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Double-Stranded RNA Dependent Kinase R Regulates Antibacterial Immunity in Sepsis

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Keywords
Double-stranded RNA dependent kinase R · Bacterial sepsis · Interleukin-1

Abstract
Double-stranded RNA dependent kinase R (PKR) is originally identified as an intracellular sensor of viral infection, but its role in bacterial infection remains largely unknown. Here we report that PKR was an important regulator of antibacterial immunity in sepsis. Genetic deletion of PKR or pharmacological inhibition of its kinase activity markedly increased bacterial loads, organ injury, and mortality in polymicrobial infection induced by cecal ligation and puncture (CLP). In contrast, PKR deficiency or inhibition did not affect bacterial loads, organ injury, or mortality when mice were systemically challenged with *Escherichia coli*, an abundant microbe in the gastrointestinal tract. PKR deficiency or inhibition markedly decreased the release of interleukin (IL)-1β after CLP. Defect in IL-1 signaling phenocopied PKR deficiency or inhibition in CLP-induced bacterial sepsis. Taken together, these findings identified a critical role of the PKR signaling pathway in antibacterial immunity.

Introduction

Double-stranded RNA dependent kinase R (PKR) is an interferon (IFN)-inducible serine/threonine kinase, which contains 2 RNA-binding domains at the C-terminal and a kinase domain at the N-terminal [1–4]. Upon binding to the double-stranded (ds) RNA molecules, PKR uncovers its intramolecular dimerization domain, culminating in PKR dimerization, autophosphorylation, and activation [2–4]. As dsRNA is a general by-product of viral infection, PKR was originally identified as an antiviral molecule [3–5]. PKR contributes to the antiviral defense at multiple levels: (1) activated PKR recruits and phosphorylates eIF2a, resulting in inhibition of general protein synthesis; (2) PKR enhances the production of type 1 IFNs through the interaction with IFN response factor 3 (IRF3) pathways; and (3) activated PKR regulates actin dynamics and abrogates viral entry into cells by inhibiting gelsolin, a key actin-modifying protein [4–6]. We and others found that PKR promotes pyroptosis through physical interaction with inflammasome components

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and enhances apoptosis [1, 7–9]. Notably, both apoptosis and pyroptosis are important strategies for the host to directly eliminate the intracellular virus [10, 11]. Owing to its important antiviral roles, most viruses have devised mechanisms to subvert PKR functions [3–5].

Although huge progress has been made in understanding how PKR responses to viral infections, little is known about the roles of PKR in antibacterial defenses. During the case of studying how PKR regulates inflammasome activation in response to various pathogen-associated molecular patterns (PAMPs), we unexpectedly found that *Escherichia coli*, which are abundant Gram-negative bacteria in the gastrointestinal tract, could induce robust PKR autophosphorylation [1]. This observation prompted us to investigate whether PKR plays a role in antibacterial defenses, especially in the context of intestinal bacterial infection. In current study, we found that genetic deletion of PKR or pharmacological inhibition of its kinase activity markedly increased bacterial loads, organ injury, and mortality in intestinal polymicrobial infection. PKR deficiency or inhibition significantly decreased the release of interleukin (IL)-1β. Defect in IL-1 signaling phenocopied PKR deficiency or inhibition in intestinal polymicrobial infection. Thus, the PKR signaling pathway is critical for intestinal antibacterial defense.

Materials and Methods

Mice

The PKR-deficient mice and their wild-type (WT) littermate controls are in the C57BL/6 background as described previously [12]. All mice were bred in the animal facilities of Central South University. Experimental protocols were approved by the Institutional Animal Care and Use Committees of Central South University.

Reagents

The PKR inhibitor C16 was obtained from Abcam. Meropenem was obtained from Shenzhen Haibin Pharmaceutical Co., Ltd.

CLP Procedure

Experimental sepsis was induced by cecal ligation and puncture (CLP). Male or female mice that were 25–30 g in weight were used. The skin was disinfected with a 2% iodine tincture. Laparotomy was performed under 2% isoflurane (Piramal Critical Care) with oxygen. To cause mortality ranged from 10 to 30% after CLP, 25% of the cecum was ligated and punctured twice with a 20-gauge needle. Saline (1 mL) was given subcutaneously for resuscitation immediately after operation. Mice were monitored daily by signs of a moribund state for lethality. For detection of TNF-α and IL-6 serum level and gene expression in the intestine, mice were sacrificed at 4 h after CLP. For detection of IL-1β, IL-1α, alanine aminotransferase (ALT), and aspartate transaminase (AST), mice were sacrificed at 18 h after CLP. Lung specimens were stained with H&E. C16 was administered intraperitoneally (500 μg/kg) 1 h prior to CLP. Meropenem at the dose of 50 μg/kg was administered immediately after CLP and twice a day. The same vehicle was given to the sham operated mice.

E. coli Injection

Approximately 10^8 CFU of *E. coli* dissolved in 100-μL phosphate-buffered solution (PBS) were injected intravenously into mice. Control mice underwent an injection with the same volume of PBS. After injection, animals were allowed to access to food and water freely and were observed for mortality or conducted examinations at predetermined time intervals.

Macrophages Depletion

WT and PKR-deficient mice were injected intraperitoneally with the first dose at 400 μL of liposome-encapsulated clodronate or control lipsome (N.van Rooijen) 48 h before CLP and then were injected intravenously with the second dose at 250 μL of liposome-encapsulated clodronate or control lipsome 24 h before CLP.

Bacterial Counts

Bacterial loads were measured in the lung, the liver, the blood, and the spleen. The homogenates of these organs or the blood were gradiently diluted in sterile saline and incubated at 37°C for 16 h on Luria-Bertani agar plates. Colony-forming units (CFU/mL·g) were counted as bacterial loads.

ELISA Assay

IL-1β, IL-1α, IL-6, and TNF-α levels of serum samples were determined using ELISA kits as described in the protocol of the manufacturer (eBioscience).

Fig. 1. PKR activity is critical for antibacterial defense. **a** Kaplan Meier survival curves for the indicated genotypes of mice subjected to either mild CLP or sham operation. **b** The AST and ALT levels from the mice of indicated genotypes subjected to either mild CLP or sham operation. Serum samples were collected 18 h after CLP operation. **c** Histology of the lungs from the mice of indicated genotypes ($n = 6$) subjected to either mild CLP or sham operation. The lungs were collected 18 h after CLP operation and then stained with H&E. **d** Kaplan Meier survival curves for the mice subjected to the sham operation or the mice subjected to mild CLP with intraperitoneal C16 (500 μg/kg) or DMSO injection. **e** The AST and ALT levels from the mice subjected to the sham operation or the mice subjected to mild CLP with intraperitoneal C16 (500 μg/kg) or DMSO injection. Serum samples were collected 18 h after CLP operation. **f** Histology of the lungs from the mice ($n = 6$) subjected to the sham operation or the mice subjected to mild CLP with intraperitoneal C16 (500 μg/kg) or DMSO injection. The lungs were collected 18 h after the operations and then stained with H&E. Dots represent individual mice. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; ns, not significant (Student's *t* test and log-rank test for survival). PKA, double-stranded RNA dependent kinase R; CLP, cecal ligation and puncture; AST, serum aspartate transaminase; ALT, alanine aminotransferase; WT, wild type.

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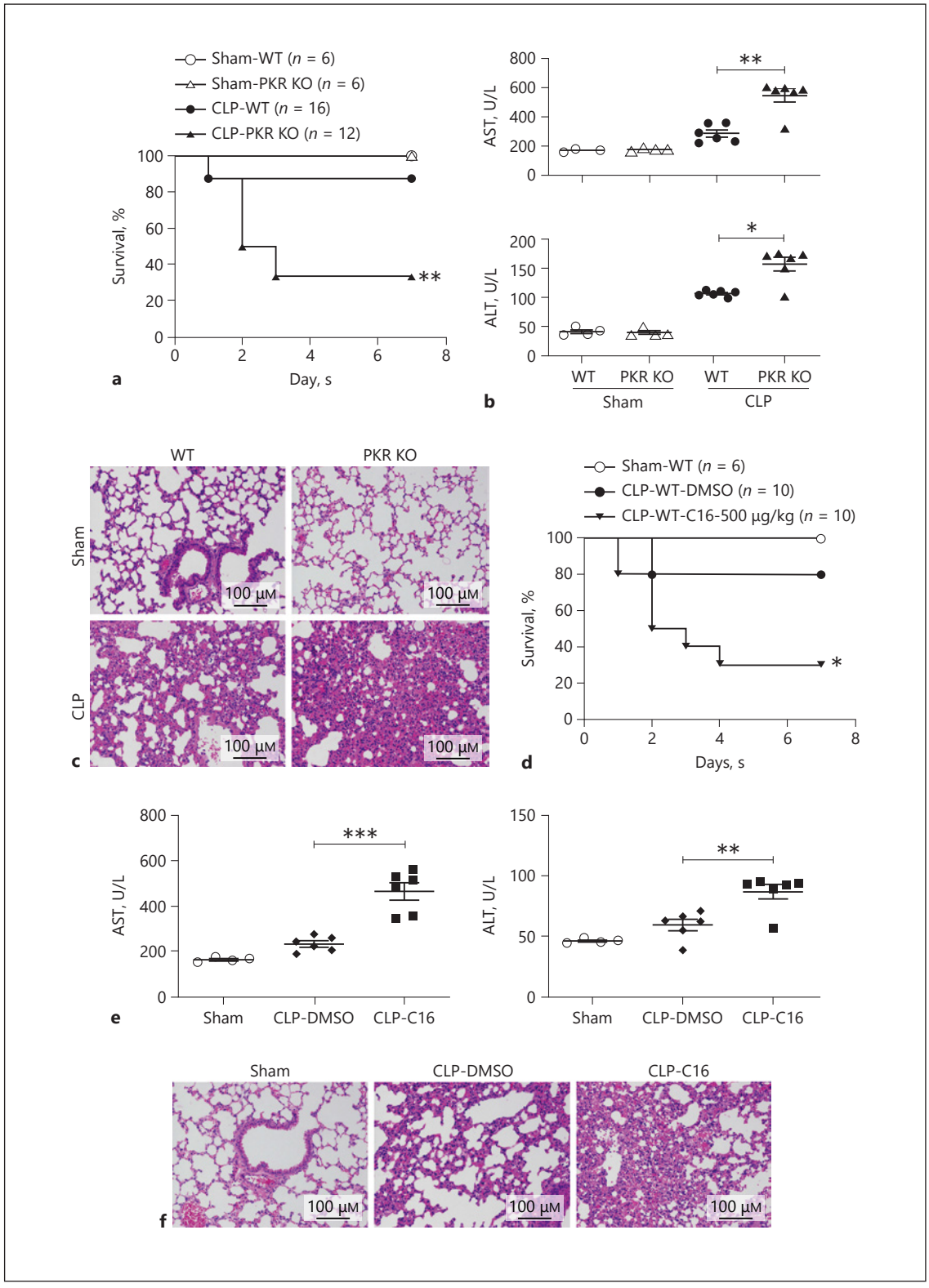

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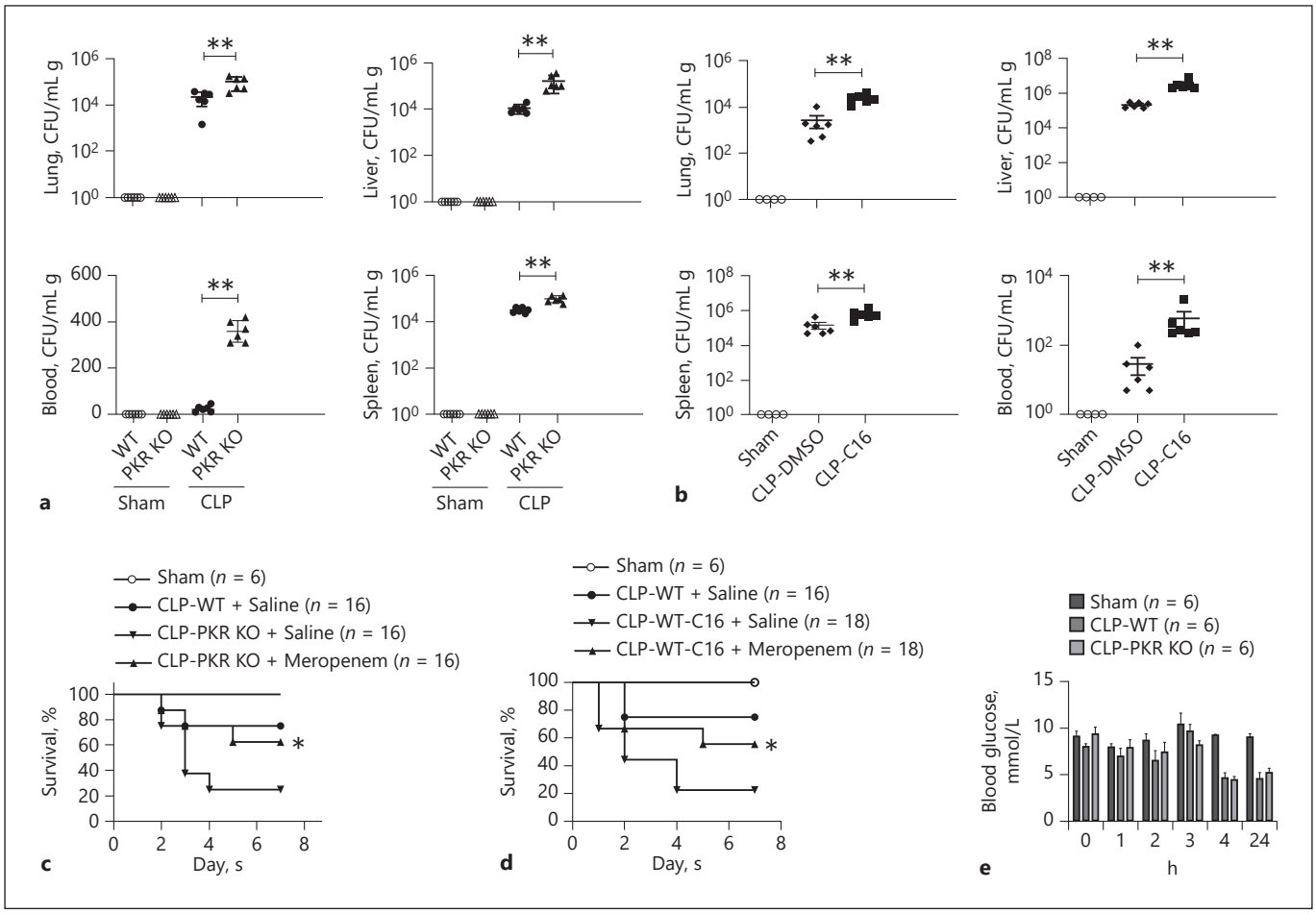

Fig. 2. PKR deficiency leads to higher bacterial loads during bacterial sepsis. **a** Bacterial load levels in the lung, liver, blood, and spleen from the mice of indicated genotypes subjected to either mild CLP or sham operation. The lung, liver, blood, and spleen were collected 18 h after the operations. **b** Bacterial load levels in the lung, liver, blood, and spleen from the mice subjected to the sham operation or the mice subjected to mild CLP with intraperitoneal C16 (500 μg/kg) or DMSO injection. The lung, liver, blood, and spleen were collected 18 h after the operations. **c** Kaplan Meier survival curves for the indicated genotype mice subjected to the sham operation or the mice subjected to mild CLP with or without intraperitoneal Meropenem injection (50 μg/kg). **d** Kaplan Meier survival curves for the mice subjected to the sham operation or the mice subjected to mild CLP with or without intraperitoneal C16 injection (500 μg/kg) or intraperitoneal Meropenem injection (50 μg/kg). **e** Blood glucose levels for the indicated genotype mice subjected to either mild CLP or sham operation. Dots represent individual mice. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant (Student's t test and log-rank test for survival). PKA, double-stranded RNA dependent kinase R; CLP, cecal ligation and puncture; WT, wild type.

Fig. 3. PKR is dispensable for bacterial clearance after intravenous injection of *E. coli*. **a** Bacterial loads in the lung, liver, blood, and spleen from the mice of indicated genotypes after receiving intravenous injection of *E. coli* (10^8 CFU) or PBS. The lung, liver, blood, and spleen were collected 18 h after the injections. **b** Bacterial loads in the lung, liver, blood, and spleen from the mice were intravenously injected with *E. coli* (10^8 CFU) or PBS and simultaneously intraperitoneally injected with C16 (500 μg/kg) injection or DMSO. The lung, liver, blood, and spleen were collected 18 h after the injections. Dots represent individual mice. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant (Student's t test). PKA, double-stranded RNA dependent kinase R; WT, wild type; PBS, phosphate-buffered solution.

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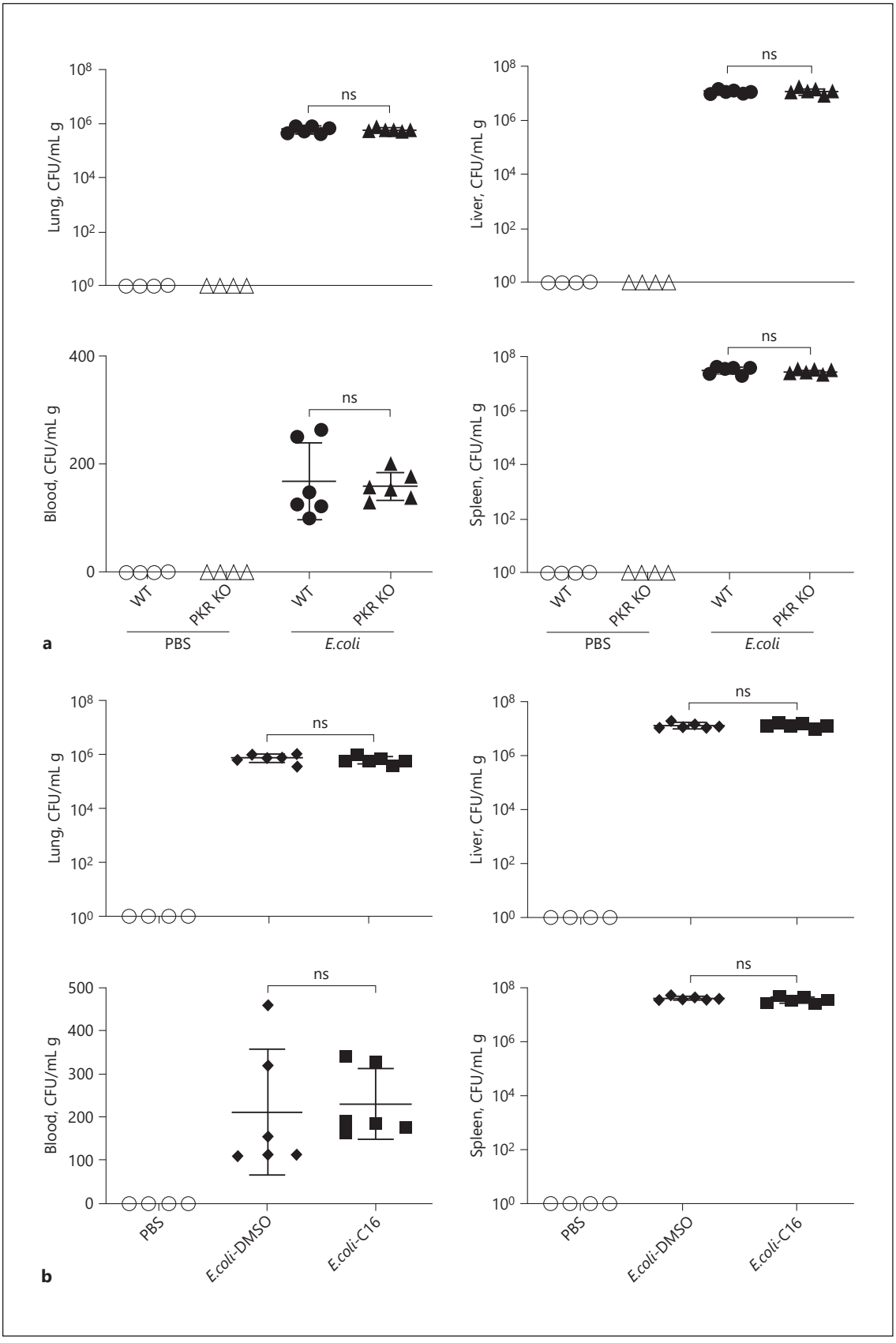
Lung, CFU/mL g
Liver, CFU/mL g
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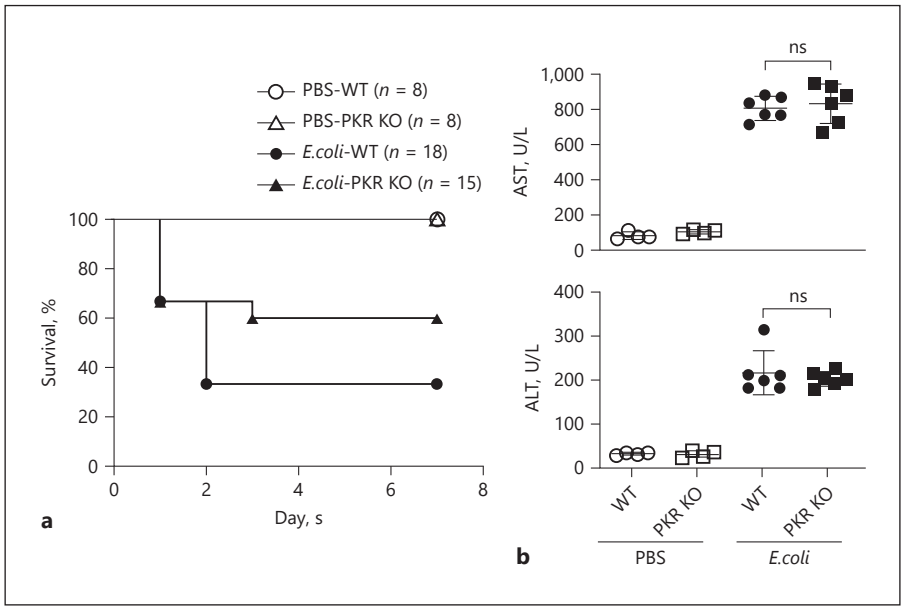

Fig. 4. PKR is dispensable for bacterial clearance after intravenous injection of *E. coli*. **a** Kaplan Meier survival curves for the indicated genotype mice after receiving intravenous injection of *E. coli* (10^8 CFU) or PBS. **b** The AST and ALT levels from mice of indicated genotypes after receiving intravenous injection of *E. coli* (10^8 CFU) or PBS. Serum samples were collected 18 h after the injection. Dots represent individual mice. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant (Student's *t* test and log-rank test for survival). PKA, double-stranded RNA dependent kinase R; AST, serum aspartate transaminase; ALT, alanine aminotransferase; WT, wild type; PBS, phosphate-buffered solution.

ALT and AST

Serum samples were collected from various types of mice, and the levels of ALT and AST in serum were measured by Automatic Biochemical Analyzer (Chemray240).

qRT-PCR

Small/large intestine tissues are collected to determine the gene expression of TNF-α and IL-6 at 4 h after CLP from WT and PKR-deficient mice. Total RNA was prepared using Trizol (Ambion, Waltham, MA, USA) according to the manufacturer's instructions, and the cDNA was synthesized from 3 μg of total RNA using reverse transcriptase (TransGen Biotech, Beijing, China). Real-time PCR was performed using SYBR Green Master Mix (Vazyme Biotech Co., Ltd., Nanjing, China) and a C1000 Touch thermal cycler (Roche). The detector was programed with the following PCR conditions: 40 cycles for 5 s denaturation at 95°C and 30 s amplification at 60°C. The mRNA level of target genes was normalized to that of GAPDH.

IL-6_2_F 5′-CCAAGAGGTGAGTGCTTCCC-3′
IL-6_2_R 5′-CTGTTGTTCAGACTCTCTCCCT-3′
TNF-α_F 5′-CCCTCACACTCAGATCATCTTCT-3′
TNF-α_R 5′-GCTACGACGTGGGCTACAG-3′
GAPDH_F 5′-TGGATTTGGACGCATTGGTC-3′
GAPDH_R 5′-TTTGCACTGGTACGTGTTGAT-3′

Isolation of Bone Marrow Neutrophils

Murine PMN from WT and PKR-deficient mice were isolated essentially as described [13]. Marrow cavities of the tibias and femurs of 8- to 12-week-old donor mice were flushed with DMEM 10% FCS media. After RBC hypotonic lysis, neutrophils were isolated by centrifugation over discontinuous Percoll (sigma, P4937) gradients at 500 g for 30 min at 28°C, consisting of 55/(vol/vol), 62 (vol/vol), and 81% (vol/vol) Percoll in PBS, and then neutrophils were harvested from the 62/81% interface. At the end of the preparation, PMN were suspended in ice-cold HBSS containing 0.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mMD-glucose (HBSS) and kept in ice until use.

Isolation of Peritoneal Macrophages

Mice in age between 7 and 12 weeks received intraperitoneal injection with 3 mL of sterile 3% thioglycollate broth to induce peritoneal macrophages. Cells were harvested after 72 h by peritoneal lavage with 5 mL of RPMI medium 1,640 (Gibco) and passed through a 40-μm cell strainer. After centrifugation (800 rpm, 5 min), the cells were resuspended in RPMI medium 1,640 containing 10% fetal bovine serum (Gibco) and 1% antibiotics (Gibco) and plated in a 24-well plate overnight.

In vitro Murine Neutrophil and Macrophage Infection Assays

Neutrophils and macrophages from WT and PKR-deficient mice were seeded at a density of 1×10^7/mL and 1×10^6/mL in RPMI 1640 supplemented with 10% fetal calf serum and then infected with *E. coli* at a multiplicity of infection (MOI) of 10. The cells were then incubated at 37°C for 2 h to allow bacterial uptake, before adding gentamicin (100 g/mL; Life Technologies) to the culture medium for the remainder of the assay to kill extracellular bacteria. The cells were then further incubated for 16 h before harvesting cell-free supernatants for cytokine assays.

Statistical Analysis

All data were analyzed using GraphPad Prism software (version 5.01). Data were analyzed using two-tailed Student's *t* test for the comparison between 2 groups. Data were analyzed using one-way ANOVA followed by the post hoc Bonferroni test for comparison between multiple groups. Survival data were analyzed using the log-rank test. A *p* value <0.05 was considered as statistically significant for all experiments. Dots represent individual mice in both vivo experiments and vitro experiments, graphs show the mean ± SD of technical replicates and are representative of three independent experiments.

Result

PKR Activity Is Critical for Antibacterial Defense

To test whether PKR activation contributes to the antibacterial defenses during bacterial infection, we subjected PKR-deficient mice, in which the kinase domain of PKR was deleted [12, 14], and their WT littermates to mild degree of CLP. Most of the WT mice survived after CLP, whereas most PKR-deficient mice succumbed to the CLP-induced polymicrobial infection (Fig. 1a). Accordingly, PKR-deficient mice had significantly higher levels of serum AST and ALT in CLP, as compared to their WT controls (Fig. 1b). Further, PKR deficiency significantly increased pulmonary leukocyte infiltration after CLP (Fig. 1c). To test whether pharmacological inhibition of PKR could impair the antibacterial defenses in bacterial sepsis, we pretreated the mice with either C16, a specific PKR inhibitor [15], or vehicle. Notably, pharmacological inhibition of PKR significantly enhanced liver and lung injury and promoted lethality after CLP (Fig. 1d–f). Taken together, these findings suggest that PKR activity is critical for antibacterial defense.

PKR Deficiency Leads to Higher Bacterial Loads during Bacterial Sepsis

To further confirm that PKR activation is important for antibacterial defenses in sepsis, we measured the bacterial loads in the lungs, livers, blood, and spleens of PKR-deficient mice and their WT littermate controls. Deletion of the PKR kinase domain led to significantly higher bacterial loads in the lung, liver, blood, and spleen (Fig. 2a). Accordingly, pharmacological inhibition of PKR markedly increased the bacterial loads in these organs after CLP (Fig. 2b). Next we determined whether the increased bacterial loads resulted from PKR deletion or inhibition promote organ injury and lethality after CLP. PKR-deficient mice were pretreated with broad-spectrum antibiotics or placebo before CLP. Administration of antibiotics rescued PKR-deficient mice from lethal sepsis (Fig. 2c). Similar observations were made in C16-treated mice (Fig. 2d). Maintaining blood glucose at certain level is essential for the host to survive bacterial infection. Thus, we next measured blood glucose levels of PKR-deficient mice and their WT controls after CLP. We observed that PKR-deficient mice and their WT controls had comparable levels of blood glucose after CLP (Fig. 2e). Together, PKR deficiency or inhibition impairs antibacterial defenses in bacterial sepsis.

PKR Is Dispensable for Bacterial Clearance after Intravenous Injection of E. coli

Next we determined whether the increased bacterial loads in PKR-deficient mice after CLP were due to the impaired capacity of bacterial clearance or the dissemination of enteric bacteria. To test this, PKR-deficient mice and their WT littermate controls were intravenously injected with the same amount of *E. coli*, an abundant microbe in the gastrointestinal tract. However, we observed that PKR-deficient mice and their WT controls had comparable bacterial loads in the lung, liver, blood, and spleen (Fig. 3a). Similar observations were made in mice treated with C16 or vehicle (Fig. 3b). PKR deficiency failed to promote lethality after intravenous injection of *E. coli* (Fig. 4a). Accordingly, PKR-deficient mice and their WT controls had comparable amount of serum levels of AST and ALT (Fig. 4b). Together with other data (Fig. 2), these findings suggested that the increased bacterial loads in PKR-deficient mice during intestinal polymicrobial infection might be due to impaired dissemination of enteric bacteria, rather than impaired capacity of bacterial clearance.

PKR Deficiency Leads to Impaired Production of IL-1 in Bacterial Sepsis

We and others previously found that PKR broadly regulate the activation of inflammasome, an intracellular protein complex that mediates the enzymatic cleavage of caspase-1 [1, 7–9]. This process leads to the maturation and release of IL-1β, which critically regulates the antibacterial defenses [16]. Thus, we next measured serum levels of IL-1β and IL-1α using CLP models. Genetic deletion of the PKR kinase domain significantly inhibited the release of IL-1β and IL-1α after CLP (Fig. 5a). Accordingly, pharmacological inhibition of PKR by C16 also led to markedly decreased serum levels of IL-1β and IL-1α (Fig. 5b). However, PKR deficiency did not affect the serum levels (Fig. 5c) of IL-6 and tumor necrosis factor (TNF), as well as the gene expression (Fig. 5d) of IL-6 and TNF in the intestine after CLP. Further, genetic deletion of the PKR kinase domain failed to affect the bacterial loads in the intestine (Fig. 5e). Together, these data demonstrated that PKR activity is critical for the release of IL-1 in experimental bacterial sepsis.

Defect in IL-1 Signaling Phenocopied PKR Deficiency in Bacterial Sepsis

Next we determined whether the impaired IL-1 signaling phenocopied PKR deficiency in bacterial sepsis. IL-1R KO mice and their WT controls were subjected to mild

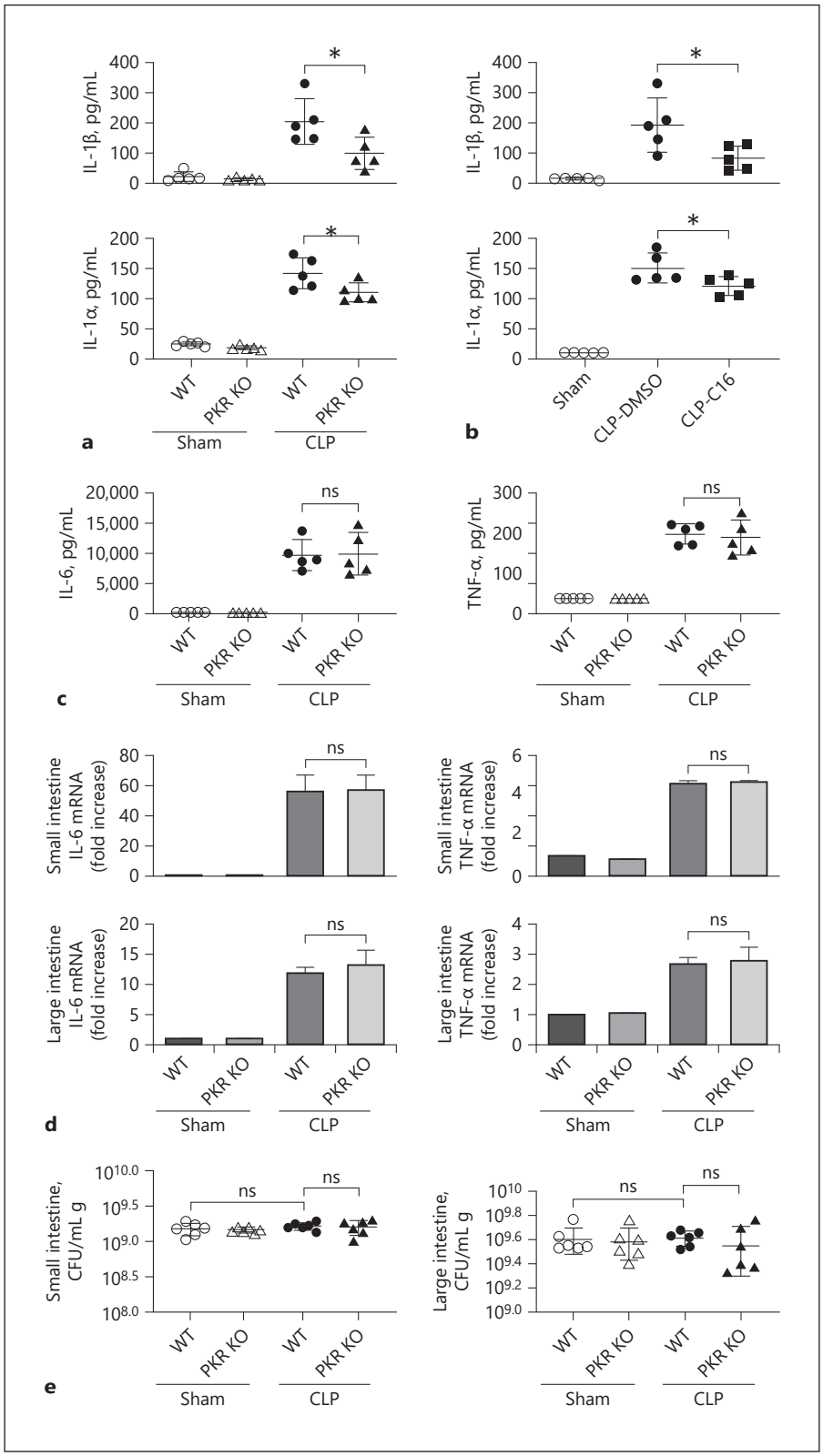

Fig. 5. PKR deficiency leads to impaired production of IL-1β in bacterial sepsis. **a** Serum IL-1β and IL-1α levels from the mice of indicated genotypes subjected to either mild CLP or sham operation. Serum samples were collected 18 h after the operations. **b** Serum IL-1β and IL-1α levels from mice subjected to either mild CLP or sham operation after using the C16 inhibitor. Serum samples were collected 18 h after the operations. **c** Serum levels of IL-6 and TNF from the mice of indicated genotypes subjected to either mild CLP or sham operation. Serum samples were collected 4 h after the operations. **d** Gene expression in the intestine of IL-6 and TNF from the mice of indicated genotypes subjected to either mild CLP or sham operation. Intestine tissues were collected 4 h after the operations. **e** Bacterial load levels in the intestine from the mice of indicated genotypes subjected to either mild CLP or sham operation. Dots represent individual mice. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant (Student's *t* test). PKA, double-stranded RNA dependent kinase R; IL, interleukin; CLP, cecal ligation and puncture; WT, wild type.

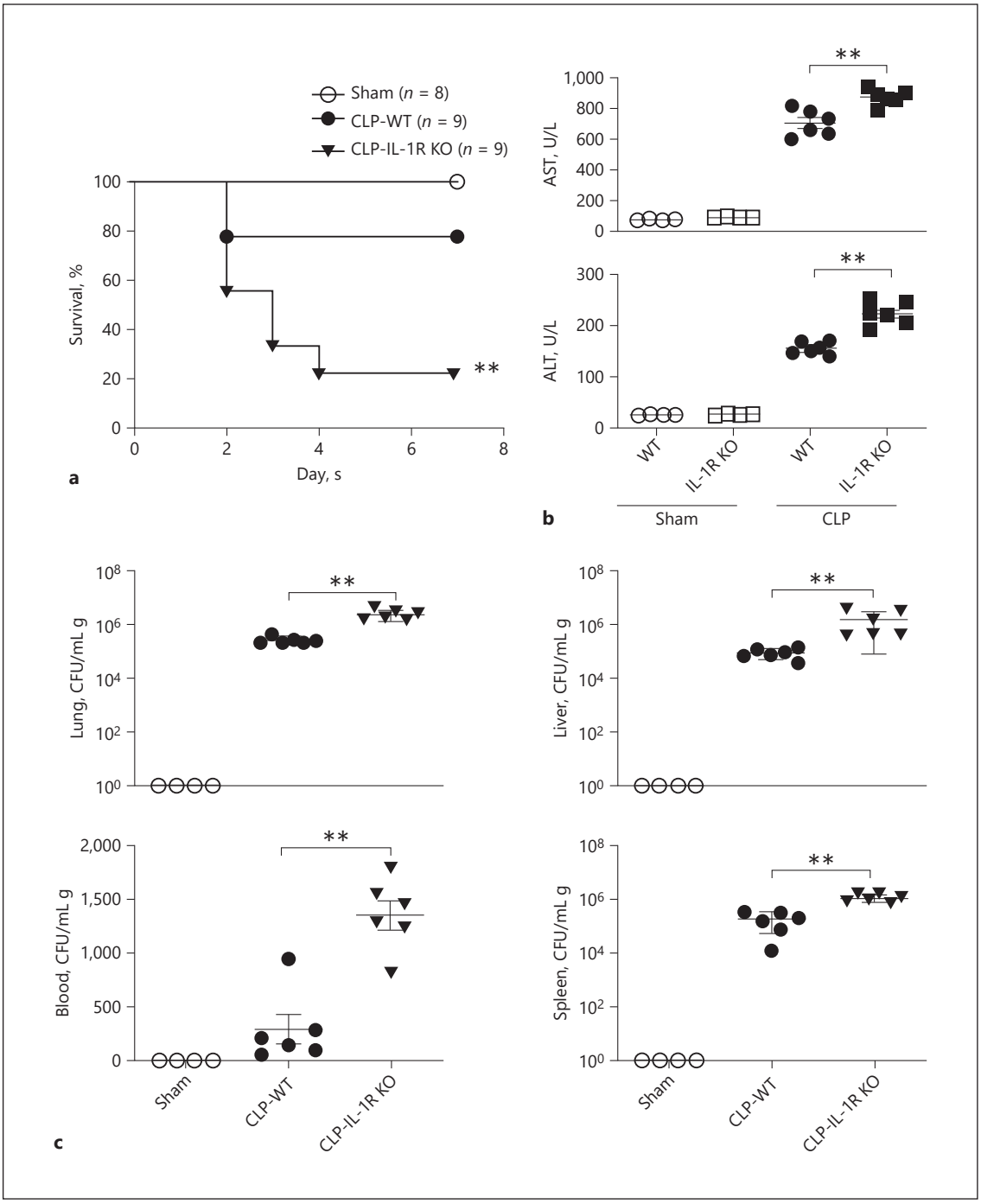

Fig. 6. Defect in IL-1 signaling phenocopied PKR deficiency in bacterial sepsis. **a** Kaplan Meier survival curves for the indicated genotype mice subjected to either mild CLP or sham operation. **b** The AST and ALT levels from the mice of indicated genotypes subjected to either mild CLP or sham operation. Serum samples were collected 18 h after the operations. **c** Bacterial load levels in the lung, liver, blood, and spleen from the mice of indicated genotypes subjected to either mild CLP or sham operation. The lung, liver, blood, and spleen were collected 18 h after the operations. Dots represent individual mice. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant (Student's *t* test and log-rank test for survival). PKA, double-stranded RNA dependent kinase R; CLP, cecal ligation and puncture; AST, serum aspartate transaminase; ALT, alanine aminotransferase; WT, wild type.

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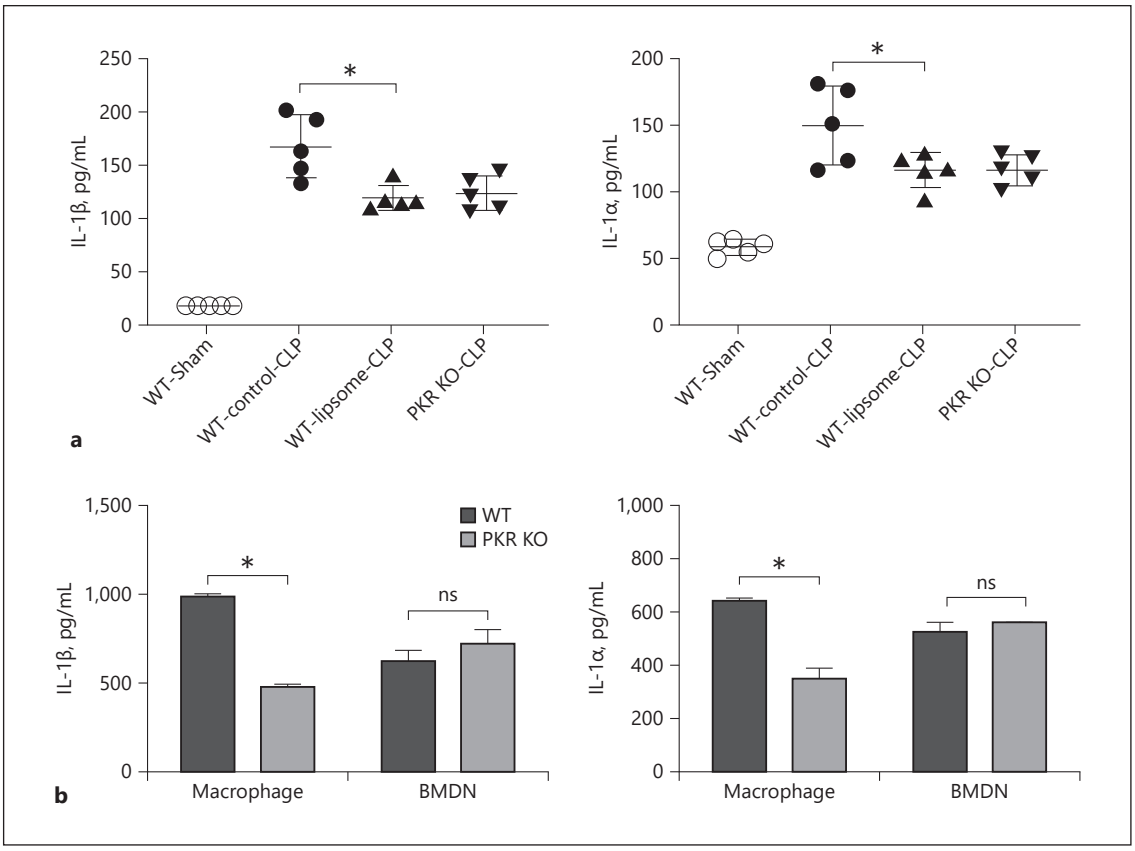

Fig. 7. PKR in macrophages is important for IL-1 secretion. **a** Serum IL-1β and IL-1α levels from the mice with or without macrophage depletion subjected to either mild CLP or sham operation. Serum samples were collected 18 h after the operations. Dots represent individual mice. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant (Student's *t* test). **b** ELISA for IL-1β and IL-1α in the supernatants of neutrophils or macrophages from WT and PKR-deficient mice infected with *E. coli* at an MOI of 10 for 2 h uptake and then killed by gentamicin (100 g/mL). Cell-free supernatants were collected after 16 h further incubation. Graphs show the mean ± SD of technical replicates and are representative of 3 independent experiments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant (Student's *t* test). PKA, double-stranded RNA dependent kinase R; IL, interleukin; CLP, cecal ligation and puncture; WT, wild type; MOI, multiplicity of infection.

CLP, in which most of the WT mice were survived, and deletion of IL-1R significantly promoted CLP-induced lethality (Fig. 6a). IL-1R KO mice displayed significantly higher serum levels of AST and ALT in CLP, as compared to those of their WT controls (Fig. 6b). Further, defect in IL-1 signaling was associated with significantly increased bacterial loads in the lung, liver, blood, and spleen (Fig. 6c). Collectively, defect in IL-1 signaling phenocopied PKR deficiency during intestinal polymicrobial infection.

PKR in Macrophages Is Important for IL-1 Secretion

We and others previously show that macrophages constitutively express PKR [17] and that PKR is critical for IL-1 secretion during sepsis [1]. To determine whether PKR-dependent IL-1 secretion mainly occurs in macrophages during sepsis, we depleted macrophages in vivo by using liposomes-clodronate. Administration of liposomes-clodronate markedly decreased IL-1β and IL-1α secretion and significantly increased the bacterial loads after CLP (Fig. 7a). To further support the notion that macrophage PKR is important for the IL-1 secretion in bacterial sepsis, we stimulated mouse macrophages or neutrophils isolated from WT or PKR-deficient mice with *E. coli*. Deletion of the PKR kinase domain significantly reduced IL-1β and IL-1α secretion in macrophages but not neutrophils (Fig. 7b). Collectively, these findings clearly suggest that PKR in macrophages is important for IL-1 secretion.

Discussion

In this study, we showed for the first time that PKR is an important regulator of intestinal antibacterial immunity. The antiviral function of PKR was discovered more than 30 years ago [18]. The best-known model of how PKR combats viral infection is that autophosphorylated PKR recruits and phosphorylates its substrate eIF2a upon recognizing viral double-stranded RNA, resulting in inhibition of general protein synthesis [2–4]. Though PKR is originally identified as an intracellular viral RNA sensor, bacterial endotoxin (lipopolysaccharide [LPS]), the major cell-wall component of Gram-negative bacteria, is also able to induce PKR phosphorylation and activation through toll-like receptor (TLR) 4 and its adapter molecule TIR-domain-containing adapter-inducing interferon-β (TRIF). In this scenario, LPS-induced PKR phosphorylation is essential for bacteria-induced macrophage death [17]. As macrophages are important innate immune cells, this study implicates that PKR might regulate antibacterial immunity. However, the roles of PKR in antibacterial defenses at the organism level are yet to be found. Here we showed that PKR activity is critical for the antibacterial defense during experimental sepsis.

In this context, PKR regulates the production of IL-1β, which plays important roles in preventing the dissemination of enteric bacteria after CLP through neutrophil recruitment. Intriguingly, PKR deficiency failed to affect the bacterial clearance and the mortality when *E. coli* were directly injected into the veins, suggesting that PKR is important for the antibacterial defenses in the peritoneal cavity but dispensable for bacterial clearance during bacteremia. By using *E. coli*-induced peritonitis model, we previously found that PKR deficiency significantly inhibits neutrophil recruitment in the peritoneal cavity [1]. Macrophages are abundant in the peritoneal cavity. It is noteworthy that PKR in macrophages is important for IL-1 secretion, which leads to subsequent neutrophil recruitment. As neutrophil influx is critical for bacterial clearance in tissues, our data suggest an important role of peritoneal macrophage PKR in the antibacterial defenses during bacterial peritonitis. In this context, Gram-negative bacteria induce PKR activation in peritoneal macrophages through TLR4, leading to IL-1 secretion and subsequent neutrophil recruitment, which prevents the dissemination of bacteria.

The maturation and release of IL-1β is mediated by caspase-1, which is activated by intracellular protein complexes, termed inflammasomes [1, 7]. The well-characterized inflammasomes include NLRP1, NLRP3, AIM2, and NLRC4, all of which contribute to the antibacterial immunity [19–22]. However, different types of inflammasomes response to distinct stimuli during bacterial infection [19–22]. For examples, NLRP1 is activated by anthrax lethal toxin or MDP. The AIM2 or NLRC4 inflammasome responds to intracellular bacterial DNA or flagellin, respectively. Further, Gram-negative bacteria activate the NLRP3 inflammasome through caspase-11 [23, 24]. We and others previously showed that PKR regulates the activation of multiple types of inflammasomes [1, 7–9]. It is unlikely that the inflammasome components are the substrate of PKR [1]. However, the autophosphorylation of PKR could increase the affinity between PKR and inflammasomes, which is required for PKR to promote the activation of multiple types of inflammasomes [1, 7]. One remaining unsolved question is as follows: which type of inflammasome is activated by PKR in intestinal polymicrobial infection? As the 4 types of inflammasomes are all expressed in the gastrointestinal tract [21, 25–27], it is possible that PKR might signal through multiple types of inflammasomes during intestinal polymicrobial infection. Taken together, our study identifies a critical role of the PKR-inflammasome pathway in the antibacterial immunity.

Acknowledgement

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Statement of Ethics

Experimental protocols were approved by the Institutional Animal Care and Use Committees of Central South University.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Yiting Tang conceived the project, designed the experiments, supervised the study, and wrote the paper; Yanliang Yang and Lingli Xie designed and performed the experiments, analyzed the data and made the figures; Yanjun Zhong, Xiaoli Zhong, Ran Meng, and Qianqian Xue assisted in the in vivo experiments; Kai zhao and Fang liang assisted in data interpretation and edited the manuscript..

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