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Burkholderia cenocepacia O polysaccharide chain contributes to caspase-1-dependent IL-1β production in macrophages

Sheetal Kotrange, Benjamin Kopp,† Anwari Akhter,* Dalia Abdelaziz,* Arwa Abu Khweek,* Kyle Caution,* Basant Abdulrahman,* Mark D. Wewers,* Karen McCoy,† Clay Marsh,* Slade A. Loutet,‡ Ximena Ortega,‡ Miguel A. Valvano,‡ and Amal O. Amer*,1*

*Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Center for Microbial Interface Biology and the Department of Internal Medicine, Ohio State University, Columbus, Ohio, USA; †Division of Pediatric Pulmonology, Nationwide Children's Hospital, Columbus, Ohio, USA; and ‡Centre for Human Immunology, Department of Microbiology and Immunology, University of Western Ontario, London, Ontario, Canada

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ABSTRACT

Burkholderia cenocepacia infections in CF patients involve heightened inflammation, fatal sepsis, and high antibiotic resistance. Proinflammatory IL-1β secretion is important in airway inflammation and tissue damage. However, little is known about this pathway in macrophages upon _B. cenocepacia_ infection. We report here that murine macrophages infected with _B. cenocepacia_ K56-2 produce proinflammatory cytokine IL-1β in a TLR4 and caspase-1-mediated manner. We also determined that the OPS (O antigen) of _B. cenocepacia_ LPS contributes to IL-1β production and pyroptotic cell death. Furthermore, we showed that the malfunction of the CFTR channel augmented IL-1β production upon _B. cenocepacia_ infection of murine macrophages. Taken together, we identified eukaryotic and bacterial factors that contribute to inflammation during _B. cenocepacia_ infection, which may aid in the design of novel approaches to control pulmonary inflammation. _J. Leukoc. Biol._ 89: 481–488; 2011.

Introduction

Macrophages represent a first-line defense against pathogens; however, the response of macrophages to invading pathogens may contribute to the destructive, inflammatory process in the lungs of CF patients [1]. Nearly 85% of the deaths in CF are a result of progressive inflammation because of respiratory infections [2, 3]. Therefore, anti-inflammatory intervention should assume a larger role in CF management until a cure for CF is discovered.

One way for macrophages to recognize pathogens is through recognition by TLRs and NLRs. TLRs activate MAPKs via the MyD88 and TRIF adaptor molecules. Through these kinase pathways, TLRs activate transcription factors that regulate the expression of various host defense genes, including IL-8, IL-6, IL-1, and TNF-α [4].

NLRs respond to microbial components by initiating the assembly of the inflammasome and the proteolytic activation of caspase-1 to generate the active forms of proinflammatory cytokines IL-1β and IL-18 and pyroptosis [5, 6]. Pyroptosis, or caspase 1-dependent cell death, is inherently inflammatory and is triggered by various pathological stimuli, such as stroke, heart attack, or cancer, and is crucial for controlling microbial infections [7]. Ultimately, tight regulation of caspase-1 activation is important to control the magnitude of the innate immune response and protect the host from possible damaging effects, such as heightened inflammation, which may lead to fatal sepsis.

Strains of the Bcc and in particular, *Burkholderia cenocepacia* and *Burkholderia multivorans*, have become a serious threat to CF patients as a result of their ability to cause lung infections with rapid and severe inflammation, necrotizing pneumonia, and sometimes fatal septicemia [8]. *B. cenocepacia* strains are also capable of patient-to-patient transmission and display resistance to nearly all clinically useful antibiotics [9]. The bacterial determinants associated with poor clinical outcome in CF patients are not clear. Bcc bacteria are extracellular, opportunistic pathogens, which can also become intracellular in eu-

Abbreviations: Bcc=*Burkholderia cepacia* complex, BMDM=bone marrow-derived macrophage, CF=cystic fibrosis, CFTR=cystic fibrosis transmembrane conductance regulator, Ct=comparative threshold, Kdo=3-*deoxy*-D-*manno*-octulosonic acid, NLR=nucleotide-binding oligomerization domain-like receptor, OPS=O polysaccharide chain, OS=oligosaccharide, RCN=relative copy number, RT-qPCR=real-time quantitative PCR, TRIF=Toll/IL-1R-domain-containing adaptor-inducing IFN-β

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1. Correspondence: Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Center for Microbial Interface Biology and the Department of Internal Medicine, Ohio State University, Biological Research Tower, 460 W. 12th Ave., Room 1014, Columbus, OH 43210, USA. E-mail: amal.amer@osumc.edu

karyotic cells such as amoebae, epithelial cells, and human macrophages [10–14].

LPS is a major component of the surface of Gram-negative bacteria. LPS consists of lipid A, core OS, and in some bacteria, O-specific polysaccharide or O antigen [15]. Lipid A is embedded in the outer leaflet of the outer membrane and accounts for the endotoxic activity of LPS [16]. The core OS moiety has inner-core and outer-core regions. The inner-core OS typically consists of one to three Kdo residues linked to the lipid A and three L-*glycero*-D-*manno*-heptose residues linked to the first Kdo [15]. The outer-core region typically consists of eight to 12 branched sugars linked to heptose II of the inner core. The O antigen is the outermost component of the LPS and consists of a repeating OS that is highly variable in terms of chemical composition, structure, and antigenicity [17].

In this study, we have used *B. cenocepacia* mutants carrying various core OS truncations to explore the roles of LPS components and host factors in inflammatory cytokine IL-1β production in murine macrophages in response to *B. cenocepacia*. We report that the LPS O antigen in this bacterium and host caspase-1 and TLR4 are important modulators of inflammatory responses in macrophages.

MATERIALS AND METHODS

Bacterial strains and culture

B. cenocepacia strain K56-2 was isolated from a CF patient. This strain is from the same ET12 lineage as the prototypic epidemic strain J2315 [18]. SAL1 is a heptoseless mutant of K56-2, which was generated by insertional mutagenesis [19]. Other isogenic mutants of *B. cenocepacia* K56-2, namely XOA3, XOA7, and XOA8 [20], carry various core OS truncations (see Supplemental Fig. 1). The mutant XOA3 has an insertional mutation in the *wbxE* gene that encodes a glycosyltransferase involved in O antigen synthesis, resulting in the production of lipid A-core OS and a partial O antigen unit [21] (Supplemental Fig. 1). This mutation recreates the same LPS phenotype as observed in strain J2315 [21], whose structure has been reported recently [22]. The mutant XOA7 has an inactivated *waaL* gene and therefore, harbors a truncated O antigen [20]. The structure of the core OS in the XOA8 strain reveals a major truncation as a result of mutation in the *wabO* gene, which encodes a putative glycosyltransferase responsible for the glucosylation of HepI (Supplemental Fig. 1). All bacterial strains were grown in LB broth at 37°C overnight with high-amplitude shaking.

BMDMs

All animal experiments were performed according to protocols approved by the Animal Care Use Committee of the Ohio State University College of Medicine (Columbus, OH, USA). WT C57BL/6, MyD88$^{-/-}$, TRIF$^{-/-}$, TLR3$^{-/-}$, and TLR4$^{-/-}$ mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Caspase-1$^{-/-}$ mice were obtained from Dr. Amy Hise (Case Western Reserve University, Cleveland, OH, USA). ΔF508 mice were obtained from Case Western Reserve University and housed in the Ohio State University vivarium. Mice homozygous for the *Cftr* mutation were provided with Colyte® (Schwarz Pharmaceuticals, Mequon, WI, USA) in their drinking water and the irradiated diet of Teklad 7960. BMDMs were isolated from femurs of 6- to 12-week-old mice and were cultured in IMDM containing 10% heat-inactivated FBS, 20% L cell-conditioned medium, 100 U/ml penicillin, and 100 mg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. After 5 days of incubation, cells were collected and plated in six-well plates or in 24-well plates in IMDM containing 10% heat-inactivated FBS [23]. About 95% of the BMDMs obtained possess the CSF-1R, 93–98% proliferate in response to CSF-1, and 90% of cells die on removal of CSF-1 from the serum-containing medium. This latter observation reflects the absence in these cultures of contaminating, fibroblast-like cells [24]. Macrophages were infected with *B. cenocepacia* K56-2 or corresponding mutants at a MOI of 10.

Immunoblotting

Macrophages were stimulated with *B. cenocepacia* K56-2, and the culture supernatant was removed. The cells were washed twice with PBS and lysed in lysis buffer solution (150 mM NaCl, 10 mM Tris, pH 7.4, 5 mM EDTA, 1 mM EGTA, and 0.1% Nonidet P-40), supplemented with 1× protease inhibitor mixture (Roche Applied Science, Indianapolis, IN, USA). The cell lysate was centrifuged for the removal of any insoluble material. The protein concentration was adjusted to 20 ug. After clarification, samples were denatured with SDS buffer and boiled for 10 min; proteins were separated on SDS-15% polyacrylamide gel and transferred to a PVDF membrane (Bio-Rad Laboratories, Hercules, CA, USA). Membranes were immunoblotted against IL-1β (antibody kindly provided by M. D. Wewers), and pro-IL-1β protein was detected with secondary anti-rabbit antibody conjugated to HRP, followed by ECL reagents (Amersham ECL Western blotting detection reagents, GE Healthcare-Life Sciences, UK).

ELISA

Macrophages were infected with *B. cenocepacia* K56-2 for 24 h, and the culture supernatant was collected, centrifuged, and stored at –20°C until assayed for cytokine content. The activity of IL-1β, IL-6, and TNF-α in the supernatant was determined by specific sandwich ELISA following the manufacturer's protocol (R&D Systems, Minneapolis, MN, USA) and as described previously [25].

RT-qPCR

Total RNA was extracted from macrophages infected for 24 h with *B. cenocepacia* K56-2 or the appropriate mutants by the phenol and guanidine isothiocyanate (TRIzol reagent)-chloroform method (Invitrogen Life Technologies, Carlsbad, CA, USA). One to 2 μg RNA was reverse-transcribed into cDNA by the Taqman MicroRNA RT kit (Applied Biosystems, Foster City, CA, USA) at 16°C for 30 min, 42°C for 30 min, and 85°C for 5 min. cDNA (26 ng) was used for qPCR with Power SYBR Green PCR master mix in the Step One Plus RT-PCR system (Applied Biosystems) at 95°C for 10 min, 95°C for 15 s, and 60°C for 1 min for 40 cycles for IL-1β and mouse GAPDH. The target gene Ct values were normalized to the Ct values of the housekeeping gene, GAPDH, and expressed as RCN, as described earlier [26]. Primer sequences used in the study for mouse IL-1β were 5′-CCTGTGTTTTCCTCCTTGCCT-3′ and 5′-GCCTAATGTCCCCTTGAATCAA-3′, and those for mouse GAPDH were 5′-GCAAAGTGGAGATTGTTGCCAT-3′ and 5′-CCTTGACTGTGCCGTTGAATTT-3′.

Cell death detection

WT macrophages were infected with *B. cenocepacia* parent strain K56-2 or mutants SAL1, XOA3, XOA7, and XOA8 for 24 h. Cell lysates from infected macrophages were prepared and used for in vitro quantification of apoptosis. Histone-associated DNA fragments were detected using the cell death detection ELISA^{plus} photometric enzyme immunoassay kit from Roche Applied Science to the specifications of the manufacturer [25]. Every sample was performed in triplicate; mean ± SD was calculated for each sample after subtraction of the blank from each reading. Results are displayed as fold increase from the untreated cells.

RESULTS

B. cenocepacia K56-2 induces caspase-1 and IL-1β mRNA in murine macrophages

To understand the role of the inflammasome in macrophages infected with *B. cenocepacia*, we quantified mRNA of different

inflammasome components by RT-qPCR. Primary WT mouse macrophages infected with *B. cenocepacia* K56-2 for 4 h were processed by RT-qPCR for open array analysis (BioTrove, Cambridge, MA, USA). Among different inflammasome components, the caspase-1 message was increased sixfold ($P<0.001$; **Fig. 1A**), and the IL-1β message was increased 20-fold in response to *B. cenocepacia* when compared with uninfected controls ($P<0.05$; Fig. 1B). Accordingly, protein levels of pro-IL-1β were increased in macrophages when infected with *B. cenocepacia* (Fig. 1F). As activation of the inflammasome is accompanied by the release of mature IL-1β into macrophage culture supernatants, we tested whether WT murine macrophages release mature IL-1β in response to *B. cenocepacia* K56-2 infection. Macrophages were infected at a MOI of 10 for 24 h, and culture supernatants were examined for the presence of active IL-1β by ELISA as indicated in Materials and Methods. Fig. 1C shows that infection with K56-2 induces the release of high levels of IL-1β. Therefore, *B. cenocepacia* provokes a strong IL-1β response in murine macrophages.

IL-1β induction and release in *B. cenocepacia* K56-2-infected murine macrophages are mainly MyD88- and partially TLR4-dependent

The induction of the IL-1β message can be TLR-mediated [27]. Accordingly, to discern the identity of TLR involved in the innate immune response to *B. cenocepacia* K56-2, infections were also carried out in macrophages lacking MyD88 or TRIF, and then IL-1β production was examined at 24 h postinfection. MyD88 is an adaptor molecule for most TLRs except TLR 3, whereas TRIF is an adaptor molecule for TLR3 and -4. Compared with infected WT macrophages, MyD88$^{-/-}$ macrophages did not significantly up-regulate the IL-1β message [eightfold difference ($P<0.05$); Fig. 1B] and only released negligible amounts of IL-1β (Fig. 1C). Also, infected TRIF$^{-/-}$ macrophages showed less up-regulation of the IL-1β message [3.5-fold difference ($P<0.05$); Fig. 1D] and released 50% less IL-1β (Fig. 1E). Western blot analysis demonstrated that pro-IL-1β protein levels were increased in infected WT macrophages but reduced in macrophages lacking MyD88 or TRIF (Fig. 1F). These data demonstrate that TLR3 or TLR4 is required for induction of IL-1β by *B. cenocepacia* K56-2.

As MyD88 and TRIF are adaptor molecules for TLR4, we examined the activation and release of IL-1β in macrophages lacking TLR4. TLR3$^{-/-}$ macrophages were used as a control. TLR4$^{-/-}$ macrophages released less IL-1β into culture supernatants when infected with K56-2 for 24 h (**Fig. 2A**). However, TLR3$^{-/-}$ macrophages released levels of IL-1β that were similar to those of infected WT macrophages (Fig. 2A). The induction of the IL-1β message was compromised in TLR4$^{-/-}$ macrophages compared with WT macrophages ($P<0.01$; Fig. 2B). IL-1β protein analysis by Western blots showed that pro-IL-1β is induced in WT macrophages and to a lesser extent, in macrophages lacking TLR4 (Fig. 2C). Therefore, the up-regulation and the expression of pro-IL-1β in response to *B. cenocepacia* K56-2 involve TLR4.

B. cenocepacia K56-2 O antigen polysaccharide contributes to IL-1β production and pyroptotic cell death of infected macrophages

As TLR4 is required for IL-1β production by *B. cenocepacia*, and TLR4 interacts with LPS, we examined the effect of *B. cenocepacia* mutants lacking different portions of the LPS molecule on IL-1β production (Supplemental Fig. 1). WT macrophages were infected with K56-2 and the mutant SAL1. Infection with the heptoseless LPS strain SAL1 resulted in a ninefold reduction ($P<0.05$) in IL-1β release compared with WT macrophages (**Fig. 3A**). SAL1 induction of IL-1β message was also defective ($P<0.05$; Fig. 3B). Yet, SAL1 induced the production of IL-6 and TNF-α similar to parent strain K56-2 (Fig. 3C and D).

To pinpoint which portion of the LPS molecule is important for induction of IL-1β production and release, we took advantage of a panel of mutants carrying various LPS truncations (Supplemental Fig. 1) [21, 28]. The macrophages infected with XOA3, XOA7, and XOA8 mutants secreted less IL-1β compared with the parent strain K56-2 (**Fig. 4A**). However, IL-6 and TNF-α production in response to XOA3, XOA7, and XOA8 was comparable with that of K56-2-infected macro-

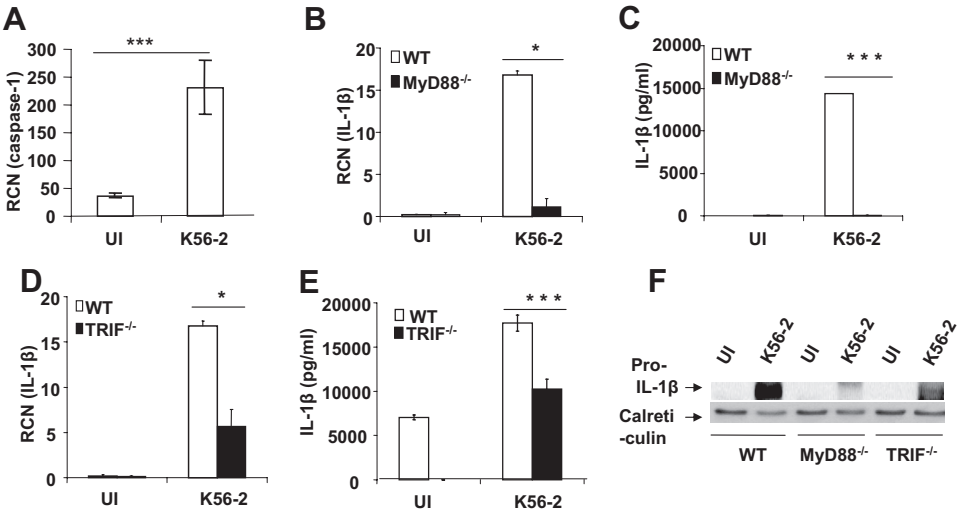

Figure 1. IL-1β is induced by *B. cenocepacia* K56-2 and requires host MyD88 and TRIF. BMDMs from WT (C57BL/6), MyD88$^{-/-}$, and TRIF$^{-/-}$ mice were uninfected (UI) or infected with clinical isolate *B. cenocepacia* K56-2, and the cells were lysed in TRIzol. RNA was extracted and analyzed by RT-qPCR for caspase-1 (A) and IL-1β (B and D). Murine BMDMs from WT and MyD88$^{-/-}$ (C) and TRIF$^{-/-}$ (E) were uninfected or infected with *B. cenocepacia* K56-2 clinical isolate, and the culture supernatants were assayed for the release of mature IL-1β (C and E). The results are expressed as the mean of triplicate samples ± SD, representative of three independent experiments. *$P < 0.05$; ***$P < 0.001$. Samples similar to those described above were lysed and analyzed by Western blot for the expression of pro-IL-1β (F).

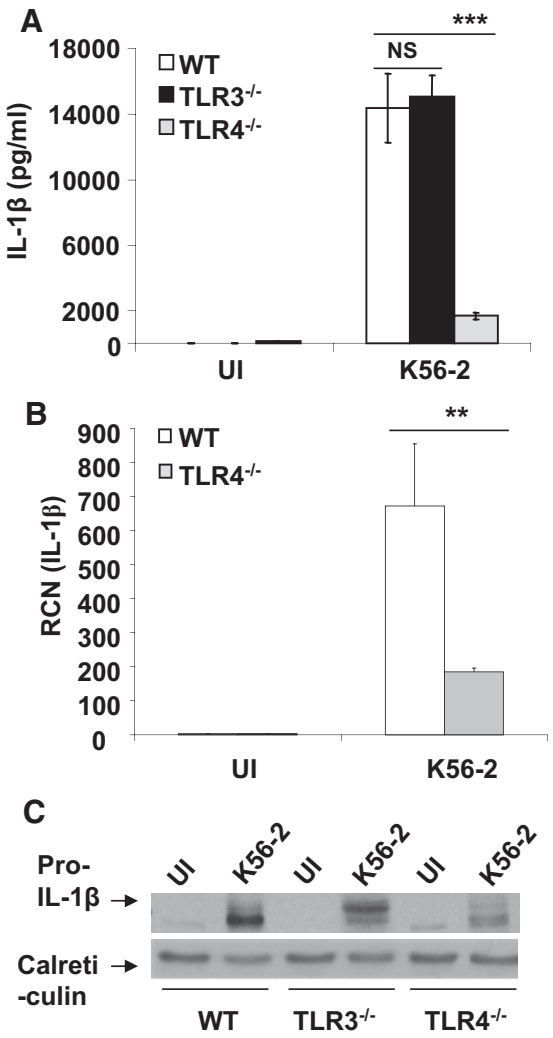

Figure 2. IL-1β is induced by *B. cenocepacia* K56-2 and uses host TLR4. Murine BMDMs from WT and TLR3$^{-/-}$ and TLR4$^{-/-}$ were uninfected or infected with *B. cenocepacia* K56-2 clinical isolate. and the supernatants were analyzed for the release of mature IL-1β (A). BMDMs from WT and TLR4$^{-/-}$ mice were uninfected or infected with clinical isolate *B. cenocepacia* K56-2, and the cells were lysed in TRIzol. RNA was extracted and analyzed by RT-qPCR for IL-1β (B). The results are expressed as the mean of triplicate samples ± SD, representative of three independent experiments. $^{**}P < 0.01$; $^{***}P < 0.001$. Cell lysates from WT, TLR3$^{-/-}$, and TLR4$^{-/-}$ BMDM were infected with K56-2 and then analyzed by Western blots for pro-IL-1β expression (C). Blots are representative of more than three independent experiments.

phages, except for XOA7, which provoked much less IL-6 production than the rest of the mutants (Supplemental Fig. 2A and B). The XOA3 mutant lacks a portion of the O antigen while maintaining the rest of the LPS molecule intact (Supplemental Fig. 1). This mutation recreates the same LPS phenotype as observed in strain J2315 [21], of which the structure has been reported recently [22]. Taken together, we concluded that *B. cenocepacia* O antigen is required for IL-1β production through host TLR4.

We next examined apoptosis induction in WT macrophages in response to the parent strain K56-2 and the derived LPS mutants XOA3, XOA7, and XOA8 [7]. Fig. 4C shows that the truncation of the O antigen diminished apoptotic cell death ($P<0.01$). Taken together, our data demonstrate that TLR4 contributes to IL-1β production in response to *B. cenocepacia*, however the contribution of another unidentified, MyD88-dependent TLR cannot be excluded.

Caspase-1 is essential for IL-1β production by macrophages in response to *B. cenocepacia* K56-2 infection

NLRs respond to microbial components through the activation of caspase-1 [6], which becomes activated by cleavage to generate an enzymatically active heterodimer composed of 10 and 20 kDa chains [6, 29]. Upon activation, caspase-1 cleaves pro-IL-1β and pro-IL-18 to generate biologically active cytokines [6, 29]. To determine if IL-1β activation in response to *B. cenocepacia* requires caspase-1, WT macrophages and caspase-1$^{-/-}$ macrophages were infected with *B. cenocepacia* K56-2 for 24 h, and cytokines were evaluated in cell supernatants (**Fig. 5**). The amount of IL-1β produced by K56-2-infected macrophages lacking caspase-1 was almost undetectable when compared with WT macrophages (Fig. 5A). IL-6 produced by caspase-1$^{-/-}$ macrophages was similar to that of WT cells (Fig. 5B). TNF-α levels were decreased in macrophages lacking caspase-1, which could be a secondary effect for the lack of IL-1β (Fig. 5C). Therefore, caspase-1 is essential for IL-1β production by macrophages during *B. cenocepacia* infections, suggesting an involvement of the inflammasome.

The ΔF508 mutation in the CFTR gene increases IL-1β release in response to *B. cenocepacia* K56-2 infection

As *B. cenocepacia* infects mainly CF patients, and a recent study demonstrated that TLR4 expression is increased on monocytes and macrophages derived from CF patients [30], we examined IL-1β release from primary murine macrophages derived from WT mice and compared them with those derived from mice harboring a ΔF508 mutation. WT and ΔF508 BMDM were infected with K56-2, and cell supernatants were examined for cytokine release (**Fig. 6**). Notably, IL-1β release was increased significantly in the presence of the ΔF508 mutation ($P<0.05$; Fig. 6A), as well as IL-6 and TNF-α production ($P<0.001$; Fig. 6B and C). These results suggest that the CFTRΔF508 mutation contributes to an exacerbated, proinflammatory response by macrophages (Fig. 6B and C).

DISCUSSION

The hallmark of infection in the CF lung is severe inflammation, which destroys the lung tissue [1, 31–33]. Infections by Bcc bacteria, in particular, *B. cenocepacia*, can also lead to necrotizing pneumonia resulting in death [34]. Although the role of macrophages in CF has been recognized [1, 31, 32, 35], the host receptors and key bacterial determinants

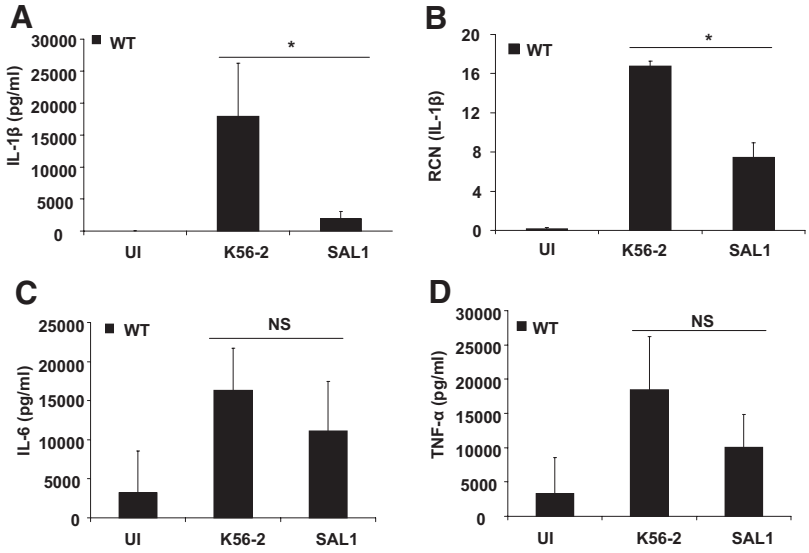

Figure 3. Heptoseless *B. cenocepacia* K56-2 mutant defectively induces IL-1β. BMDMs from WT macrophages were uninfected or infected with K56-2 or with SAL1 for 24 h, and the supernatants were analyzed for the release of mature IL-1β (A) and the induction of IL-1β mRNA (B). Culture supernatants were also analyzed for IL-6 (C) and TNF-α (D). The results are expressed as the mean of three independent experiments (performed in triplicate) ± SD. *$P < 0.05$; NS = not significant.

associated with the heightened inflammatory state and poor clinical outcome in CF patients are not well understood.

Several studies showed that purified *B. cenocepacia* LPS stimulates the respiratory burst response of human neutrophils to *B. cenocepacia* infections [36]. *B. cenocepacia* and *B. multivorans* lysates or LPS derived from them induce significant proinflammatory cytokine production and TNF-α production from U937 macrophages compared with LPS from other lung pathogens including *Pseudomonas aeruginosa* [36–39]. Using three different LPS preparations, Shimomura et al. [40] demonstrated that highly purified LPS is not recognized by TLR4 and weakly induces IL-1β. On the other hand, Bamford et al. suggested that highly purified *B. cenocepacia* LPS is recognized by TLR4 and mediates NF-κB activation in human monocytic cell lines [11]. Another study with purified *B. cenocepacia* LPS showed that LPS and lipid A are responsible for cytokine production, such as IL-6 and TNF-α [37, 41–44], whereas the OPS prevents phagocytosis by macrophages and adhesion to epithelial cells [28].

IL-1β is an important inflammatory cytokine associated with tissue destruction and pyroptosis [7]. To produce IL-1β, macrophages need a double stimulation, one through TLR ligands, which induce gene transcription, and another through NLR agonists, which activate caspase-1 via the inflammasome complex [27]. It is essential to discern the role of IL-1β during *B. cenocepacia* infection, as BAL of infected CF patients shows high levels of IL-1β. With the aid of a panel of *B. cenocepacia* LPS mutants, we show here that *B. cenocepacia* O antigen contributes to the induction of the IL-1β message within infected macrophages, as the truncation of the O antigen polysaccharide induces less IL-1β production. It is unlikely that the decrease in IL-1β is a result of reduced uptake of the organism, as several studies demonstrated that the internalization of strains that lack O antigen was unaltered or increased compared with their isogenic smooth parent strain [28, 44]. Concurrently, similar to previous studies, we also found that a heptoseless LPS mutant, lacking outer-core and O antigen, did not alter the TNF response to *B. cenocepacia* [44]. Notably, the O antigen also adds to macrophage death, which explains the severe damage observed in the lung of *B. cenocepacia*-infected patients.

In epithelial cells, TLR5 but not TLR2 or TLR4 regulates *B. cenocepacia*-induced lung epithelial inflammatory responses [45]. Others have reported that TLR4 and TLR5 are potential

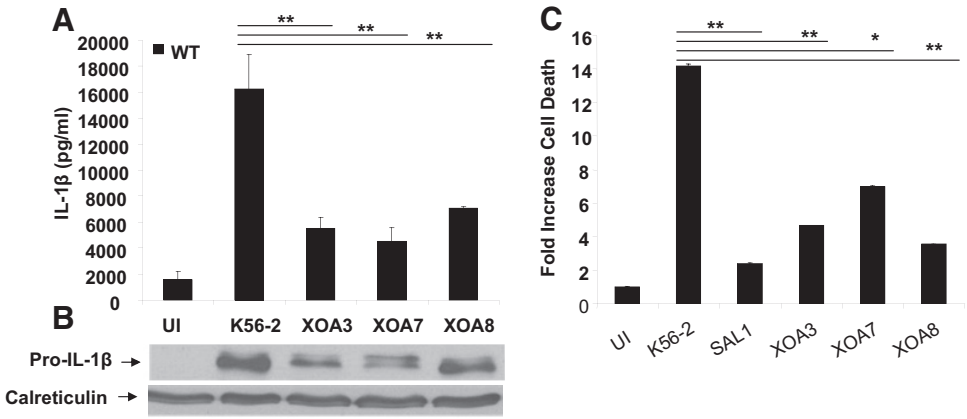

Figure 4. *B. cenocepacia* K56-2 OPS truncations reduce IL-1β production. Murine BMDMs from WT were uninfected or infected with K56-2 or the OPS mutants XOA3, XOA7, and XOA8 for 24 h, and then culture supernatants were analyzed for IL-1β (A), and cell lysates were analyzed by Western blots for the expression of pro-IL-1β protein (B) and for apoptosis (C). The results are representative of three independent experiments and expressed as the mean of triplicate samples ± SD. *$P < 0.05$; **$P < 0.01$.

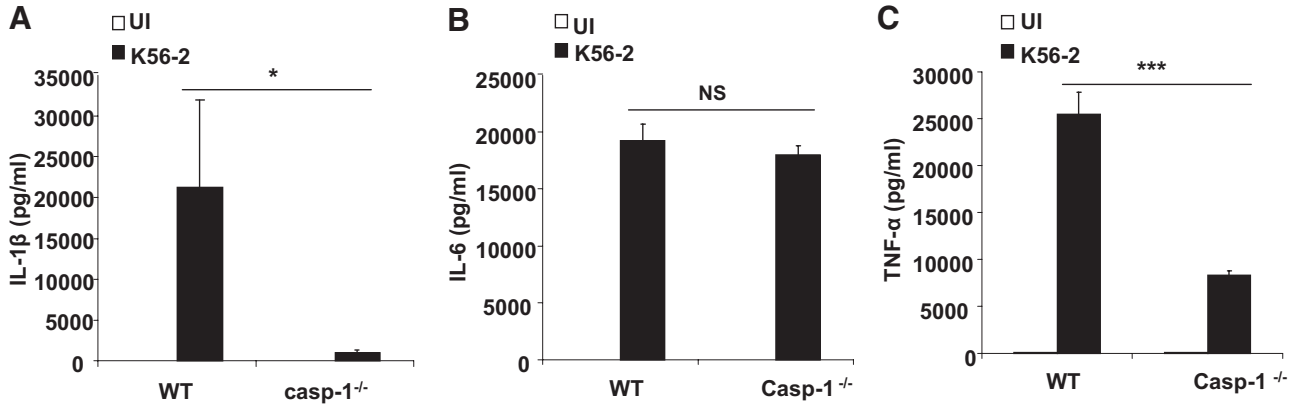

Figure 5. Caspase-1 is required for IL-1β activation in response to *B. cenocepacia* K56-2. BMDMs from WT or caspase-1$^{-/-}$ mice were uninfected or infected with K56-2 for 24 h. Then, culture supernatants were analyzed for the release of mature IL-1β (A), IL-6 (B), and TNF-α (C). The results are expressed as the mean of three independent experiments ± SD. *$P < 0.05$; ***$P < 0.001$.

receptors for *B. cenocepacia* LPS and flagella, respectively, and bacteria–receptor interactions result in NF-κB activation and IL-8 secretion in epithelial cells [39]. Our data demonstrate that in macrophages, TLR4 senses *B. cenocepacia* LPS, leading to up-regulation of the pro-IL-1β message. IL-1β production was absent in MyD88$^{-/-}$ macrophages yet diminished in TRIF$^{-/-}$ and TLR4$^{-/-}$ macrophages. Taken together, our data demonstrate that TLR4 is a major factor for IL-1β production in response to *B. cenocepacia*, yet another unidentified, MyD88-dependent TLR is also required. Ultimately, these data suggest that developing means for the tight regulation of IL-1β and pyroptotic cell death can decrease the tissue damage observed in the lungs of CF patients when infected with *B. cenocepacia*.

Once up-regulated, IL-1β is then activated and released in a caspase-1-dependent manner [27]. As caspase-1 activation typically requires the assembly and activation of the inflammasome complex, it is conceivable that an unidentified cytosolic receptor senses *B. cenocepacia* in the cytosol and brings the inflammasome components together, leading to caspase-1 activation that cleaves pro-IL-1β to its active form. Given that intracellular *B. cenocepacia* remains in the phagocytic vacuole, a cytosolic activation of inflammasome components would imply that the bacterial effectors are released from the phagocytic vacuole through membrane damage of the vacuole or active secretion mediated by T3SS, T4SS, or T6SS, as we are investigating currently.

It is more evident that CFTR dysfunction is associated with increased inflammatory responses by means of several pathways, connected at least in part with the accumulation of abnormal CFTR protein forms, especially in the case of the ΔF508 mutation [46, 47]. Notably, monocytes and macrophages derived from CF patients express more TLR4 than those obtained from non-CF counterparts [30]. Here, we demonstrate that macrophages harboring the ΔF508 mutation produced exacerbated levels of IL-1β in response to *B. cenocepacia*. It is possible that this is a result of increased expression of TLR4 in macrophages derived from mice harboring the ΔF508 mutation. Yet, the exact mechanism of CFTR regulation of IL-1β secretion remains to be delineated.

In summary, we have found that macrophage activation of the proinflammatory cytokine IL-1β during *B. cenocepacia* infection of macrophages requires TLR4 and caspase-1. This

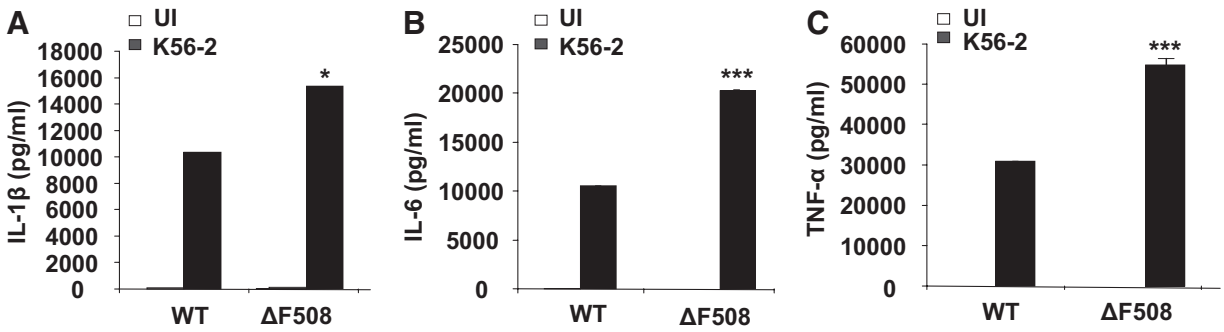

Figure 6. ΔF508 mutation enhances IL-1β production during *B. cenocepacia* K56-2 infection. BMDMs from WT mice and BMDM harboring the ΔF508 mutation were uninfected or infected with the *B. cenocepacia* K56-2 clinical isolate for 24 h, and the culture supernatants were analyzed for IL-1β (A), IL-6 (B), and TNF-α (C). Data are representative of three independent experiments ± SD. *$P < 0.05$; ***$P < 0.001$.

inflammatory reaction is in response to *B. cenocepacia* O antigen. Therefore, several major factors could help bridge the path to new, anti-inflammatory targets aimed at increasing survival in CF patients with *B. cenocepacia* infections.

AUTHORSHIP

S.K. and B.K. designed, performed, analyzed, and interpreted data and contributed to the writing of the manuscript. A.A., D.A., A.A.K., K.C., and B.A. contributed to the performance of experiments and editing of the manuscript. M.D.W., K.M., and C.M. contributed to the editing of the manuscript. M.A.V. supplied the *B. cenocepacia* clinical isolates and their corresponding mutants (produced by S.A.L. and X.O.) and contributed to the interpretation of results and editing of the manuscript. A.O.A. designed the experiments, analyzed and interpreted the results, and wrote the manuscript.

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