AMERICAN SOCIETY FOR MICROBIOLOGY | Infection and Immunity®

HOST RESPONSE AND INFLAMMATION

Caspase-11 Plays a Protective Role in Pulmonary *Acinetobacter baumannii* Infection

Wei Wang,[a] Yue Shao,[a] Shengjun Li,[b] Na Xin,[a] Tingxian Ma,[a] Chenghai Zhao,[a] Min Song[c]

Department of Pathophysiology, College of Basic Medical Science, China Medical University, Shenyang, China[a]; Department of Immunology, College of Basic Medical Science, China Medical University, Shenyang, China[b]; Department of Pathology, College of Basic Medical Science, China Medical University, Shenyang, China[c]

ABSTRACT Activation of caspase-11 by some Gram-negative bacteria triggers the caspase-1/interleukin 1β (IL-1β) pathway, independent of canonical inflammasomes. *Acinetobacter baumannii* is a Gram-negative, conditionally pathogenic bacterium that can cause severe pulmonary infection in hospitalized patients. *A. baumannii* was revealed to activate canonical and noncanonical inflammasome pathways in bone marrow-derived macrophages (BMDMs). Pulmonary infection of caspase-11$^{-/-}$ mice with *A. baumannii* showed that caspase-11 deficiency impaired *A. baumannii* clearance, exacerbated pulmonary pathological changes, and enhanced susceptibility to *A. baumannii*. These data indicate that the caspase-11-mediated innate immune response plays a crucial role in defending against *A. baumannii*.

KEYWORDS caspase-11, caspase-1, inflammasome, *A. baumannii*

Received 17 May 2017 **Returned for modification** 2 June 2017 **Accepted** 27 July 2017

Accepted manuscript posted online 31 July 2017

Citation Wang W, Shao Y, Li S, Xin N, Ma T, Zhao C, Song M. 2017. Caspase-11 plays a protective role in pulmonary *Acinetobacter baumannii* infection. Infect Immun 85:e00350-17. https://doi.org/10.1128/IAI.00350-17.

Editor Andreas J. Bäumler, University of California, Davis

Address correspondence to Chenghai Zhao, zhaochenghai1@sina.com, or Min Song, minsongli@sina.com.

Innate immune cells, such as macrophages, recognize invasive pathogens through pattern recognition receptors (PRRs). NOD-like receptors (NLRs), belonging to the PRRs, recruit pro-caspase-1 to assemble inflammasomes (1–3). Pro-caspase-1 is autocleaved into active caspase-1, which further cleaves pro-interleukin 1β (IL-1β) and pro-IL-18 to form mature IL-1β and IL-18. Activation of NLRs/caspase-1 inflammasomes is usually called the canonical inflammasome pathway. The NLRP3 inflammasome is one of the most important canonical inflammasomes and can be activated by a variety of microorganisms, including bacteria, viruses, and fungi (4–6).

In recent years, another member of the inflammatory caspase family, caspase-11, has been demonstrated to activate caspase-1/IL-1β independently of NLRs, which is called the noncanonical inflammasome pathway (7–9). Gram-negative bacteria are the major factors in caspase-11 activation, and lipopolysaccharide (LPS) has been identified as a cytoplasmic ligand for caspase-11 (7–9). Activation of both caspase-1 and caspase-11 can induce cell pyroptosis.

As a Gram-negative, conditionally pathogenic bacterium, *Acinetobacter baumannii* can cause severe skin, pulmonary, or urinary tract infection in hospitalized patients (10–12). It has been revealed that *A. baumannii* can activate Toll-like receptor 4 (TLR4), another cellular membrane PRR, as well as NF-κB (13–15). However, it is unclear whether *A. baumannii* can activate the caspase-11 pathway. In the present study, caspase-11 knockout (*caspase-11*$^{-/-}$) mice were infected with *A. baumannii* to explore the role of caspase-11 in pulmonary *A. baumannii* infection.

RESULTS

***A. baumannii* can activate the canonical and noncanonical inflammasome pathways in BMDMs.** To investigate whether *A. baumannii* can activate the canonical

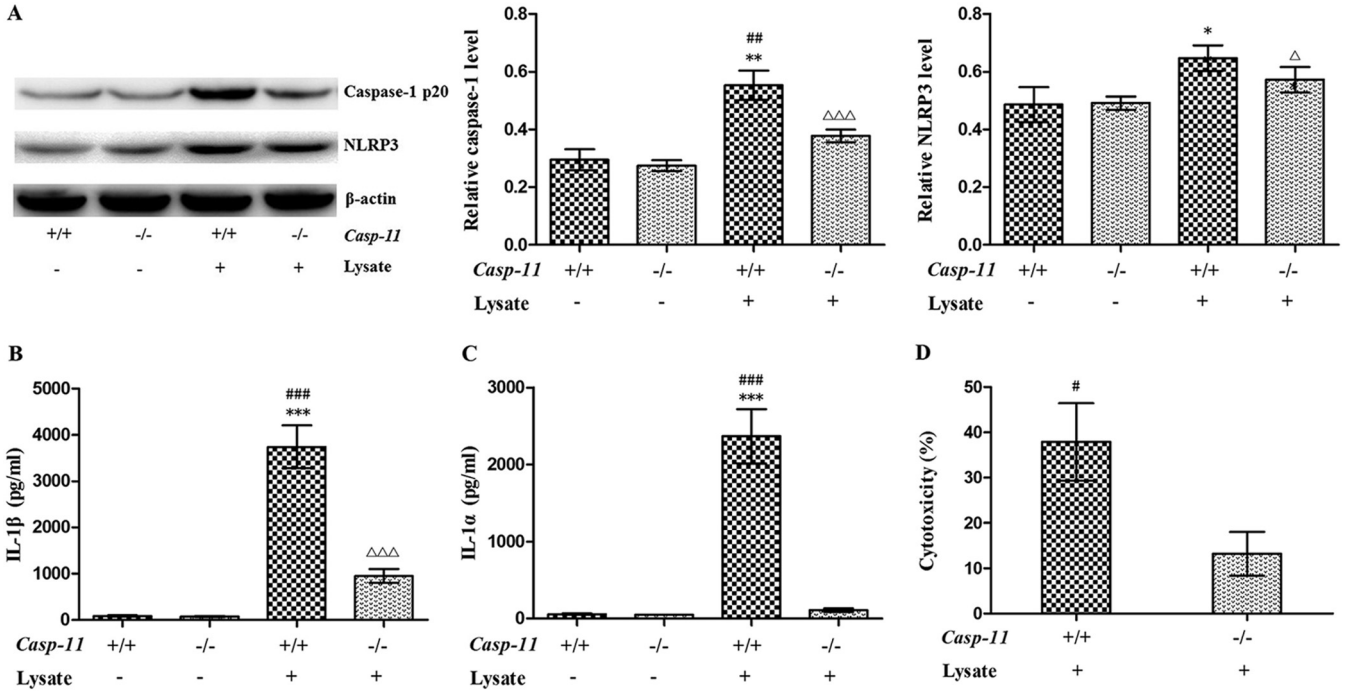

FIG 1 Activation of the NLRP3 inflammasome pathway and the noncanonical inflammasome pathway by *A. baumannii* in BMDMs. Lysate from 1×10^8 *A. baumannii* bacteria was used to treat 1×10^6 BMDMs. (A) Caspase-1 and NLRP3 expression in BMDMs was detected by Western blotting. (B and C) IL-1β and IL-1α secretion was measured by ELISA. (D) Cytotoxicity was analyzed with a lactate dehydrogenase kit. *, $P < 0.05$ versus *casp-11*$^{+/+}$ lysate$^-$; **, $P < 0.01$ versus *casp-11*$^{+/+}$ lysate$^-$; ***, $P < 0.001$ versus *casp-11*$^{+/+}$ lysate$^-$; #, $P < 0.05$ versus *casp-11*$^{-/-}$ lysate$^+$; ##, $P < 0.01$ versus *casp-11*$^{-/-}$ lysate$^+$; ###, $P < 0.001$ versus *casp-11*$^{-/-}$ lysate$^+$; △, $P < 0.05$ versus *casp-11*$^{-/-}$ lysate$^-$; △△△, $P < 0.001$ versus *casp-11*$^{-/-}$ lysate$^-$. *casp-11*, *caspase-11*. The data are expressed as means ± SD, and all the experiments were performed three times.

and noncanonical inflammasome pathways, bone marrow-derived macrophages (BMDMs) from wild-type (*caspase-11*$^{+/+}$) and *caspase-11*$^{-/-}$ mice were treated with *A. baumannii* lysate for 12 h. Caspase-1 and NLRP3 expression and IL-1β secretion were upregulated in both *caspase-11*$^{+/+}$ BMDMs and c*aspase-11*$^{-/-}$ BMDMs, indicating that *A. baumannii* can activate NLRP3 inflammasomes even though caspase-11 is deficient (Fig. 1A and B). However, induction of caspase-1 expression and IL-1β secretion was partially abrogated, and induction of IL-1α was completely abrogated, in *caspase-11*$^{+/+}$ BMDMs when caspase-11 was knocked out (Fig. 1A to C); furthermore, the cytotoxicity of *A. baumannii* for *caspase-11*$^{-/-}$ BMDMs was significantly lower than that for *caspase-11*$^{+/+}$ BMDMs (Fig. 1D), suggesting that *A. baumannii* can also activate the noncanonical inflammasome pathway.

Caspase-11 deficiency impairs *A. baumannii* clearance. Both *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ mice were infected with *A. baumannii* (2×10^8 bacteria). The number of CFU in bronchoalveolar lavage fluid (BALF) from *caspase-11*$^{-/-}$ mice was much higher than in BALF from *caspase-11*$^{+/+}$ mice 6, 24, and 72 h after infection (Fig. 2A). Consistently, the number of CFU in lung tissue of *caspase-11*$^{-/-}$ mice was also higher than in that of *caspase-11*$^{+/+}$ mice 6, 24, and 72 h after infection (Fig. 2B). Moreover, bacterial burdens in spleens and livers of *caspase-11*$^{-/-}$ mice were also higher than those in *caspase-11*$^{+/+}$ mice at the above-mentioned time points (Fig. 2C and D).

Caspase-11 deficiency aggravates the pulmonary inflammatory response to *A. baumannii* infection. There were fewer neutrophils in BALF from *caspase-11*$^{-/-}$ mice than in BALF from *caspase-11*$^{+/+}$ mice 6 and 24 h after infection but notably more than in BALF from *caspase-11*$^{+/+}$ mice 72 h after infection (Fig. 3A). Consistently, myeloperoxidase (MPO) activity in lung tissue of *caspase-11*$^{-/-}$ mice was lower than that in *caspase-11*$^{+/+}$ mice 6 and 24 h after infection but remarkably higher than that in *caspase-11*$^{+/+}$ mice 72 h after infection (Fig. 3B). Levels of IL-1β in BALF from *caspase-11*$^{-/-}$ mice were lower at 6 h but higher at 24 and 72 h after infection than in BALF

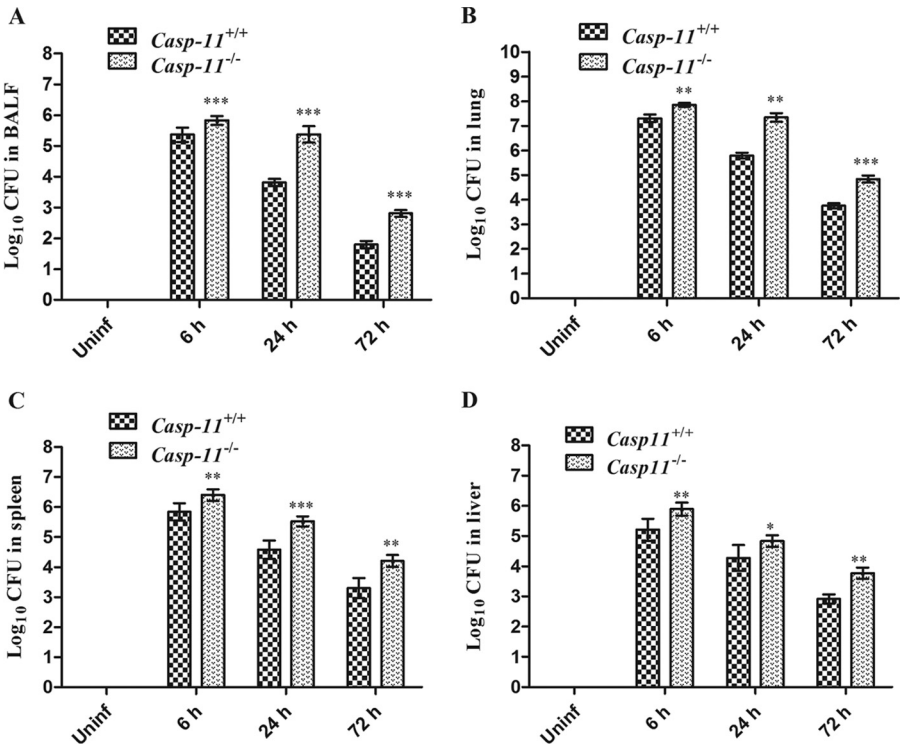

FIG 2 Bacterial burdens in *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ mice. (A and B) CFU in BALF and lungs of *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ mice infected with 2×10^8 *A. baumannii* bacteria. (C and D) CFU in spleens and livers of *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ mice infected with 2×10^8 *A. baumannii* bacteria. *, $P < 0.05$ versus *casp-11*$^{+/+}$; **, $P < 0.01$ versus *casp-11*$^{+/+}$; ***, $P < 0.001$ versus *casp-11*$^{+/+}$. *casp-11*, *caspase-11*; Uninf, uninfected. The data are expressed as means ± SD. Each group contained 5 mice.

from *caspase-11*$^{+/+}$ mice (Fig. 3C). Moreover, BALF from *caspase-11*$^{-/-}$ mice contained more IL-6 and tumor necrosis factor alpha (TNF-α) but less IL-1α than BALF from *caspase-11*$^{+/+}$ mice at each time point (Fig. 3D to F).

Caspase-11 deficiency exacerbates pulmonary pathological changes caused by *A. baumannii* infection. Pulmonary pathological changes were evaluated by hematoxylin-eosin (HE) staining. No obvious pathological change was observed in the lungs of either *caspase-11*$^{+/+}$ or *caspase-11*$^{-/-}$ mice without *A. baumannii* infection (Fig. 4, Uninf). Six hours after infection, fewer neutrophils had infiltrated into lungs of *caspase-11*$^{-/-}$ mice than into those of *caspase-11*$^{+/+}$ mice (Fig. 4, 6 h). However, 24 h after infection, lungs from both types of mice exhibited comparable pathological changes, with infiltration of large quantities of neutrophils and local consolidation (Fig. 4, 24 h). Seventy-two hours after infection, pulmonary pathological changes in *caspase-11*$^{-/-}$ were mice significantly exacerbated and were characterized by extensive neutrophil infiltration and consolidation (Fig. 4, 72 h).

Caspase-11 deficiency enhances susceptibility to *A. baumannii*. To investigate the tolerance of mice for *A. baumannii*, both *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ mice were infected with 1×10^8 or 5×10^8 *A. baumannii* bacteria. It was found that both types of mice survived infection with 1×10^8 *A. baumannii* bacteria; however, after infection with 5×10^8 *A. baumannii* bacteria, the survival rate of *caspase-11*$^{+/+}$ mice (7/8; 87.5%) was significantly higher than that of *caspase-11*$^{-/-}$ mice (2/8; 25%) (Fig. 5A). HE staining showed that 24 h after infection with 5×10^8 *A. baumannii* bacteria, *caspase-11*$^{-/-}$ mice displayed very serious pathological changes characterized by diffuse inflammatory cell infiltration and consolidation (Fig. 5B and C).

DISCUSSION

Some Gram-negative bacteria, such as *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, and *Citrobacter freundii*, have been shown to activate both the NLRP3

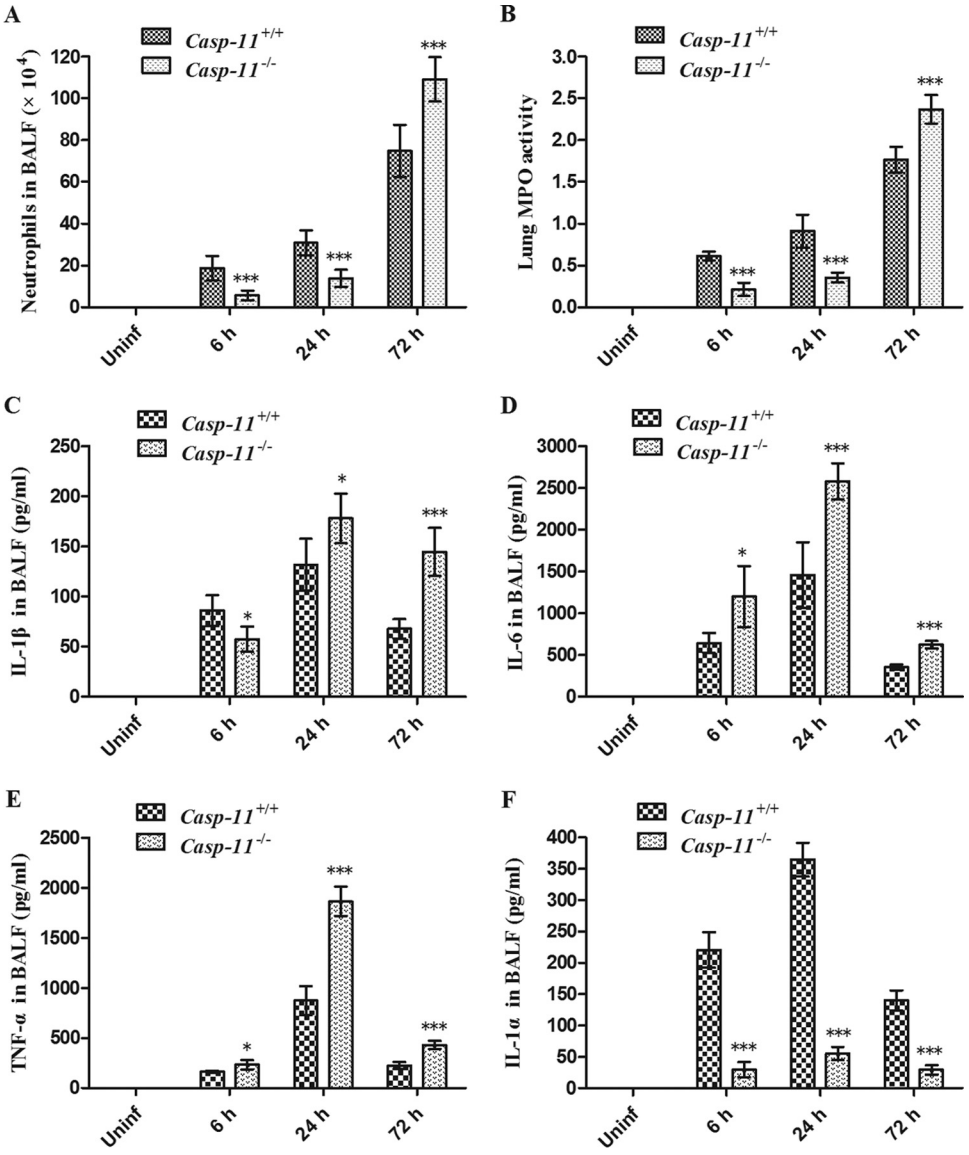

FIG 3 Pulmonary inflammatory response to *A. baumannii* in *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ mice. (A) Neutrophils in BALF from *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ mice infected with 2×10^8 *A. baumannii* bacteria. (B) MPO activity in lung tissue of *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ mice infected with 2×10^8 *A. baumannii* bacteria. (C to F) Levels of IL-1β, IL-6, TNF-α, and IL-1α in BALF from *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ mice infected with 2×10^8 *A. baumannii* bacteria. *, $P < 0.05$ versus *casp-11*$^{+/+}$; **, $P < 0.01$ versus *casp-11*$^{+/+}$; ***, $P < 0.001$ versus *casp-11*$^{+/+}$. *casp-11*, *caspase-11*; Uninf, uninfected. The data are expressed as means ± SD. Each group contained 5 mice.

inflammasome and noncanonical inflammasome pathways (8, 16–18). Our *in vitro* study showed that *A. baumannii* upregulated caspase-1 and NLRP3 expression and IL-1β secretion in both *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ BMDMs, indicating that *A. baumannii* can activate the NLRP3 inflammasome. Consistently, Kang et al. found that *A. baumannii*-induced IL-1β secretion was dependent on NLRP3, the adaptor ASC (apoptosis-associated speck-like protein containing a CARD), and caspase-1, involving the P2X7 receptor, K^+ efflux, reactive oxygen species (ROS) production, and cathepsin release (19).

In addition to the NLRP3 inflammasome, our *in vitro* study has revealed that *A. baumannii* can activate the caspase-11-mediated noncanonical inflammasome, based on the following observations. (i) Caspase-11 knockout partially abrogated *A. baumannii*-induced caspase-1 expression and IL-1β secretion. (ii) Caspase-11 knockout completely abrogated *A. baumannii*-induced IL-1α secretion. (iii) Caspase-11

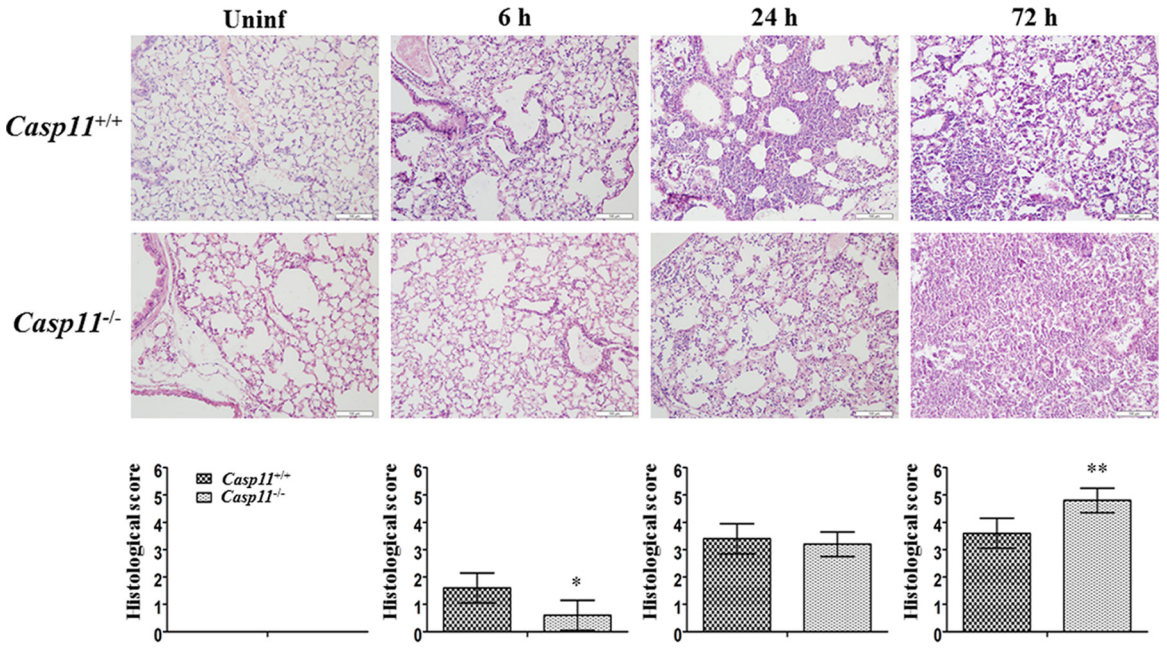

FIG 4 Pulmonary pathological changes of *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ mice. Lung tissues were stained with HE, and lung pathology was scored blindly in *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ mice after infection with 2×10^8 *A. baumannii* bacteria. *, $P < 0.05$ versus *casp-11*$^{+/+}$; **, $P < 0.01$ versus *casp-11*$^{+/+}$. *casp-11*, *caspase-11*; Uninf, uninfected. The data are expressed as means ± SD. Each group contained 5 mice.

knockout reduced the cytotoxicity of *A. baumannii* on BMDMs. Therefore, these data suggest that caspase-11 deficiency may impair the innate immune response to *A. baumannii* infection.

Actually, our *in vivo* study showed that the numbers of CFU in BALF and lung tissue from *caspase-11*$^{-/-}$ mice were much higher than those in *caspase-11*$^{+/+}$ mice 24 h or 72 h after infection with the same quantity of *A. baumannii* bacteria, indicating that caspase-11 knockout impaired the ability of macrophages to clear *A. baumannii*. Similarly, *caspase-11*$^{-/-}$ mice exhibited decreased capability to clear *Salmonella* Typhimurium in the intestine compared with *caspase-11*$^{+/+}$ mice (20). In addition, *caspase-11*$^{-/-}$ mice also displayed deficiency in *Legionella pneumophila* clearance (21).

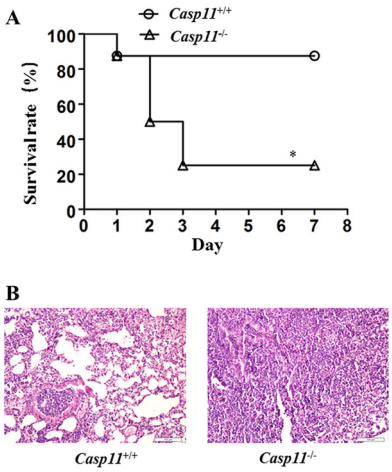

FIG 5 Survival rate of *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ mice. (A) Kaplan-Meier analysis of the survival rate of *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ mice after infection with 5×10^8 *A. baumannii* bacteria. (B) Pulmonary pathology of *caspase-11*$^{+/+}$ and *caspase-11*$^{-/-}$ mice 24 h after infection with 5×10^8 *A. baumannii* bacteria. *, $P < 0.05$ versus *casp-11*$^{+/+}$. Each group contained 8 mice.

As a consequence of deficiency in bacterial clearance, *caspase-11*$^{-/-}$ mice demonstrated exacerbated pulmonary pathological changes characterized by extensive neutrophil infiltration and consolidation for the duration of infection with *A. baumannii*. Consistently, the survival rate of *caspase-11*$^{-/-}$ mice was much lower than that of *caspase-11*$^{+/+}$ mice, indicating that caspase-11 knockout increases susceptibility to *A. baumannii* infection. The increased bacterial burden may activate NLRs or TLRs to increase cytokine release and inflammatory response.

In summary, our studies have shown that *A. baumannii* can activate the noncanonical caspase-11 inflammasome and that caspase-11 deficiency impairs pulmonary *A. baumannii* clearance, resulting in exaggerated pulmonary inflammation and consolidation. These data indicate that the caspase-11-mediated innate immune response plays a crucial role in defending against *A. baumannii* invasion.

MATERIALS AND METHODS

Animals. Six- to 8-week-old female specific-pathogen-free (SPF) caspase-11$^{-/-}$ mice (C57BL/6 background) and wild-type C57BL/6 (caspase-11$^{+/+}$) mice were studied in accordance with the Guide to the Care and Use of Experimental Animals (China). The experimental procedures were approved by the China Medical University Animal Care and Use Committee. Caspase-11$^{-/-}$ mice were purchased from Jackson Laboratory, and wild-type C57BL/6 mice were provided by the Animal Division, China Medical University.

Bone marrow-derived macrophage generation. Bone marrow was harvested from mouse femurs and tibias. BMDMs were generated by plating bone marrow cells in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 10% macrophage colony-stimulating factor (M-CSF) (L929 cell supernatant).

Bacterial culture, inoculation, and CFU measurement. *A. baumannii* (ATCC BAA-1605) bacteria were grown overnight in tryptic soy broth (TSB) at 37°C. *A. baumannii* bacteria (2×10^8) in 20 μl saline were administered to anesthetized mice intranasally. Twenty-four or 72 h after infection, mice were sacrificed for examination. To quantify CFU, suspension fluid from BALF or homogenized lung tissue was inoculated into sterile tryptic soy agar (TSA) at 37°C for 24 h.

Bacterial lysate preparation. Bacteria were collected by centrifugation at 4°C and 7,000 $\times$ *g* for 10 min and were suspended in 1 ml phosphate-buffered saline (PBS). Subsequently, the bacteria were sonicated with a JY96-IIN sonifier (Xinzhi, Ningbo, China) on ice. After centrifugation at 4°C and 13,000 $\times$ *g* for 10 min, the supernatant was filtered by using a 0.45-μm membrane (Millipore). Lysate from 1×10^8 bacteria was used to treat 1×10^6 BMDMs.

Western blotting. Equal amounts of proteins were separated on 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore) in a wet electron transfer device. The membranes were blocked in 5% bovine serum albumin (BSA) in Tris-buffered saline (TBS) containing 0.05% Tween 20 for 1 h at room temperature. The membranes were incubated with rabbit polyclonal antibody for caspase-1 (1:1,000; Abcam) or rabbit polyclonal antibody for NLRP3 (1:500; Abcam) overnight at 4°C. After being washed in TBS-Tween 20 three times, the membranes were incubated in horseradish peroxidase (HRP)-conjugated goat anti-rabbit (1:5,000) for 2 h at room temperature. An enhanced chemiluminescence (ECL) kit was used to visualize target proteins. The optical densities (ODs) of the bands were determined with Image-J software.

Bronchoalveolar lavage fluid collection and staining. BALF was collected by injecting into the lungs and withdrawing 1 ml saline. The BALF was centrifuged at 2,000 rpm for 5 min, and the supernatant was collected for enzyme-linked immunosorbent assay (ELISA) detection. Lysis buffer was added to the precipitant to eliminate red blood cells. After another centrifugation at 2,000 rpm for 5 min, the precipitant was resuspended. The suspension fluid was then smeared, and the neutrophils were counted after Wright staining.

ELISA. Levels of TNF-α, IL-1β, IL-6, and IL-1α in the supernatants of BMDMs and BALF were measured using mouse ELISA kits (eBioscience) for the cytokines according to the manufacturer's instructions. The sensitivity was 8 pg/ml for TNF-α and IL-1β and 4 pg/ml for IL-6 and IL-1α.

Cytotoxicity assay. The cytotoxicity of *A. baumannii* for BMDMs was analyzed using a lactate dehydrogenase (LDH) assay kit (Beyotime Biotechnology, China). After 12 h of infection, cell supernatants were harvested for analysis of LDH release by dying cells. The absorbance at 490 nm was measured with a plate reader spectrophotometer, and cytotoxicity values were determined based on the following equation: $(OD_{sample} - OD_{low\ control})/(OD_{high\ control} - OD_{low\ control}) \times 100$, where low control indicates BMDMs without bacterial infection and high control indicates BMDMs (without bacterial infection) treated with LDH-releasing agent.

Myeloperoxidase activity assay. MPO activity in lung tissue was measured with an MPO assay kit (Nanjing Jiancheng Corp., China) according to the manufacturer's instructions. The absorbance at 460 nm was measured with a plate reader spectrophotometer. The MPO value (units per gram of lung tissue) was determined based on the following equation: $(OD_{sample} - OD_{control})/11.3 \times$ amount of sample (in grams).

Hematoxylin-eosin staining. Lung tissue was fixed with 10% formalin and embedded in paraffin; 4-μm-thick sections were stained with HE and observed using a Leica DMRB microscope. The degree of pathology was judged blindly and scored according to the following criteria (22): 0, no reaction in alveolar walls; 1, diffuse reaction in alveolar walls, primarily neutrophilic, with no thickening of alveolar walls; 2, diffuse presence of inflammatory cells in alveolar walls with slight thickening; 3, distinct

thickening of alveolar walls due to the presence of inflammatory cells; 4, alveolar wall thickening with up to 25% of the lung consolidated; 5, alveolar wall thickening with more than 50% of the lung consolidated.

Statistical analysis. Data are expressed as means ± standard deviations (SD). Differences were analyzed by Student's *t* test or one-way analysis of variance (ANOVA). The survival rate was determined by the Kaplan-Meier method and compared by log-rank test. Statistical analysis was performed using SPSS 13.0. Differences were considered significant when the *P* value was less than 0.05.

REFERENCES

1. Sharma D, Kanneganti TD. 2016. The cell biology of inflammasomes: mechanisms of inflammasome activation and regulation. J Cell Biol 213:617–629. https://doi.org/10.1083/jcb.201602089.
2. Man SM, Kanneganti TD. 2015. Regulation of inflammasome activation. Immunol Rev 265:6–21. https://doi.org/10.1111/imr.12296.
3. Latz E, Xiao TS, Stutz A. 2013. Activation and regulation of the inflammasomes. Nat Rev Immunol 13:397–411. https://doi.org/10.1038/nri3452.
4. Marim FM, Franco MM, Gomes MT, Miraglia MC, Giambartolomei GH, Oliveira SC. 2017. The role of NLRP3 and AIM2 in inflammasome activation during Brucella abortus infection. Semin Immunopathol 39:215–223. https://doi.org/10.1007/s00281-016-0581-1.
5. Jo EK, Kim JK, Shin DM, Sasakawa C. 2016. Molecular mechanisms regulating NLRP3 inflammasome activation. Cell Mol Immunol 13:148–159. https://doi.org/10.1038/cmi.2015.95.
6. Franchi L, Munoz-Planillo R, Nunez G. 2012. Sensing and reacting to microbes through the inflammasomes. Nat Immunol 13:325–332. https://doi.org/10.1038/ni.2231.
7. Shi J, Zhao Y, Wang Y, Gao W, Ding J, Li P, Hu L, Shao F. 2014. Inflammatory caspases are innate immune receptors for intracellular LPS. Nature 514:187–192. https://doi.org/10.1038/nature13820.
8. Kayagaki N, Wong MT, Stowe IB, Ramani SR, Gonzalez LC, Akashi-Takamura S, Miyake K, Zhang J, Lee WP, Muszynski A, Forsberg LS, Carlson RW, Dixit VM. 2013. Noncanonical inflammasome activation by intracellular LPS independent of TLR4. Science 341:1246–1249. https://doi.org/10.1126/science.1240248.
9. Hagar JA, Powell DA, Aachoui Y, Ernst RK, Miao EA. 2013. Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock. Science 341:1250–1253. https://doi.org/10.1126/science.1240988.
10. Garcia-Patino MG, Garcia-Contreras R, Licona-Limon P. 2017. The immune response against Acinetobacter baumannii, an emerging pathogen in nosocomial infections. Front Immunol 8:441. https://doi.org/10.3389/fimmu.2017.00441.
11. Dexter C, Murray GL, Paulsen IT, Peleg AY. 2015. Community-acquired Acinetobacter baumannii: clinical characteristics, epidemiology and pathogenesis. Expert Rev Anti Infect Ther 13:567–573. https://doi.org/10.1586/14787210.2015.1025055.
12. Peleg AY, Seifert H, Paterson DL. 2008. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev 21:538–582. https://doi.org/10.1128/CMR.00058-07.
13. Korneev KV, Arbatsky NP, Molinaro A, Palmigiano A, Shaikhutdinova RZ, Shneider MM, Pier GB, Kondakova AN, Sviriaeva EN, Sturiale L, Garozzo D, Kruglov AA, Nedospasov SA, Drutskaya MS, Knirel YA, Kuprash DV. 2015. Structural relationship of the lipid A acyl groups to activation of murine Toll-like receptor 4 by lipopolysaccharides from pathogenic strains of Burkholderia mallei, Acinetobacter baumannii, and Pseudomonas aeruginosa. Front Immunol 6:595. https://doi.org/10.3389/fimmu.2015.00595.
14. Kim CH, Jeong YJ, Lee J, Jeon SJ, Park SR, Kang MJ, Park JH, Park JH. 2013. Essential role of Toll-like receptor 4 in Acinetobacter baumannii-induced immune responses in immune cells. Microb Pathog 54:20–25. https://doi.org/10.1016/j.micpath.2012.08.008.
15. March C, Regueiro V, Llobet E, Moranta D, Morey P, Garmendia J, Bengoechea JA. 2010. Dissection of host cell signal transduction during Acinetobacter baumannii-triggered inflammatory response. PLoS One 5:e10033. https://doi.org/10.1371/journal.pone.0010033.
16. Kailasan Vanaja S, Rathinam VA, Atianand MK, Kalantari P, Skehan B, Fitzgerald KA, Leong JM. 2014. Bacterial RNA:DNA hybrids are activators of the NLRP3 inflammasome. Proc Natl Acad Sci U S A 111:7765–7770. https://doi.org/10.1073/pnas.1400075111.
17. Broz P, Newton K, Lamkanfi M, Mariathasan S, Dixit VM, Monack DM. 2010. Redundant roles for inflammasome receptors NLRP3 and NLRC4 in host defense against Salmonella. J Exp Med 207:1745–1755. https://doi.org/10.1084/jem.20100257.
18. Lupfer CR, Anand PK, Liu Z, Stokes KL, Vogel P, Lamkanfi M, Kanneganti TD. 2014. Reactive oxygen species regulate caspase-11 expression and activation of the non-canonical NLRP3 inflammasome during enteric pathogen infection. PLoS Pathog 10:e1004410. https://doi.org/10.1371/journal.ppat.1004410.
19. Kang MJ, Jo SG, Kim DJ, Park JH. 2017. NLRP3 inflammasome mediates interleukin-1beta production in immune cells in response to Acinetobacter baumannii and contributes to pulmonary inflammation in mice. Immunology 150:495–505. https://doi.org/10.1111/imm.12704.
20. Knodler LA, Crowley SM, Sham HP, Yang H, Wrande M, Ma C, Ernst RK, Steele-Mortimer O, Celli J, Vallance BA. 2014. Noncanonical inflammasome activation of caspase-4/caspase-11 mediates epithelial defenses against enteric bacterial pathogens. Cell Host Microbe 16:249–256. https://doi.org/10.1016/j.chom.2014.07.002.
21. Akhter A, Caution K, Abu Khweek A, Tazi M, Abdulrahman BA, Abdelaziz DH, Voss OH, Doseff AI, Hassan H, Azad AK, Schlesinger LS, Wewers MD, Gavrilin MA, Amer AO. 2012. Caspase-11 promotes the fusion of phagosomes harboring pathogenic bacteria with lysosomes by modulating actin polymerization. Immunity 37:35–47. https://doi.org/10.1016/j.immuni.2012.05.001.
22. Szarka RJ, Wang N, Gordon L, Nation PN, Smith RH. 1997. A murine model of pulmonary damage induced by lipopolysaccharide via intranasal instillation. J Immunol Methods 202:49–57. https://doi.org/10.1016/S0022-1759(96)00236-0.