Immunologic Research (2019) 67:77–83
https://doi.org/10.1007/s12026-018-9039-y

ORIGINAL ARTICLE

FcRγ deficiency improves survival in experimental sepsis by down-regulating TLR4 signaling pathway

Zhi-Min Wei[1] · Zhuo Wang[2,3] · Xiao-Jian Wan[4] · Xian-Jing Li[2] · Yi-Xing Li[2] · Yang Bai[2] · Xue Yang[2] · Yong Yang[2] · Shun-Chang Jiao[1] · Zhe-Feng Liu[1]

Published online: 14 December 2018

Abstract

Fc receptor common γ signaling chain (FcRγ), a common subunit shared by Fc receptors (FcγRI, III, IV, FcαRI, and FcεRI), is an important immune regulator both in innate and adaptive immunity. Previous studies have shown that FcRγ was a potential target of inflammatory diseases, whereas the role of FcRγ in sepsis has been poorly understood. In this study, we found that deficiency of FcRγ resulted in increased survival in lipopolysaccharide (LPS)/*D*-galactosamine and *E. coli*-induced sepsis in mice. This protective effect was characterized by decreased TNF-α, IL-6, and IL-10. Further experiments in bone marrow-derived macrophages (BMDMs) in vitro also showed that FcRγ deficiency resulted in decreased production of TNF-α, IL-6, and IL-10 upon LPS stimulation. The mechanism study showed that FcRγ was physiologically associated with toll-like receptor 4 (TLR4), and tyrosine phosphorylation of FcRγ mediated TLR4 signaling pathway, followed by increased ERK phosphorylation upon LPS stimulation. Our results suggest that FcRγ might be a potential therapeutic target of sepsis.

Keywords LPS · TLR4 · FcRγ · ERK

Abbreviations

FcRγ	Fc receptor common γ signaling chain
TLR4	Toll-like receptor 4
LPS	Lipopolysaccharide
PAMP	Pathogen-associated molecule patterns
IFNs	Interferons
D-gal	*D*-galactosamine
BMDMs	Bone marrow-derived macrophages
ITAM	Immunoreceptor tyrosine-based activation motif
Syk	Spleen tyrosine kinase
PLCγ	Phospholipase C-γ
TNF-α	Tumor necrosis factor α
IL-6	Interleukin 6
IL-10	Interleukin 10
p-Tyr	Phosphorylation of tyrosine
Tyr	Tyrosine

Zhi-Min Wei and Zhuo Wang contributed equally to this work.

✉ Yong Yang
valianty@hotmail.com

✉ Shun-Chang Jiao
jiaosc@vip.sina.com

✉ Zhe-Feng Liu
Lzf1220@sina.com

[1] Department of Oncology, General Hospital of Chinese PLA, 28 Fuxing Road, Beijing 100853, China

[2] State Key Laboratory of Natural Medicines, Center for New Drug Safety Evaluation and Research, China Pharmaceutical University, Nanjing 211198, China

[3] School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China

[4] Department of Anesthesiology and Intensive Care Medicine, Changhai Hospital, Second Military Medical University, 800 Xiangyin Rd, Yangpu Qu, Shanghai, China

Introduction

Recognition of microbial-conserved pathogen-associated molecule patterns (PAMP) by the innate immune system elicits strong proinflammatory cytokines such as IL-1, TNF-α, IL-6, and type I interferons (IFNs) [1]. Activation of the innate immune system needs to be tightly regulated, because uncontrolled inflammation may have damaging effects on the host, and result in inflammatory diseases, such as septic shock [2]. Pattern-recognition receptors (PRRs) are essential components of the innate immune system, including RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), C-type

lectin receptors (CLRs), and toll-like receptors (TLRs) [3]. Among them, TLR4 is the most thoroughly investigated PRRs, because it recognizes bacterial lipopolysaccharide (LPS), which is a well-known PAMP. LPS-triggered inflammatory response is started by interacting with lipopolysaccharide-binding protein in the serum; then, LPS is transported to the cell membrane where CD14 is present. Sequentially, LPS binds to TLR4-MD2 complexes, thus initiates the activation of TLR4/MyD88 and TLR4/TRIF signaling, resulting in subsequent inflammatory cytokine production and release [4]. However, the details of the initiating process remain unclear. It had been demonstrated that there was an activation cluster in the lipid raft, which comprised TLR4, CD11b/CD18, CD14, CD36 and CD55, FcγRIIIa, and CD81 after LPS or lipoteichoic acid (LTA) stimulation [5]. The exacting role of CD11b/CD18, CD14, CD36, and CD55 has been partly explained [6–10], as well as that of the mouse FcγRIII [11].

FcRγ is a common subunit shared by Fc receptors, including FcγRI, III, IV, FcαRI, and FcεRI. Binding of FcγRI, III, and IV by IgG transduces activating signals via phosphorylation of the FcRγ immunoreceptor tyrosine-based activation motif (ITAM) and leads to activation of spleen tyrosine kinase (Syk) and downstream signaling pathways such as phosphatidylinositol-3 kinase (PI3K) and phospholipase C-γ (PLCγ). As for FcαRI, IgA is a potent stimulus for producing pro-inflammatory cytokines, especially in neutrophils [12]. IgE binds to FcεRI in mast cells and recruits early signaling proteins, including Syk, PLCγ, and linker for activation of T cells, all of which localize with engaged receptors [13]. The primary aim of this study is to investigate the role of FcRγ in the mouse model of sepsis and the mechanism of FcRγ in the regulation of LPS-induced production of cytokines. We demonstrated that FcRγ interacted with TLR4 in BMDMs and promoted the release of cytokines in vitro.

Materials and methods

Animals FcRγ-deficient ($FcR\gamma^{-/-}$) mice were purchased from the Jackson Laboratory (#017793) and were backcrossed into the C57BL/6J strain to N10. Wide-type (WT) mice were obtained from the filial generation of the mating of $FcR\gamma^{+/-}$ with C57BL/6J mice. All animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Center for New Drug Evaluation and Research, China Pharmaceutical University, Nanjing, China.

Reagent LPS (L2630) from *E. coli* O111:B4 was purchased from Sigma-Aldrich (St. Louis, MO, USA). ELISA kits of mouse IL-6 (cat#: DKW12-2060-096), TNF-α (cat#: DKW12-2720-096), and IL-10 (cat#: DKW12-2100-096) were purchased from Dakewei Biotech (Beijing, China). Recombinant murine macrophage colony-stimulating factor (M-CSF, 315-02) was purchased from PeproTech (Rocky Hill, NJ, USA) and stock solution (10 μg/mL) was stored at − 80 °C. Anti-phosphotyrosine (05–321) and γ subunit antibodies (06–727) were purchased from Millipore (Billerica, MA, USA). TLR4 antibody (ab22048) was purchased from Abcam (Cambridge, Mass. UA). β-actin antibodies (60008-1-Ig) were purchased from Proteintech (Wuhan, Hubei, China). Antibodies of p-p65 (3033L), p-Akt (308T, 13038s), p-Akt (473S, 4060L), p-ERK1/2 (4370L), p-JNK (4668S), PI3K (p85), ERK1/2 (4695S), JNK (9252L), and p65 (8242s) were purchased from Cell Signaling Technology (Danvers, MA, USA). Akt antibody (WL0003b) was purchased from Wanleibio (Dalian, Liaoning, China). Goat anti-mouse IgG HRP (sc-2005) and goat anti-rabbit IgG HRP (sc-2004) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Establishment of mouse sepsis models

The experimental sepsis model was established by intraperitoneal injection of LPS (4 mg per kg body weight) and *D*-galactosamine (500 mg per kg body weight) as described in the previous study [14]. Bacterial infection was performed by intraperitoneal injection with 1×10^7 CFU *E. coli* strain.

Quantification of TNF-α, IL-10, and IL-6 by ELISA For the in vivo studies, serum was collected at 3 and 6 h after LPS plus *D*-galactosamine treatment, and 6 and 12 h after bacterial infection. For the in vitro studies, bone marrow-derived macrophages (BMDMs) were stimulated with LPS (10 ng/mL, 100 ng/mL, and 1 μg/mL) for 24 h, and the supernatants were collected. Concentrations of TNF-α, IL-6, and IL-10 in the serum or supernatant were measured by using ELISA according to the manufacturer's protocols.

Isolation of BMDMs BMDMs were created as described in a previous study [15]. Briefly, bone marrow cells flushed from femur and tibia were cultured on flasks for 7 days in the presence of 10 ng/mL M-CSF.

Western blotting and immunoprecipitation BMDMs treated with LPS (100 ng/mL) were lysed with lysis buffer [protease inhibitor mixture (Roche Diagnostics), 1% Triton X-100, 200 mM NaCl (pH 7.4), 0.2 mM Na_3VO_4, 0.3 mM EDTA, and 10% glycerol]. Bicinchoninic acid assay (Pierce) was used to measure protein concentrations. Western blot analysis and immunoprecipitation were done as described [14].

Confocal microscopy

BMDMs were cultured at 2×10^5 cells per well in 24-well plates on sterile coverslips overnight and stimulated with LPS (100 ng/mL) for 15 min. Then, cells were fixed with 4% paraformaldehyde for 10 min at room temperature and incubated with TLR4 and FcRγ antibodies overnight. Co-location was detected by using Alexa Fluor 647-labeled goat anti-mouse IgG antibody or Alexa Fluor 488-labeled goat anti-rabbit IgG antibody (Molecular Probes). Images were obtained by using a Leica TCS SPE confocal microscope with a 1.4 numerical aperture and 63 Leica objective.

Statistical analysis

Survival curves were obtained using Kaplan-Meier method and survival rates were analyzed by log-rank test using the Prism 5 GraphPad Software. Student's *t* test was used for comparisons between two groups. Differences were considered significant at a *P* value of less than 0.05.

Results

FcRγ deficiency suppresses LPS-induced inflammatory response

To investigate the role of FcRγ in the development of sepsis, we challenged WT and FcRγ$^{-/-}$ mice with LPS and *D*-gal. FcRγ deficiency in female mice dramatically increased the survival rate in the LPS and *D*-gal-induced septic models. The survival rate was 70% in female FcRγ$^{-/-}$ mice compared with 30% in WT female mice at 16 h, and 50% in female FcRγ$^{-/-}$ mice compared with 10% in WT female mice at 80 h (Fig. 1a). Results in male mice confirmed this effect, because male FcRγ$^{-/-}$ mice showed an increased survival rate from 10 to 50% at 16 h and from 0 to 30% at 30 h (Fig. 1b). Consistent with the improvement in survival rate, FcRγ deficiency significantly decreased the production of TNF-α and IL-6 in serum (Fig. 1c, d). However, a significant decrease in IL-10 was also found in FcRγ$^{-/-}$ mice compared with WT mice (Fig. 1e). These results indicate that FcRγ deficiency blocked LPS-induced immune responses, which may contribute to the protective effect against septic shock.

Next, we examined the response of WT and FcRγ$^{-/-}$ mice to another sepsis model induced by intraperitoneal infection with Gram-negative bacteria *Escherichia coli* (*E. coli*). A total of 50% of female FcRγ$^{-/-}$ mice remained alive after 50 h whereas no WT mice survived (Fig. 2a). We repeat this experiment in male mice. Results showed that 80% of WT male mice succumbed to fatal shock within 30 h and none survived after 60 h. In contrast, 40% of male FcRγ$^{-/-}$ mice remained alive at 60 h after infection with *E. coli* (Fig. 2b). We measured the representative cytokines in response to *E. coli* and found that FcRγ$^{-/-}$ mice showed a decrease in the production of TNF-α and IL-6 (Fig. 2c, d), whereas no difference was observed between WT mice. Besides, we also detected the level of the anti-inflammatory cytokine IL-10 in WT and FcRγ$^{-/-}$ mice. Results showed that there was no difference between WT and FcRγ$^{-/-}$ mice (Fig. 2e). Moreover, FcRγ$^{-/-}$ mice had a higher bacterial load than WT mice in the spleen, liver, and lung (Fig. 2f). This is consistent with published reports that proinflammatory cytokines promote the dissemination of *E. coli* [16]. In contrast to the bacterial load, the spleen weight index of WT mice was significantly higher than those of FcRγ$^{-/-}$ mice (Fig. 2g, h). Collectively, these data suggest that FcRγ$^{-/-}$ mice develop an alleviative inflammatory innate response and are less sensitive to sepsis. As TLR4 played an essential role in sepsis [17], this data indicated that FcRγ may positively regulate TLR4-triggered immune response.

Attenuated in vitro cytokine production by FcRγ-deficient macrophages following LPS exposure

FcRγ is mainly expressed in myeloid cells and macrophage plays an essential role in endotoxic shock [18, 19]; thus, we cultured BMDMs from WT and FcRγ$^{-/-}$ mice to investigate whether FcRγ regulated TLR4-triggered inflammation in the macrophage. In accordance with in vivo observations, FcRγ$^{-/-}$ BMDMs produced less TNF-α and IL-6 in response to LPS (either in 10 ng/mL or 100 ng/mL) than did BMDMs from littermate male and female WT mice (Fig. 3a, b). However, FcRγ$^{-/-}$ BMDMs also produced less of the anti-inflammatory cytokine IL-10 (Fig. 3c), which was consistent with a previous report wherein the activation of FcRγ led to a remodeling of the chromatin at the IL-10 locus and subsequently increased the production of IL-10 [20]. We further investigated the underlying mechanism for the effect of FcRγ deficiency on TLR4 signaling. The result showed that the intensity of phosphorylated ERK was markedly reduced in LPS-stimulated FcRγ$^{-/-}$ macrophages compared with the WT macrophages (Fig. 3d, e). However, the phosphorylation of JNK and AKT, as well as p65, remained unchanged (Fig. 3d, e). These data suggest that FcRγ deficiency may diminish TLR4-triggered production of cytokines by decreasing the activation of ERK in macrophages.

TLR4 signaling activates FcRγ

To study the physiological relevance of FcRγ signals in regulating the TLR4 response, we examined whether TLR4 ligation leads to activation of FcRγ. BMDMs from WT mice were treated with LPS for 15 and 30 min; then, the cell lysates were immunoprecipitated with anti-FcRγ antibody and analyzed

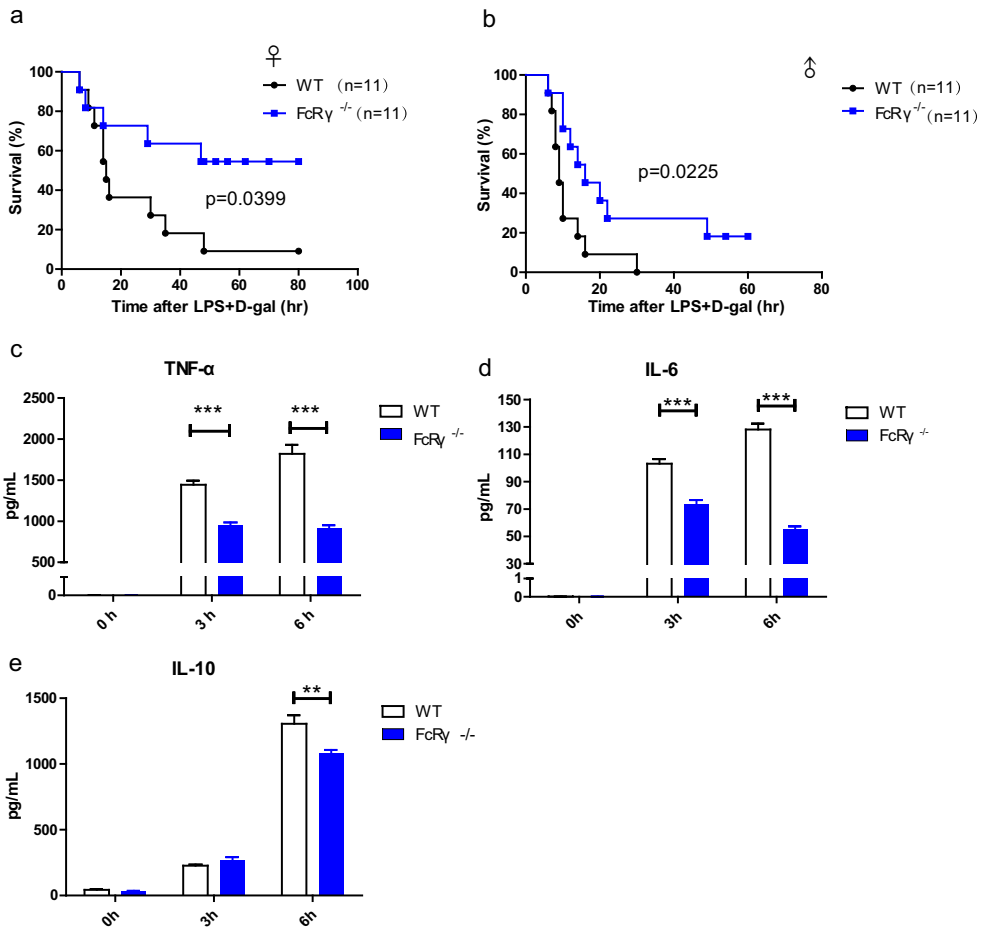

Fig. 1 FcRγ deficiency protects LPS-induced sepsis. **a**, **b** Survival rate of WT and FcRγ$^{-/-}$ female and male mice ($n = 11$ per genotype), monitored every hour after lethal challenge with LPS and *D*-gal. **c**–**e** Concentrations of TNF-α, IL-6, and IL-10 in serum after 0, 3, 6 h from WT and FcRγ$^{-/-}$ mice injected intraperitoneally with LPS and *D*-gal ($n = 11$). $^{*}P < 0.05$ vs WT mice, $^{**}P < 0.01$ vs WT mice, $^{***}P < 0.001$ vs WT mice

for tyrosine phosphorylation (p-Tyr) and TLR4. The results showed that BMDMs from WT mice exhibited rapid p-Tyr of the FcRγ-subunit, as indicated by increased bands compared with those in untreated BMDMs in the Western blotting (Fig. 4a). Moreover, reverse direction immunoprecipitation using anti-TLR4 antibody followed by Western blot analysis for FcRγ showed that FcRγ was associated with TLR4 in the presence or absence of LPS (Fig. 4b). Then, we obtained the distribution of TLR4 and FcRγ upon LPS stimulation by using the confocal microscopy. The result showed TLR4 and FcRγ co-localized in both rested and LPS-stimulated macrophages (Fig. 4c). These data suggest that FcRγ physically binds to TLR4 and can be activated upon LPS stimulation.

Discussion

Sepsis is a systemic inflammatory response syndrome, caused by life-threatening infections; it is characterized by tissue damage and multisystem organ failure [21]. However, except for antibiotics, current strategies to treat excessive inflammation remain ineffective. Therefore, it is extremely important to discover alternative treatments. Recent therapies that aim to inhibit the pro-inflammatory cytokine storm, including blood purification, CD28 antagonistic peptide (AB103), and soluble recombinant human thrombomodulin (ART-103), have been proposed [22–24].

FcRγ may serve as an activating molecule or an inhibitory receptor by recruiting Syk and SHP-1, respectively, depending upon different stimuli [25]. In the present study, FcRγ-deficient macrophages and mice were used to describe that FcRγ functioned as an activating molecule under stimulation with LPS in vitro and in vivo. FcRγ combines with TLR4 physiologically and LPS stimulation causes phosphorylating of Tyr within the ITAM region, followed by activation of the intracellular signaling cascades. TLR4 signaling is initiated by the dimerization of TLR4 and recruits TNF receptor-associated factor 6 subsequently [26]. Based on our findings, the multimerization of TLR4 and FcRγ is possibly required for the engagement of intracellular signaling pathways. This is consistent with the report that the ligation of FcRγ-subunit results in inhibition of pro-inflammatory cytokine production by monocytes in response to LPS [27]. However, Ching-Liang et al. demonstrated that lack of FcRγ increased TLR responses in dendritic cells, which included the stimuli of zymosan (which stimulates via TLR2), LPS (TLR4), poly

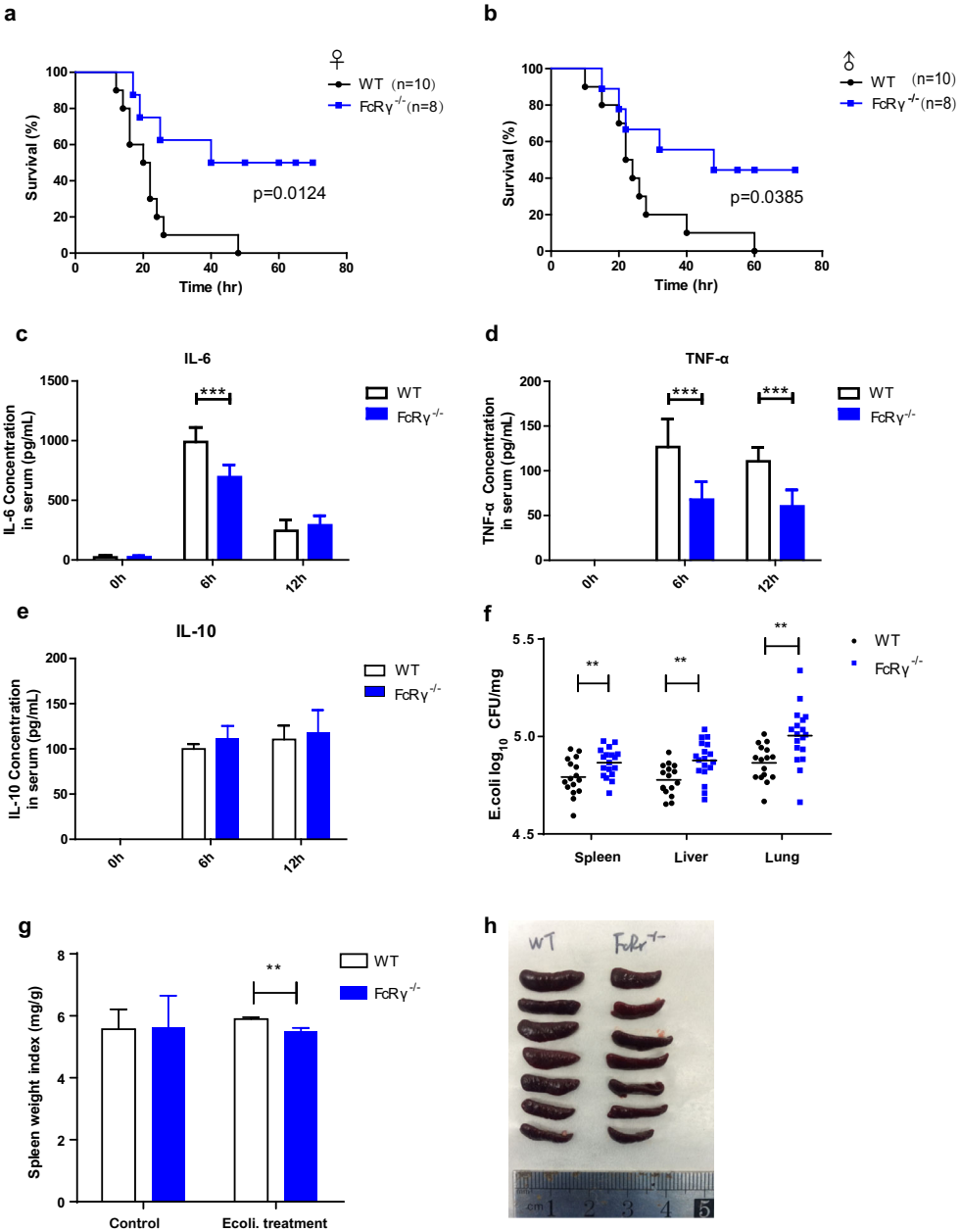

Fig. 2 FcRγ-deficient mice are more resistant to *E. coli* infection. **a**, **b** Survival rate of WT and FcRγ$^{-/-}$ female and male mice, monitored every hour after intraperitoneally injected with 1×10^7 *E. coli.* **c**–**e** Concentrations of TNF-α, IL-6, and IL-10 in serum after 0, 6, and 12 h from WT and FcRγ$^{-/-}$ male mice injected intraperitoneally with *E. coli.* **f** Bacterial load in the spleen, liver, and lung of WT and FcRγ$^{-/-}$ male mice injected peritoneally with 1×10^7 *E. coli*, assessed 12 h after infection. **g** Spleen weight index of WT and FcRγ$^{-/-}$ male mice with or without *E. coli* infection. **h** Photo of spleen. **P* < 0.05 vs WT mice, ***P* < 0.01 vs WT mice, *** *P* < 0.001 vs WT mice

(I:C) (TLR3), flagellin (TLR5), and CpG DNA (TLR9) [28]. Thus, how FcRγ exhibits different roles between macrophages and DC is unclear. The reduction of IL-10 in the absence of FcRγ in DC is consistent with our results, which is dependent on Syk-mediated intracellular signaling [29]. Furthermore, phagocytosis is closely linked to inflammatory response; however, the precise mechanism of this coupling remains obscure [30]. FcRγ is required for phagocytosis, which is essential for its subsequent cytokine expression. This may partly explain the decreased cytokine expression in the absence of FcRγ. Previous findings by Zanoni et al. suggest a role for FcRγ in regulating TLR4 endocytosis, which initiated TLR4-TRIF-IRF3 signaling [7]. FcRγ deficiency impaired the recruitment of Syk and PLCγ2, followed by the decreased production of IFNβ. Thus, FcRγ may regulate the TLR4-MyD88 and TLR4-TRIF signaling cascade.

The titer of IgG1 is significantly different between male and female; thus, we use different genders of mice to perform the in vivo experiment [31]. The results showed that male mice were dead more quickly than female mice both in WT and FcRγ$^{-/-}$ mice. This result suggested that IgG1 may involve in the development of sepsis.

In summary, our results have demonstrated that tyrosine phosphorylation of FcRγ mediated TLR4 signaling pathway in macrophage. This working model explains why FcRγ-

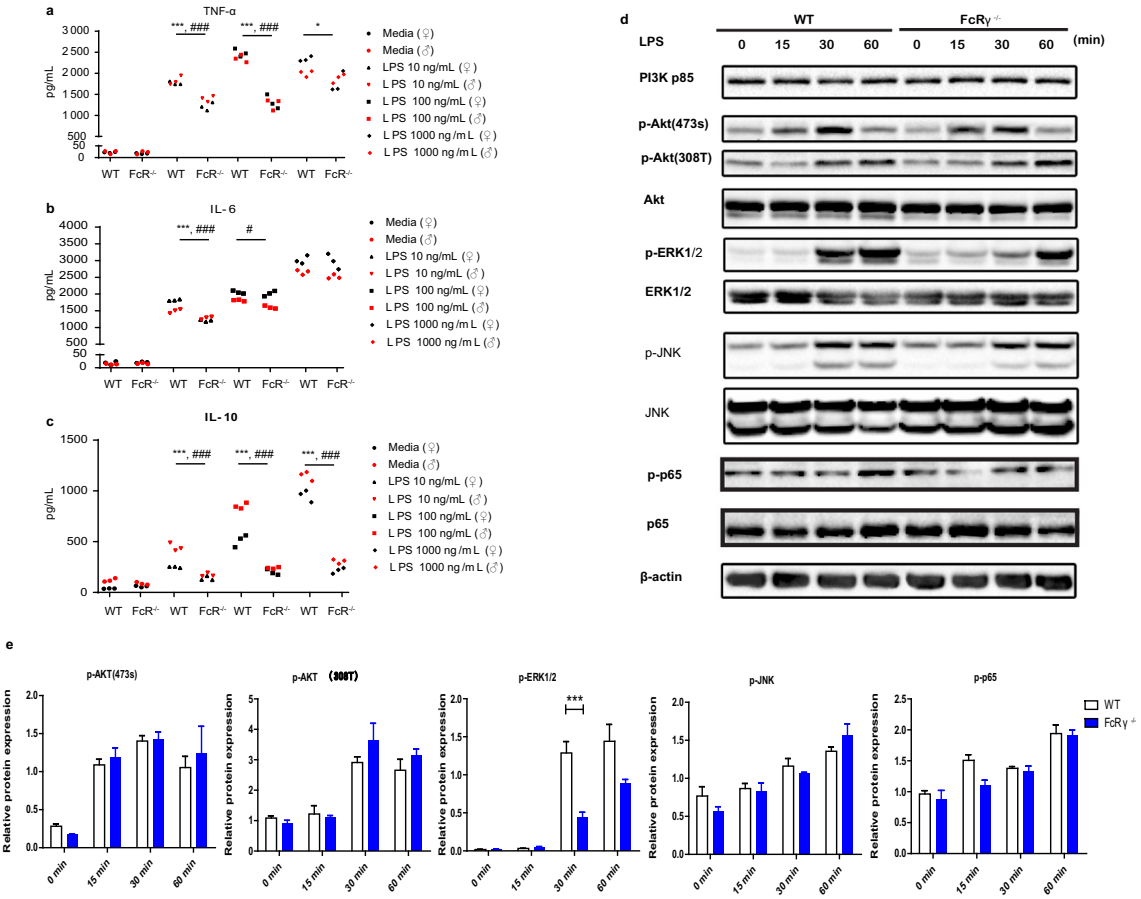

Fig. 3 FcRγ deficiency decreases TLR4 triggered production of cytokines through decreased activation of ERK. **a**–**c** Concentrations of TNF-α, IL-6, and IL-10 in supernatants of female and male BMDMs from WT and FcRγ$^{-/-}$ mice stimulated with LPS. **d** WT and FcRγ$^{-/-}$ BMDMs were stimulated with 100 ng/mL LPS for 15, 30, and 60 min and cell lysates were analyzed for the level of phosphorylated or total protein expression with indicated antibodies; β-actin served as a loading control. **e** The densitometric analyses of the bands for phosphorylated AKT (473s), AKT (308T), ERK1/2, JNK, and p65 were shown ($n = 3$). *$P < 0.05$, FcRγ$^{-/-}$ female BMDMs vs WT female BMDMs; *** $P < 0.001$, FcRγ$^{-/-}$ female BMDMs vs WT female BMDMs; #$P < 0.05$, FcRγ$^{-/-}$ male BMDMs vs WT male BMDMs; ### $P < 0.001$, FcRγ$^{-/-}$ male BMDMs vs WT female BMDMs

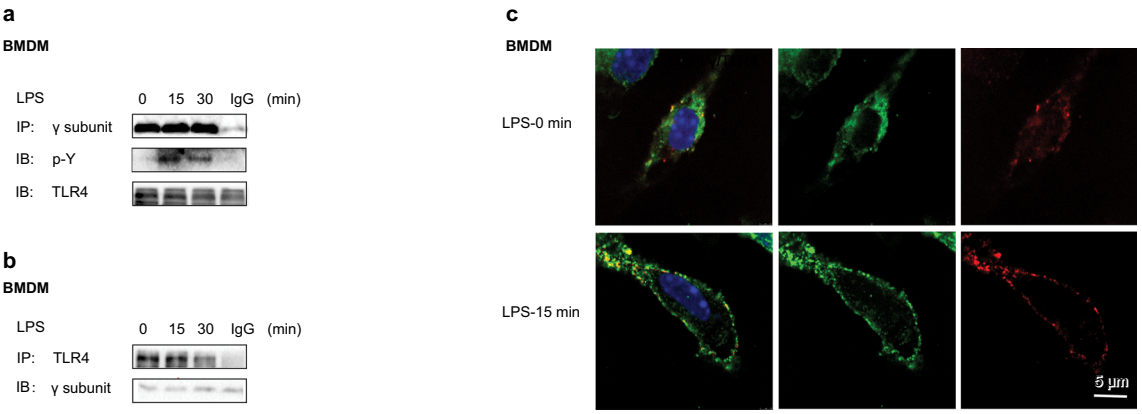

Fig. 4 FcRγ associates with TLR4 physiologically and is activated upon LPS stimulation. **a** Immunoblot analysis of TLR4 and phosphorylated tyrosine (p-Tyr) in anti-FcRγ immunoprecipitates of lysates of cells stimulated with 100 ng/mL LPS at indicated time. **b** Immunoblot analysis of TLR4 and FcRγ in anti-TLR4 immunoprecipitates of lysates of cells stimulated with 100 ng/mL LPS at indicated time. **c** BMDMs were stimulated with LPS (100 ng/mL) for 15 min and processed for confocal microscopy to detect the presence of TLR4 and FcRγ (scale bar: 5 μm)

deficient mice display less susceptibility to endotoxic shock. Our findings suggest that targeting FcRγ could be an interesting approach for management of sepsis in humans.

Funding information This work was supported in part by the National Science Foundation of China (Nos. 91529304, 81473230, and 81673468) and the Natural Science Foundation of Jiangsu Province (No. BK20170732).

Compliance with ethical standards All animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Center for New Drug Evaluation and Research, China Pharmaceutical University, Nanjing, China.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Miller SI, Ernst RK, Bader MW. LPS, TLR4 and infectious disease diversity. Nat Rev Microbiol. 2005;3(1):36–46.
2. Liew FY, Xu D, Brint EK, O'Neill LA. Negative regulation of toll-like receptor-mediated immune responses. Nat Rev Immunol. 2005;5(6):446–58.
3. Kugelberg E. Pattern recognition receptors: curbing gut inflammation. Nat Rev Immunol. 2014;14(9):583.
4. Triantafilou M, Triantafilou K. Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. Trends Immunol. 2002;23(6):301–4.
5. Pfeiffer A, Bottcher A, Orso E, Kapinsky M, Nagy P, Bodnar A, et al. Lipopolysaccharide and ceramide docking to CD14 provokes ligand-specific receptor clustering in rafts. Eur J Immunol. 2001;31(11):3153–64.
6. Han C, Jin J, Xu S, Liu H, Li N, Cao X. Integrin CD11b negatively regulates TLR-triggered inflammatory responses by activating Syk and promoting degradation of MyD88 and TRIF via Cbl-b. Nat Immunol. 2010;11(8):734–42.
7. Zanoni I, Ostuni R, Marek LR, Barresi S, Barbalat R, Barton GM, et al. CD14 controls the LPS-induced endocytosis of toll-like receptor 4. Cell. 2011;147(4):868–80.
8. Zanoni I, Tan Y, Di Gioia M, Springstead JR, Kagan JC. By capturing inflammatory lipids released from dying cells, the receptor CD14 induces Inflammasome-dependent phagocyte Hyperactivation. Immunity. 2017;47(4):697–709 **e3**.
9. Chen Y, Huang W, Yang M, Xin G, Cui W, Xie Z, et al. Cardiotonic steroids stimulate macrophage inflammatory responses through a pathway involving CD36, TLR4, and Na/K-ATPase. Arterioscler Thromb Vasc Biol. 2017;37(8):1462–9.
10. Zhang X, Kimura Y, Fang C, Zhou L, Sfyroera G, Lambris JD, et al. Regulation of toll-like receptor-mediated inflammatory response by complement in vivo. Blood. 2007;110(1):228–36.
11. Rittirsch D, Flierl MA, Day DE, Nadeau BA, Zetoune FS, Sarma JV, et al. Cross-talk between TLR4 and FcgammaReceptorIII (CD16) pathways. PLoS Pathog. 2009;5(6):e1000464.
12. Aleyd E, Heineke MH, van Egmond M. The era of the immunoglobulin a fc receptor FcalphaRI; its function and potential as target in disease. Immunol Rev. 2015;268(1):123–38.
13. Wakefield DL, Holowka D, Baird B. The FcepsilonRI Signaling Cascade and Integrin Trafficking Converge at Patterned Ligand Surfaces. Mol Bio Cell. 2017; 28(23):3383–96.
14. Li X, Wang D, Chen Z, Lu E, Wang Z, Duan J, et al. Galphai1 and Galphai3 regulate macrophage polarization by forming a complex containing CD14 and Gab1. Proc Natl Acad U S A. 2015;112(15):4731–6.
15. Voss OH, Murakami Y, Pena MY, Lee HN, Tian L, Margulies DH, et al. Lipopolysaccharide-induced CD300b receptor binding to toll-like receptor 4 alters signaling to drive cytokine responses that enhance septic shock. Immunity. 2016;44(6):1365–78.
16. Haziot A, Ferrero E, Köntgen F, Hijiya N, Yamamoto S, Silver J, et al. Gram-negative bacteria in CD14-deficient mice. Immunity. 1996;4:407–14.
17. Li X, Wang Z, Zou Y, Lu E, Duan J, Yang H, et al. Pretreatment with lipopolysaccharide attenuates diethylnitrosamine-caused liver injury in mice via TLR4-dependent induction of Kupffer cell M2 polarization. Immunol Res. 2015;62(2):137–45.
18. Rosadini CV, Kagan JC. Early innate immune responses to bacterial LPS. Curr Opin Immunol. 2017 Feb;44:14–9.
19. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol. 2013;13(12):862–74.
20. Lucas M, Zhang X, Prasanna V, Mosser DM. ERK activation following macrophage FcgammaR ligation leads to chromatin modifications at the IL-10 locus. J Immunol. 2005;175(1):469–77.
21. Angus DC, van der Poll T. Severe sepsis and septic shock. New Engl J Med. 2013;369(21):2063.
22. Kang JH, Super M, Yung CW, Cooper RM, Domansky K, Graveline AR, et al. An extracorporeal blood-cleansing device for sepsis therapy. Nat Med. 2014;20(10):1211–6.
23. Ramachandran G, Kaempfer R, Chung CS, Shirvan A, Chahin AB, Palardy JE, et al. CD28 homodimer interface mimetic peptide acts as a preventive and therapeutic agent in models of severe bacterial sepsis and gram-negative bacterial peritonitis. J Infect Dis. 2015;211(6):995–1003.
24. Vincent JL, Ramesh MK, Ernest D, LaRosa SP, Pachl J, Aikawa N, et al. A randomized, double-blind, placebo-controlled, phase 2b study to evaluate the safety and efficacy of recombinant human soluble thrombomodulin, ART-123, in patients with sepsis and suspected disseminated intravascular coagulation. Crit Care Med. 2013;41(9):2069–79.
25. Ivashkiv LB. Cross-regulation of signaling by ITAM-associated receptors. Nat Immunol. 2009;10(4):340–7.
26. Song DH, Lee JO. Sensing of microbial molecular patterns by toll-like receptors. Immunol Rev. 2012;250(1):216–29.
27. Chu CL, Yu YL, Shen KY, Lowell CA, Lanier LL, Hamerman JA. Increased TLR responses in dendritic cells lacking the ITAM-containing adapters DAP12 and FcRgamma. Eur J Immunol. 2008;38(1):166–73.
28. Grazia Cappiello M, Sutterwala FS, Trinchieri G, Mosser DM, Ma X. Suppression of Il-12 transcription in macrophages following fc gamma receptor ligation. J Immunol. 2001;166(7):4498–506.
29. Rogers NC, Slack EC, Edwards AD, Nolte MA, Schulz O, Schweighoffer E, et al. Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. Immunity. 2005;22(4):507–17.
30. Ravetch J, Aderem A. Phagocytic cells. Immunol Rev. 2007;219:5–7.
31. Martinsson K, Carlsson L, Kleinau S, Hultman P. The effect of activating and inhibiting fc-receptors on murine mercury-induced autoimmunity. J Autoimmun. 2008 Aug;31(1):22–9.