Gene 628 (2017) 267–274

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

# Porcine *CD14* gene silencing partially inhibited the bacterial immune response mediated by TLR4 signaling pathway

Chaohui Dai[a,1], Haifei Wang[a,1], Guoqiang Zhu[b,c], Shenglong Wu[a,c], Wenbin Bao[a,c,*]

[a] Key Laboratory for Animal Genetics, Breeding, Reproduction and Molecular Design of Jiangsu Province, College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu 225009, China
[b] College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu 225009, China
[c] Joint International Research Laboratory of Agriculture & Agri-Product Safety, Yangzhou University, Jiangsu, Yangzhou 225009, China

## ARTICLE INFO

*Keywords:*
Pig
*CD14*
Gene silencing
TLR4 signaling pathway
*E. coli*
Immune response

## ABSTRACT

Cluster of differentiation antigen 14 (CD14) is the membrane receptor protein in Toll-like Receptor 4 (TLR4) signaling pathway, which plays an important regulation role in not only innate immune response but also adaptive immune response. In this study, the pig kidney epithelial cell (PK15) line with *CD14* gene silencing mediated by lentivirus was established and cells of CD14-RNAi and NC group were exposed to three kinds of *Escherichia coli* (*E. coli* F18ab, *E. coli* F18ac and *E. coli* K88ac) and LPS. Then qPCR and western blot were used to detect expression levels of TLR4 signaling pathway-related genes. Finally, ELISA was used to detect the level of proinflammatory cytokines in the cell culture supernatant. The results showed that the expression level of TLR4 signaling pathway-related genes in the entire signal pathway had obvious increases when cells were exposed to the stimulation induced by *E. coli* and LPS. In addition, the expression levels of CD14-RNAi group were overall significantly lower than NC group ($P < 0.05$ or $P < 0.01$), which was the same with the release levels of proinflammatory cytokines. This study revealed that pig *CD14* gene silencing partially inhibited immune response to *E. coli* F18 invasion mediated by TLR4 signaling pathway.

## 1. Introduction

Toll-like receptors (TLRs) were found in drosophila's Toll proteins, which can not only participate in regulating the formation of dorsal ventral polarity in embryonic drosophilas but also directly mediate drosophila's natural (inherent) immune response to microbial infection (Medzhitov, 2001). Toll-like receptors gene family belongs to the type I transmembrane protein receptors in mammals, which widely distributes in the organization such as gastrointestinal tract and respiratory tract; TLRs play an important role in not only adaptive immune but also innate immune (Beutler, 2005). TLR4, as the main receptor for recognizing the bacterial lipopolysaccharide (LPS) and signaling pathway mediated by TLR4, belongs to signal transduction pattern recognition receptor (PRR) in TLRs family and plays an important role in a variety of inflammatory reactions (Poltorak et al., 1998).

Cluster of differentiation antigen 14 (CD14), an essential protein in TLR4 signaling pathway, is a kind of specific surface marker of monocytes, neutrophils and macrophages cells; it belongs to one of members of the cell surface glycoprotein family and has been proved to be a high affinity receptor of LPS which is endotoxin of gram-negative bacterium (Wright et al., 1990). The main biological activities of CD14 are as a receptor of LPS to recognize and combine with LPS or LPS/LBP complexes and mediate LPS inflammatory cells reaction. When the concentration of LPS is low ($\leq 100$ ng/mL), its effect on the activation of cells is completely mediated by its receptor CD14; and when concentration is high, its activation is in part mediated by CD14 (Perera et al., 1997). Its action mechanism is approximately when gram-negative bacteria invades the body, a variety of bacterial products such as LPS and lipoteichoic acid (LTA) mediated by CD14 signaling pathway activates immunocompetent cells like mononuclear macrophages and endothelial cells, which leads to release of cytokines and other

*Abbreviations:* CD14, cluster of differentiation antigen 14; mCD14, membrane CD14; sCD14, soluble CD14; TLR4, Toll-like receptor 4; MyD88, myeloid differentiation protein; IFN-α, interferon-alpha; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1β; MD2, myeloid differentiation protein 2; PRR, pattern recognition receptor; *E. coli*, *Escherichia coli*; LPS, lipopolysaccharide; LBP, LPS-binding protein; LTA, lipoteichoic acid; qPCR, quantitative real-time PCR; ATCC, American Type Culture Collection; DMEM, Dulbecco's modified eagle medium; FBS, fetal bovine serum; SYBR, Synergy Brands, Inc; GPI, glycosylphosphatidyl phthalide lipids; MHC, major histocompatibility complex

* Corresponding author at: College of Animal Science and Technology, Yangzhou University, 48 East Wenhui Ave, Yangzhou, Jiangsu 225009, China.
*E-mail address:* wbbao@yzu.edu.cn (W. Bao).

[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.gene.2017.07.055
Received 14 January 2017; Received in revised form 26 April 2017; Accepted 19 July 2017
Available online 20 July 2017
0378-1119/ © 2017 Elsevier B.V. All rights reserved.

inflammatory mediators and finally presents the response to the bacterial infection (Chen, 2000). Plenty of clinical studies have indicated that the expression level of *CD14* gene has a close relation with human sepsis, traumatic disease, disease of immune system, diseases of the blood system, digestive system diseases and so on (Carrillo et al., 2001; Frey et al., 1992; Gluck et al., 2001; Nockher and Scherberich, 1997; Sakr et al., 2008). Fung and Mollnes (2012) has found that the combined use of complement inhibitors and the inhibitor of CD14 signaling pathway was a kind of effective method for the prevention and treatment of inflammation caused by endotoxin LPS. Diarrhea and edema disease in weaned piglets are the important infectious diseases that can lead to the deaths of weaned piglets, which causes enormous economic loss to the pig industry. The enteropathogenic *Escherichia coli* (EPEC) is the main pathogenic factor of these two kinds of diseases, which results in intestinal diseases mainly through the release of endotoxin LPS. CD14 is the major receptor for recognition of LPS, so functional analysis of *CD14* gene could be conducive to investigate the molecular mechanism of diarrhea and edema disease in weaned piglets caused by EPEC.

Pig *CD14* gene is located on chromosome 2 q28, including two exons and one intron and encoding 373 amino acid sequence, whose structure has high homology with humans, mice, rabbits, horses and cattle. Liu et al. (2008) analyzed the polymorphism of pig *CD14* gene and found that there were three potential polymorphic loci (− 61, 587 and 1246) in porcine *CD14* gene, and they analyzed the relationship between three kinds of genotypes at − 61 site and parts of the immune traits like IgG and DTH. Sun et al. (2015) found that under the condition of stimulation induced by LPS, the transcription of *CD14* gene in pig small intestinal epithelial cells (IPEC-J2) significantly raised with extending of time, which showed that LPS was released in the IPEC-J2 cells with *E. coli* infection and *CD14* gene played an essential role in mediating the inflammatory reactions and immune response infected by *E. coli* with releasing LPS. In our previous study, transcriptome analysis of duodenum of *E. coli* F-18 resistant and sensitive full sibs in Meishan weaning piglets was conducted and we found that TLR4 signaling pathway and *CD14* gene were associated with resistance to *E. coli* in weaning piglets (Wu et al., 2016). In order to investigate the immune regulation roles of TLR4 signaling pathway and *CD14* gene in the process of *E. coli* infection, a PK15 cell line with stable *CD14* gene silencing was established in this study. Then three kinds of *E. coli* (F18ab, F18ac and K88ac) and LPS with three kinds of concentration (50 ng/mL, 100 ng/mL and 1000 ng/mL) were used to stimulate cells to detect the expression changes of key genes in the TLR4 signaling pathway and the release levels of proinflammatory factor in downstream, which aims to explore the influence of *CD14* gene silencing on the immune response mediated by TLR4 signaling pathway so as to provide a basis for further function and mechanism research of *CD14* gene.

## 2. Materials and methods

### 2.1. Reagents and materials

The pig kidney cell line (PK15) was obtained from the American Type Culture Collection (ATCC, USA). *E. coli* F18ab, *E. coli* F18ac, and *E. coli* K88ac fimbriae standard strains were provided by the veterinary laboratory at the Institute of Microbiology, University of Pennsylvania. Fetal bovine serum (FBS) and Dulbecco's modified eagle medium (DMEM) were purchased from Gibco (NY, USA). Trizol was purchased from Invitrogen (CA, USA). HiScript® II Q Select RT SuperMix for qPCR and AceQ® qPCR SYBR® Green Master Mix were purchased from Vazyme Biotech Co., Ltd. (Nanjing, China). Total protein extraction kit and BCA protein detection kit were purchased from Nanjing Keygen Technology Development Co., Ltd. (Nanjing, China). Primary antibodies-CD14 (1:400), MyD88 (1:800), IFN-α (1:600), IL-1β (1:600), TLR4 (1:1000), TNF-α (1:1000) and β-actin (1:4000)-were purchased from Abcam (Cambridge, UK). Second antibody (IgG-HRP, 1:3000) was purchased from Jackson (PA, USA). LPS was purchased from Sigma-Aldrich (MO, USA). Porcine IL-1β and TNF-α ELISA kits were purchased from AssayPro (MO, USA).

### 2.2. Primer design and sequence synthesis

The qPCR primers of TLR4 signaling pathway related genes were designed based on the sequences of these genes from the pig available in the GenBank database using Primer Express 2.0 software (Table 1). All of primers were synthesized by Sangon Biotech (Shanghai, China) Co., Ltd.

### 2.3. Establishment of PK15 cell line with CD14 gene silencing

PK15 cells were incubated with DMEM culture medium containing 10% FBS in an incubator under the conditions of 37 °C and 5% $CO_2$. When the coverage of cells reached ~80%, transfections were performed with four lentivirus vector constructed in our previous research (Wu et al., 2016) by pGLV3-CD14-1, pGLV3-CD14-2, pGLV3-CD14-3 and pGLV3-CD14-4, a negative control group pGLV3- NC and a blank control group without lentivirus infection, each treatment had three repeats. Infected cells were cultured at 37 °C in a 5% $CO_2$ incubator overnight and then cells expressing green fluorescent protein were observed after culture for 24 h. 10 μg/mL puromycin was used to screen the positive cells every 24 h and cell total RNA and protein were extracted for analyzing the transcription and translation level to detected the interference of *CD14* gene. Then several positive monoclonal cells were picked to establish the PK15 cell line with *CD14* gene silencing.

### 2.4. E. coli stimulation in PK15 cells of CD14-RNAi group and NC group

PK15 cells of CD14-RNAi group and NC group were inoculated to 12-well plates at a density of $5.0 \times 10^5$ cells per well, and cells were incubated for 24 h in an incubator under the conditions of 37 °C and 5% $CO_2$. *E. coli* F18ab, *E. coli* F18ac, and *E. coli* K88ac fimbriae standard strains were respectively inoculated to LB culture medium and incubated for 12 h at a rotating speed of 200 rev/min on a shaking table. After centrifugation for 5 min at a rotating speed of 4000 rev/min, the supernatant of three kinds of *E. coli* were filtered through 0.22 μm filters to collect the bacteria supernatant and PBS buffer solution was used to resuspend the bacteria precipitation and then wash and centrifuge for three times. Cells with CD14-RNAi group and NC group were exposed to the bacteria supernatant of *E. coli* F18ab, *E. coli* F18ac, and *E. coli* K88ac for 4, 8 and 12 h, three parallel replicates were used for each group.

### 2.5. LPS induce in PK15 cells of CD14-RNAi group and NC group

When the cells of CD14-RNAi group and NC group were 80% confluent, they were exposed to 50 ng/mL, 100 ng/mL and 1000 ng/mL LPS (Sigma-Aldrich, MO, USA) for 2, 4 and 6 h, three parallel replicates were used for each group. At the same time, CD14-RNAi cells and NC cells without stimulation treatment were set as control group respectively. Then cell total RNA and protein were extracted for transcript and translation levels of TLR4 signaling related genes. And cells culture supernatants were collected for ELISA analysis.

### 2.6. Quantitative real-time PCR (qPCR)

Total RNA was extracted using the Trizol Reagent (Takara, China), according to the manufacturer's instructions. RNA was reversed to cDNA and was used as a template to detect and analyze the transcript levels of *CD14* and TLR4 signaling related genes. The 50-μL reaction mixture for cDNA synthesis contained the following: 10 μL 5× HiScript® II Q RT SuperMix, 2000 ng total RNA, and RNase-free $H_2O$ to make up the final volume of 50 μL. The reaction was carried out at

Download English Version:

https://daneshyari.com/en/article/5589190

Download Persian Version:

https://daneshyari.com/article/5589190

Daneshyari.com