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Caspase-11 deficiency impairs neutrophil recruitment and bacterial clearance in the early stage of pulmonary *Klebsiella pneumoniae* infection

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ABSTRACT

Klebsiella pneumoniae (*K. pneumoniae*) is a gram-negative pathogen, and Klebsiella pneumonia is one of the most common nosocomial infections. Canonical NLRP3 and NLRC4 inflammasome was found involved in innate immune response against *K. pneumoniae*, but the role of caspase-11 in *K. pneumoniae* infection remains undefined. It was shown that *Caspase-11* knockout blocked *K. pneumoniae*-induced IL-1α and IL-1β secretion and pyroptosis of bone marrow-derived macrophages (BMDMs). Furthermore, *caspase-11*$^{-/-}$ mice exhibited impaired neutrophil recruitment and bacterial clearance in the early stage of *K. pneumoniae* infection, accompanied by a reduction in IL-1α production. Moreover, IL-1α neutralizing antibody pretreatment was found to inhibit neutrophil recruitment and bacterial clearance of wild-type mice. Together, these data suggest that caspase-11/IL-1α pathway plays an important role in defending against *K. pneumoniae* by recruiting neutrophils in the early stage of infection.

1. Introduction

Innate immune system plays an essential role in protection from microorganism infection. Activation of canonical inflammasome is one of the important immune responses to bacteria, viruses and other pathogens. Assembly of canonical inflammasome involves some pattern recognition receptors (PRRs), such as NOD-like receptor (NLR), which recruits pro-caspase-1 with or without adaptor protein (Latz et al., 2013; Man and Kanneganti, 2015; Sharma and Kanneganti, 2016). Subsequently, pro-caspase-1 is auto-cleaved into active caspase-1, and the latter further cleaves pro-IL-1β and pro-IL-18 to form mature IL-1β and IL-18. Cytokine release as well as immune cell recruitment attributes to inflammatory response and pathogen clearance.

In addition to canonical inflammasome, some gram-negative bacteria can activate pro-caspase-1 via LPS, which binds to another inflammatory caspase-caspase-11. Binding of caspase-11 with LPS similarly cleaves pro-caspase-1, forming mature IL-1β and IL-18, therefore, caspase-11 is also considered as a cytosolic PRR (Hagar et al., 2013; Kayagaki et al., 2013; Shi et al., 2014). Activation of caspase-11 also induces IL-1α release, and this process is not related to caspase-1. Contrary to IL-1β, the role of IL-1α in innate immune response and bacterial clearance has not been enough explored and elucidated.

Klebsiella pneumoniae (*K. pneumoniae*) is a gram-negative pathogen, which can lead to both community and nosocomial pneumonia, urinary tract infection, liver abscess and sepsis, and Klebsiella pneumonia is one of the most common nosocomial infections (Broberg et al., 2014). It has been shown that canonical NLRP3 and NLRC4 inflammasome is involved in innate immune response against *K. pneumoniae* (Cai et al., 2012; Willingham et al., 2009). In the present study, caspase-11 knockout (*caspase-11*$^{-/-}$) mice were infected with *K. pneumoniae* to explore the role of caspase-11 in pulmonary *K. pneumoniae* infection.

2. Materials and methods

2.1. Animals

6–8-week-old specific pathogen free (SPF) *caspase-11* knockout (caspase-11$^{-/-}$) mice and wild-type C57BL/6 (*caspase-11*$^{+/+}$) mice were used according to Guide to the Care and Use of Experimental Animals (China). Experimental procedures were approved by China Medical University Animal Care and Use Committee. Caspase-11$^{-/-}$ mice (C57BL/6 background) were provided by the Jackson Laboratory, and wild-type C57BL/6 mice were obtained from Animal Division China Medical University.

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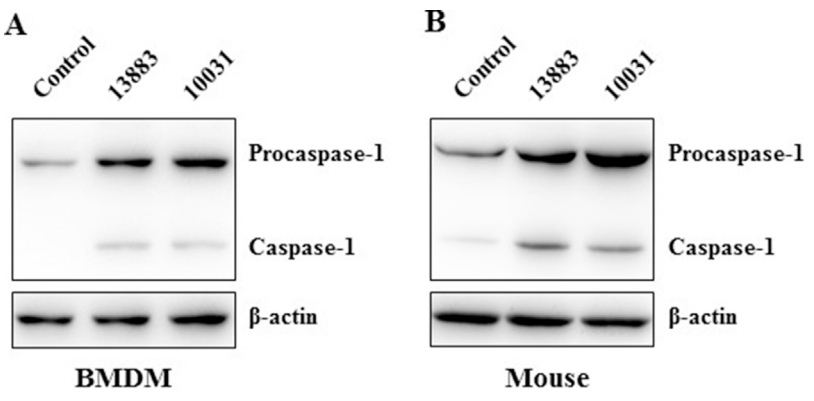

Fig. 1. Activation of capase-11 by *K. pneumoniae* in *caspase-11*$^{+/+}$ BMDMs and mice. (A) *K. pneumoniae* 10031/13883 induced procaspase-11 and caspase-11 expression in *caspase-11*$^{+/+}$ BMDMs. (B) *K. pneumoniae* 10031/13883 induced procaspase-11 and caspase-11 expression in *caspase-11*$^{+/+}$ mice. All the experiments were performed three times. Each group contained 5 mice.

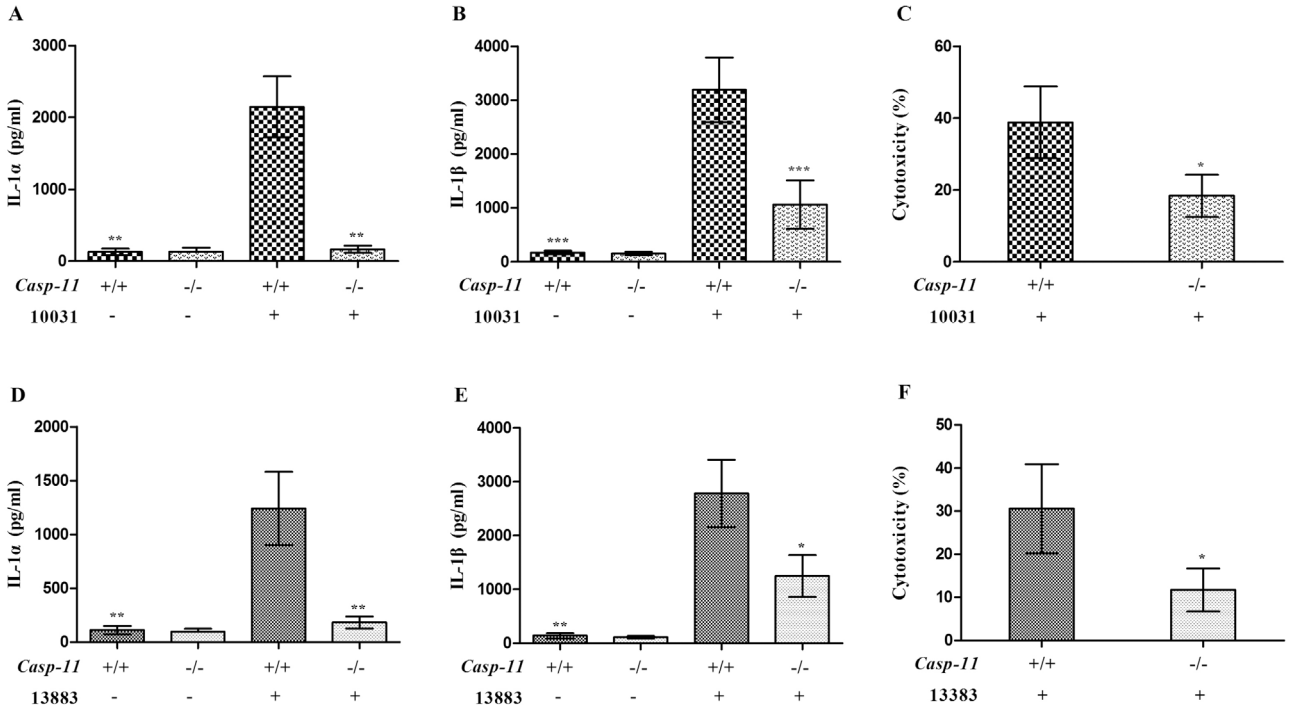

Fig. 2. Blockade of *K. pneumoniae*-induced IL-1α and IL-1β secretion in *caspase-11*$^{-/-}$ BMDMs. (A, B) ELISA detection showed that *caspase-11* knockout blocked *K. pneumoniae* 10031 −induced IL-1α and IL-1β secretion by BMDMs. (C) Lactate dehydrogenase assay revealed that *caspase-11* knockout blocked *K. pneumoniae* 10031 –induced pyroptosis of BMDMs. (D, E) ELISA detection showed that *caspase-11* knockout blocked *K. pneumoniae* 13883 –induced IL-1α and IL-1β secretion by BMDMs. (F) Lactate dehydrogenase assay revealed that *caspase-11* knockout blocked *K. pneumoniae* 13883 –induced pyroptosis of BMDMs. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, *vs casp-11*$^{+/+}$ 10031/13883(+). *casp-11*: *caspase-11*. Data are expressed as mean ± SD, and all the experiments were performed three times.

2.2. Bone marrow-derived macrophage generation

Bone marrows were harvested from mouse femur and tibia. To generate bone marrow-derived macrophages (BMDM), bone marrow cells were plated in DMEM with 10% FBS and 10% M-CSF (L929 cell supernatant).

2.3. Bacteria culture, inoculation and CFU measurement

K. pneumoniae (ATCC 10031, 13883) were grown overnight in TSB at 37 °C. 2×10^7 *K. pneumoniae* in 20 μl saline were administrated to anesthetized mice intranasally. At indicated time point, mice were sacrificed for examination. To quantify CFU (colony-forming units), suspension fluid from BALF or homogenized lung tissue was inoculated in sterile TSA at 37 °C for 24 h.

2.4. Neutralizing antibody treatment

IL-1α neutralizing antibody (Abcam, 50 μg or 100 μg per mice) or IL-1β neutralizing antibody (Abcam, 50 μg or 100 μg per mice) was used to treat *caspase-11*$^{+/+}$ mice by peritoneal injection 2 h before *K. pneumoniae* infection.

2.5. Bronchoalveolar lavage fluid collection and staining

1 ml saline was injected and withdrawn into the lungs to collect bronchoalveolar lavage fluid (BALF), which was centrifuged at 2000 rpm for 5 min. The supernatants were collected for ELISA detection. After elimination of red blood cells, the precipitant was resuspended, and the suspension fluid was smeared. The number of neutrophils was counted with Wright staining.

2.6. Western blot

Equal amount of protein was separated on 10% SDS-polyacrylamide gels and transferred to PVDF membranes in a wet electron transfer device. The membranes were blocked in 5% BSA in TBS containing 0.05% Tween 20 for 1 h at room temperature, and then incubated with