Latz, and K. A. Fitzgerald. 2009. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* 458: 514–518.
15. Saiga, H., S. Kitada, Y. Shimada, N. Kamiyama, M. Okuyama, M. Makino, M. Yamamoto, and K. Takeda. 2012. Critical role of AIM2 in *Mycobacterium tuberculosis* infection. *Int. Immunol.* 24: 637–644.
16. Rathinam, V. A., Z. Jiang, S. N. Waggoner, S. Sharma, L. E. Cole, L. Waggoner, S. K. Vanaja, B. G. Monks, S. Ganesan, E. Latz, et al. 2010. The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat. Immunol.* 11: 395–402.
17. Flint, J. L., J. C. Kowalski, P. K. Karnati, and K. M. Derbyshire. 2004. The RD1 virulence locus of *Mycobacterium tuberculosis* regulates DNA transfer in *Mycobacterium* smegmatis. *Proc. Natl. Acad. Sci. USA* 101: 12598–12603.
18. Bohsali, A., H. Abdalla, K. Velmurugan, and V. Briken. 2010. The non-pathogenic mycobacteria *M. smegmatis* and *M. fortuitum* induce rapid host cell apoptosis via a caspase-3 and TNF dependent pathway. *BMC Microbiol.* 10: 237.
19. Stanley, S. A., S. Raghavan, W. W. Hwang, and J. S. Cox. 2003. Acute infection and macrophage subversion by *Mycobacterium tuberculosis* require a specialized secretion system. *Proc. Natl. Acad. Sci. USA* 100: 13001–13006.
20. Kurenuma, T., I. Kawamura, H. Hara, R. Uchiyama, S. Daim, S. R. Dewamitta, S. Sakai, K. Tsuchiya, T. Nomura, and M. Mitsuyama. 2009. The RD1 locus in the *Mycobacterium tuberculosis* genome contributes to activation of caspase-1 via induction of potassium ion efflux in infected macrophages. *Infect. Immun.* 77: 3992–4001.
21. Converse, S. E., and J. S. Cox. 2005. A protein secretion pathway critical for *Mycobacterium tuberculosis* virulence is conserved and functional in *Mycobacterium* smegmatis. *J. Bacteriol.* 187: 1238–1245.
22. Ohol, Y. M., D. H. Goetz, K. Chan, M. U. Shiloh, C. S. Craik, and J. S. Cox. 2010. *Mycobacterium tuberculosis* MycP1 protease plays a dual role in regulation of ESX-1 secretion and virulence. *Cell Host Microbe* 7: 210–220.
23. Liu, Y. C., D. P. Simmons, X. Li, D. W. Abbott, W. H. Boom, and C. V. Harding. 2012. TLR2 signaling depletes IRAK1 and inhibits induction of type I IFN by TLR7/9. *J. Immunol.* 188: 1019–1026.