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Radix Sophorae Flavescentis of *Sophora flavescens* Aiton inhibits LPS-induced macrophage pro-inflammatory response via regulating CFHR2 expression

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ABSTRACT

Ethnopharmacological relevance: Long-term chronic inflammation often leads to chronic diseases. Although *Sophora flavescens* has been shown to have anti-inflammatory properties, its detailed molecular mechanism is still unknown.

Aim of study: This study investigated the effect of *Radix Sophorae Flavescentis* on the LPS-induced inflammatory response in macrophages.

Materials and methods: LPS was used to induce the peritoneal macrophages to simulate the inflammatory environment *in vitro*. Different concentrations of *Radix Sophorae Flavescentis*-containing (medicated) serum were used for intervention. The peritoneal macrophages were identified by using hematoxylin-eosin and immunofluorescence staining. ELISA was used to measure the TNF-α and IL-6 expression to determine the concentration of LPS. ELISA and Western blot (WB) were used to detect the PGE2 and CFHR2 expression in each group, respectively. The lentiviral vector for interference and overexpression of the CFHR2 gene was constructed, packaged, and transfected into LPS-induced macrophages. The transfection efficiency was verified by WB. Then, ELISA was used to detect the TNF-α, PGE2, and IL-6 expression. WB was used to detect the CFHR2, iNOS, COX-2, TLR2, TLR4, IFN-γ, STAT1, and p-STAT1 expression.

Results: The primary isolated cells were identified as macrophages. The LPS-treated macrophages exhibited significantly higher expression of PGE2 and CFHR2, and the inflammatory factors TNF-α and IL-6, as well as iNOS, COX-2, TLR2, TLR4, IFN-γ, STAT1, and p-STAT1 expression compared with the control group ($P < 0.05$). The TNF-α, PGE2, and IL-6 levels, as well as CFHR2, iNOS, COX-2, TLR2, TLR4, IFN-γ, STAT1, and p-STAT1 expression were considerably lower in the LPS-induced+10% medicated-serum group, LPS-induced+20% medicated-serum group, and shCFHR interference group compared with the LPS group ($P < 0.05$).

Conclusion: Radix Sophorae Flavescentis might mediate CFHR2 expression and play an important role in inhibiting the LPS-induced pro-inflammatory response of macrophages. *Radix Sophorae Flavescentis* could be a potential treatment for LPS-induced related inflammatory diseases.

1. Introduction

As the first response to infection and tissue damage, inflammation can activate the human immune system; however, long-term chronic inflammation can result in chronic diseases such as diabetes and atherosclerosis (Chen et al., 2017), caused by proinflammatory cytokines (Baniyash et al., 2014). In the process of inflammatory activation, macrophages can be recruited to the sites of inflammation and play a crucial role in the signal activation of various cellular inflammatory cascades (Lee et al., 2021). The macrophages activation is mediated by the pattern recognition receptor (PRR), which identifies various microbial pathogens on their surface (Lv et al., 2017), including intracellular signal cascade, transcriptional activation, and cytokine expression (Hedl et al., 2020). One of the most studied PRRs is the toll-like receptor

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(TLR) family. So far, 10 functional TLRs (TLR1-10) have been found (Patin et al., 2019), among which TLR2 and TLR4 are known to mediate host immune response (Silk et al., 2017). After binding to specific ligands, TLR2/TLR4 receptors trigger downstream signaling cascades that eventually result in increased levels of inflammatory mediators (Lee et al., 2016), such as nitric oxide (NO), prostaglandin E2 (PGE2), inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2), and pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 (Zou et al., 2013).

Recent research found that complement factor H-related protein 2 (CFHR2) is a human immune-related recombinant protein and a member of the family that assists in the activation of the complement system. This protein originates from the filtration membrane of the glomerulus and the lymphatic system, and plays an important role in immune regulation and inflammation suppression (Siegel et al., 2010). Studies have found that CFHR2, as an important complement protein in immunity, can enrich the accumulation of a variety of inflammatory factors and play an important regulatory role in malignant tumors, pathogenic infections, and inflammatory responses (Eberhardt et al., 2013). For instance, CFHR2 expression can affect the occurrence of acute anterior uveitis (Huang et al., 2016). Furthermore, CFHR2 expression is widely used in the defense against pathogen invasion and pulmonary arterial hypertension associated with congenital heart disease (Zhang et al., 2016). However, the molecular mechanisms and pathways of action of CFHR2 in regulating inflammation are still unclear.

The JAK/STAT signaling pathway is an important pathway for cytokine signaling. It is involved in cell proliferation, differentiation, apoptosis, and self-regulation, and plays an important role in various diseases such as tumors and inflammation (Hedl et al., 2020; Lee et al., 2021). Under physiological conditions, the JAK/STAT signaling pathway is tightly regulated by genes, however, abnormal activation of the JAK/STAT pathway can lead to the occurrence of diseases. JAK/-STAT can be activated in various ways, including cytokines, hormones, inflammatory factors TNF-α, IL-17a, and Toll like receptors (Huang et al., 2016; Lee et al., 2021). As an important complement protein, CFHR2 can stimulate the expression of various cytokines, and also plays an important regulatory role in lymphocyte development (Siegel et al., 2010; Zhang et al., 2016). Therefore, we speculate that CFHR2 can activate the JAK/STAT signaling pathway and affect the occurrence of inflammation by stimulating a variety of inflammatory factors, such as IL-6 and TNF-α.

Radix Sophorae Flavescentis ("Kushen") is the dried root of the leguminous plant *Sophora flavescens* Ait. of the family Fabaceae. Bitter in taste and cold in nature, it has the effect of promoting immature heat to mature, promoting sweating, calming endogenous wind and removing meridian obstruction. It is mainly used for immature fever, cold, gout, rheumatism, measles, scarlet fever and other diseases (Li et al., 2021). It has been widely used to treat ulcerative colitis, which is characterized by recurring episodes of inflammation and a relatively long disease course (Chen et al., 2020). Studies have reported that its main components are matrine and oxymatrine, which act as a two-way immunomodulator by stimulating lymphocyte proliferation at low concentrations and inhibiting it at high concentrations. It has been shown that the pharmacological properties of *Sophora flavescens* can inhibit 5-lipoxygenase (LOX) and 12-LOX activities (Chi et al., 2001), reduce the production of pro-inflammatory mediators and cytokines (Li et al., 2011), and exhibit anti-inflammatory activity against mouse croton oil-induced ear edema and rat carrageenan paw edema *in vivo* (Kim et al., 2002). Although these reports have briefly elucidated the anti-inflammatory activity of *Sophora flavescens*, its detailed molecular mechanism is yet unknown.

In this study, we explored the anti-inflammatory mechanism of *Radix Sophorae Flavescentis* in lipopolysaccharide (LPS)-induced peritoneal macrophages. We elucidated the role of *Radix Sophorae Flavescentis* in inhibiting inflammatory responses by targeting CFHR2 to regulate the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway.

2. Materials and methods

2.1. Experimental animals

Two-month-old Sprague Dawley (SD) male rats weighing 180–220 g (license number: SCXK (Xiang) 2019-0004) were purchased from Hunan SJA Laboratory Animal Co., Ltd. The housing conditions were temperature 20–26 °C, and humidity 40–70%. The rats had access to food and water *ad libitum*.

2.2. Isolation of rat primary peritoneal macrophages

Six rats were euthanized by cervical dislocation. The rats were soaked in 75% alcohol for 5 min. Then, they were lifted upside down, and 10 mL of serum-free Dulbecco's Modified Eagle Medium (DMEM) medium (KeyGEN BioTECH, Jiangsu, P.R. China) was injected from the side of the abdominal cavity. The rats were placed in a supine position, the abdomen was gently rubbed for 2–3 min, and let stand for 5–7 min. Then, the abdominal cavity was exposed under aseptic condition. When the intestine became flat, and upon observation the peritoneal fluid was light yellow, about 8–9 mL of peritoneal fluid was extracted with a pipette and centrifuged. DMEM complete medium was used to adjust the cells to the desired concentration. The cells were seeded in a 6-well plate, and put in a 37 °C 5% CO_2 incubator (BPN–80CW, Shanghai Yiheng Technology Co., Ltd., Shanghai, P.R. China). After cultured for 12 h, the medium was changed to remove the non-adherent cells.

2.3. Hematoxylin-eosin (HE) staining

4% fixative solution was used to fix the climbed cells on the cell climbing slice culture plate for 15 min at room temperature (RT). Then, the cells were immersed in phosphate-buffered saline (PBS) for 3 times (3 min each), and stained with hematoxylin aqueous solution for 3 min at RT (ZSGB-Bio, Beijing, P.R. China). Subsequently, hydrochloric acid ethanol differentiation solution (Xilong Scientific Co., Ltd., Guangdong, P.R. China) was added for 15 s at RT. The cells were slightly washed with water, bluing for 15 s, rinsed with running water, and stained with eosin (Beijing Solarbio Science & Technology Co., Ltd., Beijing, P.R. China) for 3 min at RT. Then, the cells were rinsed with running water, dehydrated, cleared, mounted and examined under the microscope (CX41, Olympus Corporation, Shinjuku, Tokyo, Japan).

2.4. Immunofluorescence

The climbed cells on the cell climbing slice culture plate was soaked 3 times in PBS (3 min each), and fixed with 4% paraformaldehyde (Beijing Solarbio Science & Technology Co., Ltd., Beijing, P.R. China) for 15 min. Then, the cells were immersed 3 times in PBS (3 min each), and permeabilized with 0.5% Triton X-100 (prepared in PBS) at RT for 20 min. The culture dish was then soaked 3 times in PBS (5 min each). The excess PBS was sucked with a pipette, and 5% bovine serum albumin (BSA) (Beijing Solarbio Science & Technology Co., Ltd., Beijing, P.R. China) was added dropwise to the culture dish and blocked at 37 °C for 30 min. Then, the blocking solution was sucked with a pipette, and without washing, a sufficient amount of diluted primary antibody cluster of differentiation (CD)68 (1:200) was added dropwise and incubated at 4 °C overnight. The culture dish was washed 3 times with PBS (3 min each), and the excess liquid was sucked with a pipette. Diluted fluorescent secondary antibody cyanine 3 (Cy3) (1:200) was added dropwise, and incubated at 37 °C for 30 min. Then, the culture dish was washed 3 times with PBS (3 min each). From the steps of the addition of fluorescent secondary antibody, all subsequent procedures were carried out in the dark. 4′,6-diamidino-2-phenylindole (DAPI) (KeyGEN BioTECH, Jiangsu, P.R. China) was added dropwise and incubated for 5 min. The specimens were stained, and the excess DAPI was washed with PBS. The culture dish was blocked with 50% glycerol

(Beijing Solarbio Science & Technology Co., Ltd., Beijing, P.R. China), and the images were captured under a fluorescence microscope (BX53, Olympus Corporation, Shinjukuku, Tokyo, Japan).

2.5. ELISA

Rat TNF-α enzyme-linked immunosorbent assay (ELISA) kit (MM-0180R1), rat IL-6 ELISA kit (MM-0190R1) and rat PGE2 ELISA kit (MM-0068R1) were obtained from Meimian, Jiangsu, China. The procedure was performed according to the kit instructions. Within 15 min after adding the stop solution, the absorbance (OD value) of each well was measured in sequence at 450 nm wavelength using the automated microplate reader (WD-2102B, Beijing Liuyi Instrument Factory, Beijing, China).

2.6. Western blotting

The total protein of cells in each group was extracted using the radioimmunoprecipitation assay RIPA (C1053, Applygen Technologies Inc., Beijing, China), and centrifuged in a high-speed centrifuge (5424R, Eppendorf, Hamburg, Germany) at 10001×*g*, 4 °C for 10 min. After obtaining the supernatant, the total protein was quantified using the bicinchoninic acid (BCA) protein assay kit (Elabscience Biotechnology, Wuhan, Hebei, P.R. China). The protein samples were denatured. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (151-21-3, Xilong Scientific Co., Ltd., Guangdong, P.R. China) was performed, followed by membrane transfer at a constant current of 300 mA. The polyvinylidene fluoride (PVDF) membrane (Millipore, Burlington, MA, USA) was blocked with skimmed milk powder (Applygen Technologies Inc., Beijing, P.R. China). Incubation with the primary antibodies [internal control: mouse monoclonal anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1/2000, TA-08, ZSGB-BIO, Beijing, China); target: mouse anti-CFHR2 (1:1000, Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China), rabbit anti-iNOS (1:500, AF0199), rabbit anti-STAT1 (1:500, AF6300) and rabbit anti-p-STAT1 (1:500, AF3300) (Affinity Biosciences, Cincinnati, OH, USA), mouse anti-cyclooxygenase (COX)-2 (1:1000, bs-10411R) and rabbit anti-–IFN–γ (1:500, bs-0480R) (Bioss Inc., Woburn, MA, USA), rabbit anti-TLR2 (1:500, ab209217, Abcam PLC, Cambridge, UK), and rabbit anti-TLR4 (1:500, 19811-1-AP, Proteintech Group, Inc., Rosemont, IL, USA)] were performed overnight at 4 °C, followed by the secondary antibodies [HRP-labeled goat anti-mouse IgG(H + L) (1:2000, ZB-2305) and HRP-labeled goat anti-rabbit IgG(H + L) (1:2000, ZB-2301) (ZSGB-BIO, Beijing, China)] for 2 h at RT. The PVDF membrane (Merck Millipore, Burlington, MA, USA) was wetted with enhanced chemiluminescence (ECL) Plus (RJ239676, Thermo Fisher Scientific, Waltham, MA, USA) and viewed under the ultra-high-sensitivity chemiluminescence imaging system (Chemi Doc™ XRS+, Bio-Rad Laboratories (Shanghai) Co., Ltd., Shanghai, P.R. China). The program was run to develop imaging.

2.7. Preparation of medicated serum

Radix Sophorae Flavescentis powder decoction was purchased from Anhui Jiayou Chinese Herbal Pieces Co., Ltd., Anhui, P.R. China (medicinal herb, production batch no. 181101, produced in Chifeng, Inner Mongolia, P.R. China; a voucher specimen was deposited).

The rats in the control group received intragastric administration of distilled water for 1 week, and the rats in the low- and high-dose *Radix Sophorae Flavescentis* group received intragastric administration of low and high doses of *Radix Sophorae Flavescentis* (0.8928 g/kg, 1.3392 g/kg) for 1 week, respectively (n = 9 per group). The dosage was calculated based on the body surface area for animals, at the equivalent standard human adult dose, and determined according to the commonly used clinical doses.

Two hours after the last administration, about 10 mL of the abdominal aortic blood of the rats was aseptically collected, and the serum was obtained by centrifugation at 625×*g* (5424R, Eppendorf, Hamburg, Germany) for 10 min. The serum was inactivated in water at 56 °C for 30 min, filtered through a 0.22 μm cellulose acetate membrane (Merck Millipore, Burlington, MA, USA), bottled and stored at −80 °C for subsequent use.

2.8. Cell processing and grouping

The isolated and identified macrophages were further randomly divided into 10 groups: 1) normal control group (PBS), 2) serum normal control group (10% normal control serum), 3) model group [LPS (L8880, Beijing Solarbio Science & Technology Co., Ltd., Beijing, P.R. China) + PBS], 4) serum control group (LPS+10% normal control serum), 5) low-dose *Radix Sophorae Flavescentis* group (LPS+10% *Radix Sophorae Flavescentis*-containing serum), 6) high-dose *Radix Sophorae Flavescentis* group (LPS+20% *Radix Sophorae Flavescentis*-containing serum), 7) Vector group (LPS + Vector), 8) Lv-CFHR2 (LPS + Lv-CFHR2), 9) Lv-sh-NC (LPS + Lv-sh-NC), 10) Lv-CFHR2-sh (LPS + Lv-sh-CFHR2).

2.9. Lentiviral construction and transfection

The lentiviral construction including virus-mediated Lv-CFHR2 overexpression, Lv-CFHR2-sh virus interference and the corresponding vector were prepared by ZHBY Biotech Co., Ltd. (Shanghai, China). Among them, three types of Lv-CFHR2-sh virus interference were constructed: CFHR2-sh1 (GCTCTGAAACTCAGTCATACA), CFHR2-sh2 (GCTGGAACTAA-CAATGATAGC) and CFHR2-sh3 (GGACATCATCTGGCAACAACT), and the vector was 2.pGMLV-hU6-MCS-CMV-ZsGreen1-PGK-Puro-WPRE. The lentiviral vector kept at −80 °C was taken out, and placed in an ice bath until thawed. A culture medium containing polybrene with a final concentration of 10 μg/ml was prepared, which was used to dilute the lentiviral at an multiplicity of infection (MOI) of 50. Then, this was placed in a cell culture plate, gently mixed and put in a 37 °C 5% CO_2 incubator. After 24 h, the culture medium containing lentiviral particles was aspirated, replaced with a fresh medium, and transfected for 48 h. Western blot was used to verify the transfection efficiency.

2.10. Statistical analysis

Statistical analysis was performed using SPSS version 26.0 software (IBM Corp., Armonk, NY, USA). All experiments were conducted three times. Mean ± standard deviation (SD) was used to express the quantitative results. Two group comparison was performed using independent samples *t*-test. Multiple group comparison was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc test. Inspection level α = 0.05.

3. Results

3.1. Identification of peritoneal macrophages in rats

HE staining in Fig. 1 revealed that the cytoplasm of cells was abundant, and the deeply stained nuclei, which were located at one end of the cells, were oval, kidney-shaped, horseshoe-shaped or irregular. The cultured peritoneal macrophages were positive and appeared red fluorescence in CD68 immunofluorescence staining; the isolated cells were identified as macrophages.

3.2. Detection of TNF-α and IL-6 with ELISA to determine the LPS concentration

As shown in Fig. 2, the 600 ng/mL LPS group showed significantly higher TNF-α and IL-6 expression compared with the control group ($P < 0.05$). Therefore, the LPS concentration at 600 ng/mL was selected for

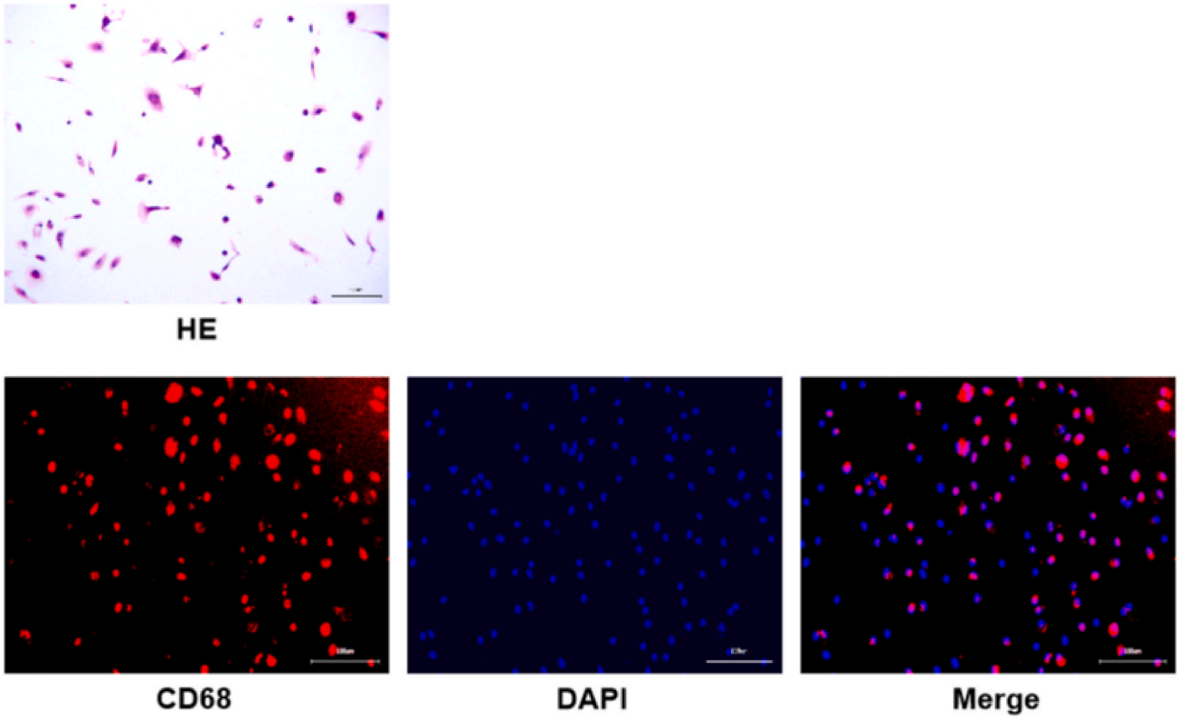

Fig. 1. Identification of peritoneal macrophages in rats (200x).

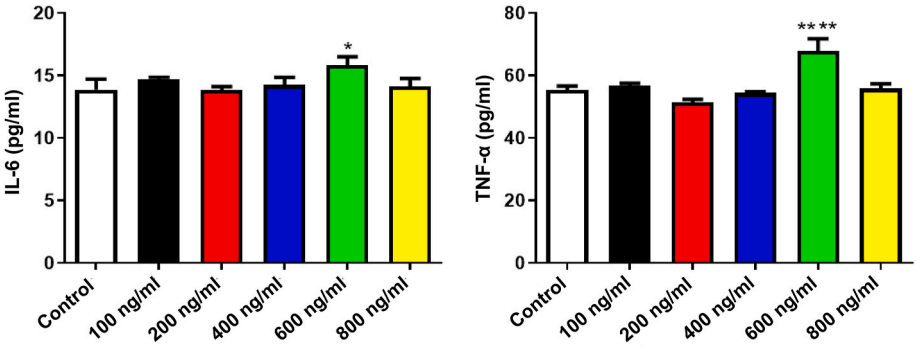

Fig. 2. TNF-α and IL-6 expression under different concentrations of LPS treatment detected by ELISA. Note: compared with the control group, *P < 0.05, ****P < 0.0001.

subsequent experiments.

3.3. The effect of LPS on PGE2 and CFHR2 expression

As demonstrated in Fig. 3, the PGE2 and CFHR2 expression in the LPS group was considerably higher than in the control group (P < 0.05).

3.4. CFHR2 gene transfection verification

As shown in Fig. 4, the expression of CFHR2 was significantly decreased in the three interference groups (P < 0.05) compared with the control group, with Lv-CFHR2-sh1 exhibiting the best interference effect. The Lv-CFHR2 group exhibited a significant increase in CFHR2 expression compared with the control group (P < 0.05).

3.5. Expression of TNF-α, PGE2 and IL-6 in cells of each group

As shown in Fig. 5, the LPS-induced group exhibited considerably higher TNF-α, PGE2, and IL-6 expression compared with the control group (P < 0.05). The TNF-α, PGE2, and IL-6 expression was considerably lower in the LPS-induced +10% medicated serum group, LPS-induced +20% medicated serum group, and Lv-CFHR2-sh interference group compared with the LPS group (P < 0.05).

3.6. Expression of CFHR2, iNOS, COX-2, TLR2, TLR4, IFN-γ, STAT1, and p-STAT1 in cells of each group

As shown in Fig. 6, the CFHR2, iNOS, COX-2, TLR2, TLR4, IFN-γ, STAT1, and p-STAT1 expression was considerably higher in the LPS-induced group compared with the control group (P < 0.05). The LPS induced +10% medicated serum group, LPS induced +20% medicated serum group, and Lv-CFHR2-sh interference group showed considerably lower levels of CFHR2, iNOS, COX-2, TLR2, TLR4, IFN-γ, STAT1, and p-STAT1 compared with the LPS group (P < 0.05).

4. Discussion

Inflammation, as a normal and important response of the body to various stimuli, plays a pivotal role in host defense (Yeom et al., 2015). Inhibiting excessive inflammatory responses is becoming an effective

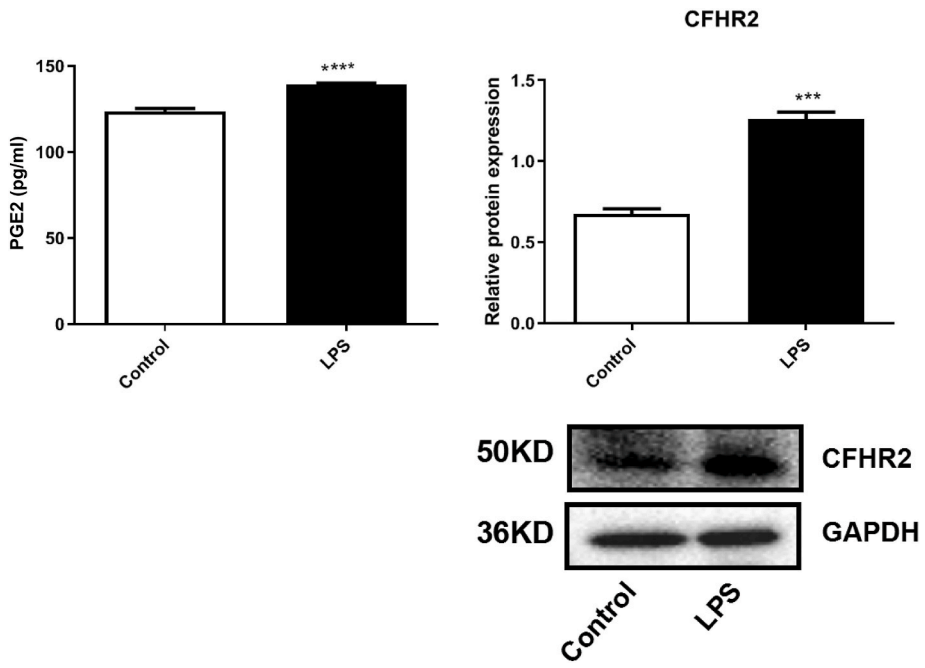

Fig. 3. The effect of LPS on PGE2 and CFHR2 expression detected by ELISA and WB, respectively. Note: compared with the control group, ***P < 0.001, ****P < 0.0001.

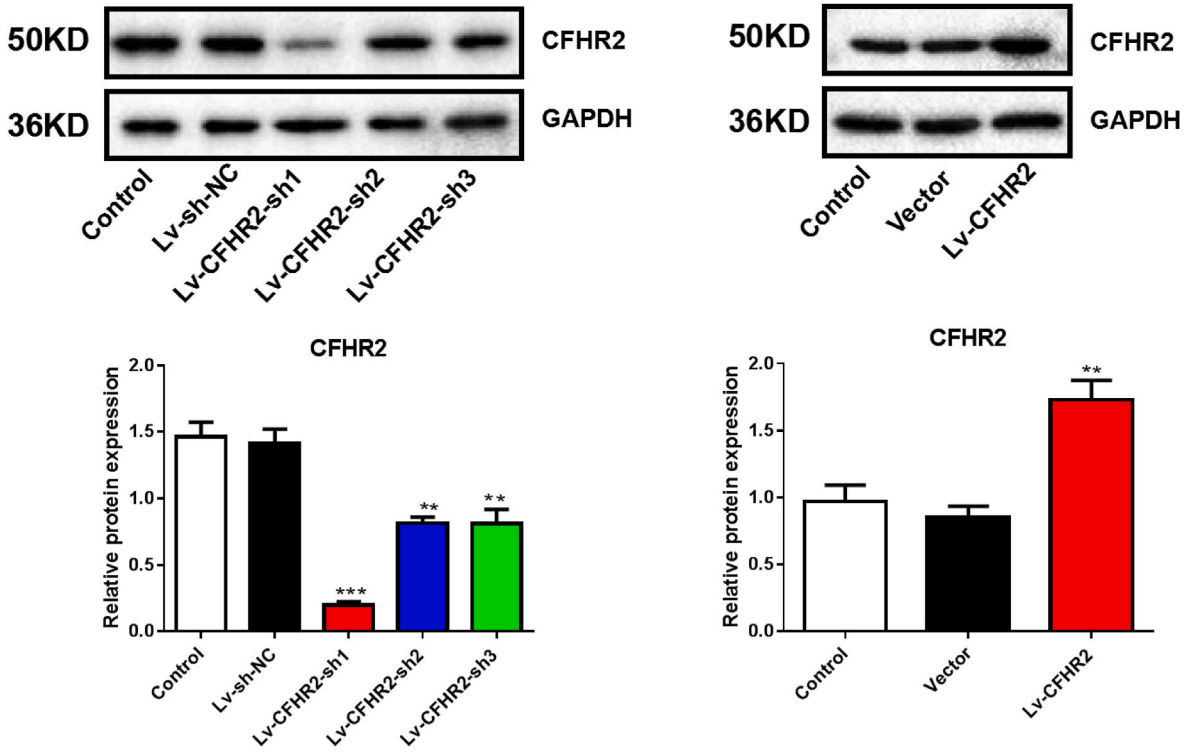

Fig. 4. CFHR2 gene transfection verification by WB. Note: compared with the control group, **P < 0.01, ***P < 0.001.

strategy to alleviate or even cure certain diseases. Macrophages play a key role in initiating and regulating host defenses including inflammation and can be activated by LPS to trigger inflammatory responses (Lee et al., 2021). Therefore, we investigated the potential mechanism of action of *Radix Sophorae Flavescentis* in inhibiting LPS-induced inflammatory mediator production by using rat peritoneal macrophages, which were more closely to the effect *in vivo*.

During the inflammation process, activated macrophages are involved in the production of a variety of proinflammatory cytokines (including TNF-α, IL-1β, IL-6 and IFN-γ) and inflammatory mediators (such as NO and PGE2) and play a crucial role, which serves as the host defense mechanism against various harmful stimuli (Shin et al., 2020). Therefore, blocking these proinflammatory mediators production may be an effective tool to prevent the development and progression of inflammatory diseases. In this study, we found that LPS could induce PGE2 and CFHR2 production in macrophages. *Radix Sophorae*

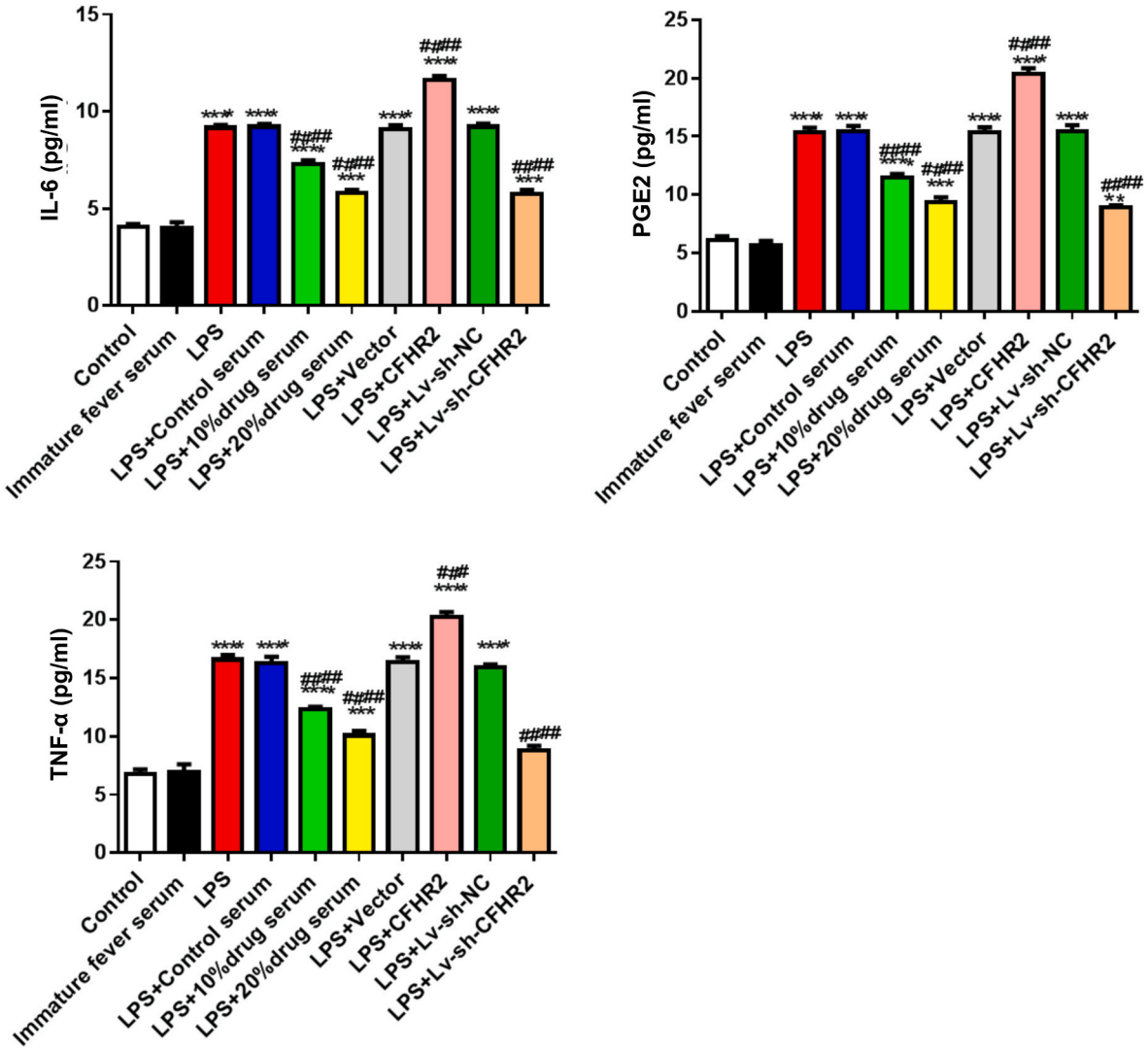

Fig. 5. TNF-α, PGE2 and IL-6 expression in cells of each group detected by ELISA. Note: compared with the control group, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; compared with LPS group, $^{\#\#\#}P < 0.001$, $^{\#\#\#\#}P < 0.0001$.

Flavescentis-medicated serum or Lv-CFHR2-sh interference could effectively inhibit TNF-α, PGE2, and IL-6 production. In addition, the iNOS, COX-2 and IFN-γ protein expression, which were the key inflammatory proteins induced by LPS, could be effectively inhibited by *Radix Sophorae Flavescentis*. Interestingly, the expression of these inflammatory proteins could also be effectively inhibited by Lv-CFHR2-sh interference. These results suggested that the production of these proinflammatory mediators could be effectively inhibited by *Radix Sophorae Flavescentis*, and this process was linked to the regulation of CFHR2 expression. Fig. 2 showed that, at 800 ng/mL, cytokine induction was lower than that at 600 ng/mL, this might probably due to the high concentration of LPS induced macrophage tolerance, resulting in a decrease in cytokines, such as TNF-α.

Many studies have shown that TLRs are key mediators of inflammatory responses and play a crucial role in both acute and chronic inflammation (Pestka and Zhou, 2006). TLRs can recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) (Barton and Medzhitov, 2003). Accumulating evidence suggests that TLR2 and TLR4 signaling not only activate the mitogen-activated protein kinase (MAPK)/nuclear factor kappa B (NF-KB) signaling pathway (Zhang et al., 2018), but also stimulate the extracellular signal-regulated kinase (ERK)/c-Jun N-terminal kinase (JNK) (Rocca et al., 2021) and JAK/STAT signaling pathways in a myeloid differentiation primary response 88 (MyD88)-independent manner (Parra-Izquierdo et al., 2021). Previous studies have demonstrated the involvement of TLR2 and TLR4 pathways in macrophage inflammation (Almutairi et al., 2021). Our results also showed that LPS could promote TLR2 and TLR4 expression in macrophages, and compared with the LPS-treated group, the use of *Radix Sophorae Flavescentis*-medicated serum could effectively inhibit the up-regulation of TLR2 and TLR4 expression. Our experiments further proved that the anti-inflammatory effect of *Radix Sophorae Flavescentis* was concentration-dependent.

Previous studies have demonstrated that the JAK/STAT signaling pathway plays a critical role in immune and inflammatory responses (Lee et al., 2021). The components of the STAT signaling pathway are the key molecular targets for the treatment of various inflammatory diseases. Specifically, it has been noted that down-regulation of STAT1 expression can inhibit LPS-induced lethality, indicating that STAT1 may play a crucial role in TLR-induced inflammation (Yang et al., 2021). STAT1 activation can translocate to the nucleus and regulate the transcription of target genes including pro-inflammatory cytokines, chemokines, and inducible enzymes such as iNOS and COX2 (Fanunza et al., 2021). Our results showed that the use of *Radix Sophorae Flavescentis* or Lv-CFHR2-sh could significantly block the phosphorylation of STAT1 induced by LPS, while overexpression of CFHR2 could promote the

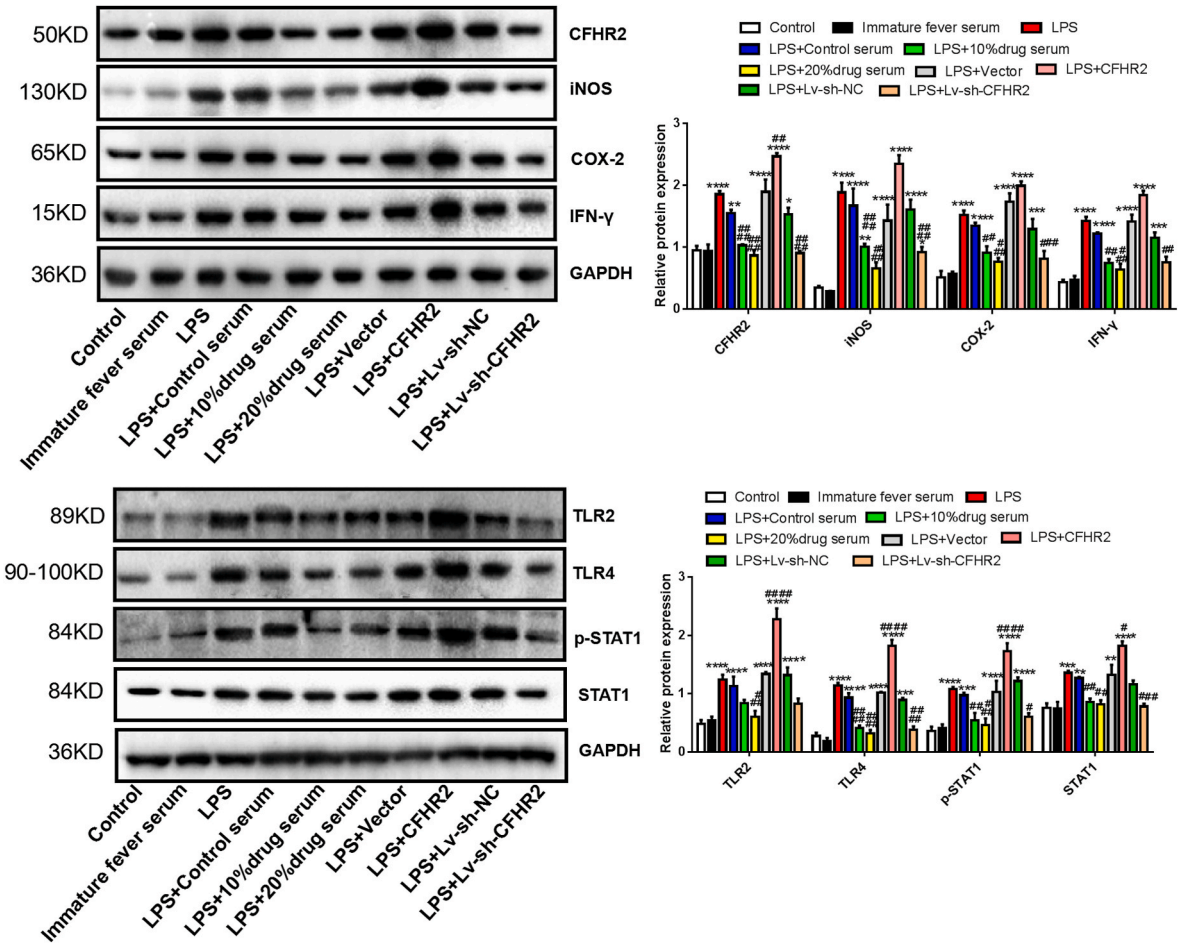

Fig. 6. CFHR2, iNOS, COX-2, TLR2, TLR4, IFN-γ, STAT1 and p-STAT1 expression in cells of each group detected by WB. Note: compared with the control group, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; compared with the LPS group, #P < 0.05, ##P < 0.01, ###P < 0.001, ####P < 0.0001.

phosphorylation of STAT1. These results suggested that *Radix Sophorae Flavescentis* might reduce the inflammatory response by inhibiting the activation of STAT signaling pathway.

Recent studies have demonstrated that CFHR2 can induce pro-inflammatory factor, activate human immune response, and play an increasingly important role in a variety of inflammatory disease models (Chen et al., 2020). However, no research has been done on the role of CFHR2 in macrophages. In this study, we found that CFHR2 was specifically up-regulated in macrophages after LPS induction; to date, no relevant studies have been conducted. The inflammatory effect on macrophages was then assessed using *Radix Sophorae Flavescentis* or Lv-CFHR2-sh. The results showed a significant reduction in NO, PGE2, TNF-α, IL-6, and other inflammatory mediators, suggesting that *Radix Sophorae Flavescentis* might mediate CFHR2 expression, and thereby blocking the production of these pro-inflammatory mediators.

Subsequent studies may further investigate whether *Radix Sophorae Flavescentis* inhibits LPS-induced proinflammatory response in experimental animals. The specific mechanism of action and signaling pathways may further be explored more in depth. Research on its toxicity may also be done. Future studies may also include principal component analysis and investigate the anti-inflammatory role of the components in *Radix Sophorae Flavescentis*.

In conclusion, *Radix Sophorae Flavescentis* could significantly reduce the inflammatory mediators NO, PGE2, TNF-α, and IL-6 production in LPS-induced rat peritoneal macrophages, and reduce the upstream TLR2 and TLR4 protein expression. Further investigation into the mechanism of action revealed that *Radix Sophorae Flavescentis* may exert these effects via regulating CFHR2 and inhibiting the activation of JAK/STAT signaling pathway. These results deepen our knowledge and understanding of the anti-inflammatory effects of *Radix Sophorae Flavescentis*, and provide some ideas for making full use of the related phytochemical resources of *Sophora flavescens*.

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Institutional review board statement

In accordance with the guidelines of the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985) and the ARRIVE criteria, all animal procedures and experimental methods used in the current study were approved by the Ethics of Animal Experiments of Inner Mongolia Minzu University.

Informed consent statement

Not applicable.

CRediT authorship contribution statement

Xiaoying Wu: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Li Li:** Visualization,

Software, Methodology, Investigation, Formal analysis, Data curation. **Jinhabure:** Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Xiaofeng:** Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Eerdunchaolu:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Abbreviations

PRR	pattern recognition receptor
TLR	Toll-like receptor
NO	nitric oxide
PGE2	prostaglandin E2
iNOS	inducible nitric oxide synthase
COX-2	cyclooxygenase-2
TNF-α	tumor necrosis factor-alpha
IL	interleukin
CFHR2	complement factor H-related protein 2
LOX	lipoxygenase
LPS	lipopolysaccharide
JAK	Janus kinase
STAT	signal transducer and activator of transcription
SD	Sprague Dawley
DMEM	Dulbecco's Modified Eagle Medium
HE	hematoxylin-eosin
RT	room temperature
PBS	phosphate-buffered saline
BSA	bovine serum albumin
CD	cluster of differentiation
Cy3	cyanine 3
DAPI	4′,6-diamidino-2-phenylindole
ELISA	enzyme-linked immunosorbent assay
PGE2	prostaglandin E2
OD	optical density
RIPA	radioimmunoprecipitation assay
BCA	bicinchoninic acid
SDS-PAGE	sodium dodecyl-sulfate polyacrylamide gel electrophoresis
PVDF	polyvinylidene fluoride
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
IFN-γ	interferon-gamma
HRP	horseradish peroxidase
IgG	immunoglobulin
(H + L)	heavy and light chains
ECL	enhanced chemiluminescence
MOI	multiplicity of infection
ANOVA	analysis of variance
PAMPs	pathogen-associated molecular patterns
DAMPs	damage-associated molecular patterns
MAPK	mitogen-activated protein kinase
NF-KB	nuclear factor kappa B
ERK	extracellular signal-regulated kinase
JNK	c-Jun N-terminal kinase
MyD88	myeloid differentiation primary response 88

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