Lipoxin A4 regulates M1/M2 macrophage polarization via FPR2–IRF pathway

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Abstract
Lipoxin A4 (LXA4) has been shown to have anti-inflammatory activity, but its underlying molecular mechanisms are not clear. Herein, we investigated the potential role of LXA4 in macrophage polarization and elucidated its possible molecular mechanism. The RAW264.7 macrophage cell line was pretreated with LXA4 with or without lipopolysaccharides (LPSs) and interleukin-4 (IL-4). In cultured macrophages, LXA4 inhibited LPS-induced inflammatory polarization, thereby decreasing the release of proinflammatory cell factors (IL-1β, IL-6, TNF-α) and increasing the release of anti-inflammatory cytokines (IL-4 and IL-10). Notably, the inhibitory effect of LXA4 on inflammatotry macrophage polarization was related to the downregulation of p-NF-κB p65 and IRF5 activity, which reduced the LPS-induced phenotypic and functional polarization of M1 macrophages via the FPR2/IRF5 signaling pathway. Moreover, LXA4 also induced the IL-4-induced polarization of M2 macrophages by promoting the FPR2/IRF4 signaling pathway. Therefore, LXA4 regulates M1/M2 polarization of macrophages via the FPR2–IRF pathway.

Keywords Lipoxin A4 · Macrophage polarization · Inflammation · FPR2–IRF

Abbreviations

| Abbreviation | Meaning |
|--------------|---------|
| Arg-1        | Arginase-1 |
| BCA          | Bicinchoninic acid |
| CCK-8        | Cell counting kit-8 |
| DMEM         | Dulbecco's modified eagle medium |
| FBS          | Fetal bovine serum |
| HRP          | Horseradish peroxidase |
| IFN-γ        | Interferon-γ |
| iNOS         | Inducible nitric oxide synthase |
| IL-4         | Interleukin-4 |
| LXA4         | Lipoxin A4 |
| LPS          | Lipopolysaccharides |
| LXs          | Lipoxygenins |
| PVDF         | Polyvinylidene difluoride |
| ROS          | Reactive oxygen species |
| TLR          | Toll-like receptor |
| CK           | Creatine kinase |
| PNC          | Penicillin |
| PFA          | Paraformaldehyde |
| TF           | Tissue factor |

Background
Macrophages participate in the onset, progression, and digestion, as well as other phases, of inflammatory events. During various phases of the inflammatory response, macrophages exhibit specific phenotypes: classically activated inflammatory (M1) or alternatively activated (M2) macrophages (Italiani and
Lipoxygenins (LXs) are endogenous lipidic mediators generated by arachidonic acid under the continuous catalysis of diverse lipoxidases. These LXs are classified into four types based on their molecular conformation (Levy and Serhan 2014), among which LX A4 (LXA4) is produced by sequential oxidative catalysis. As a negative modulator of inflammatory events, LXA4 is an anti-inflammatory agent that facilitates the regression of inflammatory events, modulates immunofunction, and stimulates tissue and cellular injury repair (Karra et al. 2015; Romano et al. 2015). In a variety of inflammation-associated illnesses, LXA4 suppresses the release of pro-inflammatory factors and the infiltration of immune cells and facilitates the chemotaxis and recruiting of macrophages, thereby reinforcing the anti-inflammatory, phagocytosis role of macrophages (Zhang et al. 2007; Zhu et al. 2020). Moreover, LXA4 suppresses ROS generation and prevents oxidation stress-mediated damage to tissues or macrophages (Zhou et al. 2007; Wu et al. 2015; Zong et al. 2016). LXA4 was demonstrated to promote the antioxidant enzymatic activity of proteins in a variety of organs and the restoration of the oxidant/antioxidant equilibrium (Chen et al. 2013; Zhao et al. 2013; Zong et al. 2017).

Herein, we explored the protective roles of LXA4 in LPS-or IL-4-stimulated macrophages (RAW264.7) and found that LXA4 inhibits IRF5 activity, promotes IRF4 activation, and downregulates p-NF-xB p65 activity through FPR2-mediated inhibition. Thus, the polarization of M1 macrophages was inhibited, while the polarization of M2 macrophages was promoted. This finding sheds light on the underlying cellular and molecular mechanisms of associated inflammatory diseases.

Materials and methods

Cell culture and treatment

The murine macrophage cell line (RAW264.7) was bought from the Cellular Bank of the CAS (Shanghai, China). RAW264.7 cells were cultured in DMEM (Invitrogen, USA) supplemented with 5% fetal bovine serum (FBS, Invitrogen), 100 U/mL PNC, and 100 μg/ml streptomycin (Invitrogen). RAW264.7 cells were inoculated into the medium with 5% FBS and cultured until about 70% confluent in 6-well cultivation dishes. The cells were cultured in medium without FBS for 24 h prior to treatment with recombinant LPS (Lot No. 5164948, Lianke Biotechnology, Hangzhou, China), 20 ng/ml IL-4 (Lot No. 081449, PeproTech, RockyHill, USA), or Lipoxin A4 methylester (LXA4,0.1 mg/kg, CAYman, USA).

Flow cytometry analysis

Cells were inoculated into 6-well plates at 1 × 106 cells/well and cultivated for 24 h as previously described. The cells were treated with LXA4 for 30 min, followed by LPS treatment for an additional 24 h. After incubating for 24 h, the cells were digested with trypsin, washed, and then resuspended in cold PBS at 1 × 106 cells/ml. The cells were incubated with an APC-conjugated monoclonal murine anti-CD86 antibody or PE-conjugated monoclonal murine CD206 antibody (all from BD Biosciences, CA, USA), or Lipoxin A4 methylester (LXA4,0.1 mg/kg, CAYman, USA). The membrane proteins CD86 and CD206 were identified via immunofluorescence staining.

ELISA for the identification of cytokine (CK) levels

A cellular suspension was prepared by inoculating RAW264.7 cells in the log-growth stage in a 6-well cultivation dish. After 24 h, the cells were subjected to pretreatment with various concentrations of LXA4 for 30 min and then cultivated with or without LPS (1 μg/ml) for 24 h. The supernatant was collected, and the levels of IL-6, IL-10, TNF-α, and iNOS were measured via ELISA as per the

Cellular activity analysis and morphology analyses

Cellular activities were evaluated via a CCK-8 analysis in a 96-well cultivation dish. Briefly, RAW264.7 cells were inoculated and exposed to various levels of LXA4 with or without LPS for 24 h. The cells were incubated with CCK-8 (10 μl) solution for 2 h at 37 °C, and then, the absorption at 450 nm was measured via a microplate spectrometer (Thermo Fisher Scientific, MA, USA). For morphology analyses, the images of cells were captured via an Olympus microscope (Shanghai, China) under 20× magnification.
supplier’s specification. The optical density (OD) was measured at 450 nm with a microplate spectrometer (Thermo Scientific).

qPCR analysis

RNA was isolated using the RNAeasy™ animal RNA separation kit with a spin column as per the supplier’s specification, and then, cDNA was reverse transcribed using PrimeScript™ RT Master Mix (Perfect Real Time) as per the supplier’s specification. The qPCR reactions were performed using TB Green™ Premix Ex Taq™ II (Tli RNaseH Plus) in an Applied Biosystems 7500 Time PCR System. The qPCR protocol was as follows: 1 cycle at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 34 s, 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. All specimens were assessed three times, and the mRNA expression was normalized to β-actin. All primer sequences used in this study are presented in Table 1. The gene expression was standardized and presented as the fold variation relative to β-actin.

Western blot analysis

Total protein lysates were prepared from cultivated cells, and the protein concentration of each sample was determined using a BCA protein analysis kit (Thermo Fisher). Equal amounts of protein (20 μg) from all specimens were resolved via SDS-PAGE and transferred onto a PVDF membrane (Bio-Rad, Hercules, CA, USA). Following blocking in 5% skim milk at 25 °C for 1 h, the membrane was incubated with primary antibodies at 4 °C overnight and then with the suitable HRP-conjugated second antibody (Thermo Fisher) for 1 h at 25 °C. The protein bands were visualized via chemiluminescent identification on autoradiographic film. For macrophage biomarkers, anti-iNOS (1:1000, Cat# ab178945, Abcam, USA), anti-CD86 (1:1000, Cat# 76755, Cell Signaling Technology (CST), MA, USA), anti-Arginase-1 (1:1000, Cat# 93668, CST), and anti-CD206 (1:1000, Cat# 24595, CST) antibodies were utilized. For the NF-κB signaling pathway, anti-NF-κB p65 (1:1000, Cat# 8242, CST) and anti-p-NF-κB p65 (1:1000, Cat# 3033, CST) antibodies were utilized. For the IRF signaling pathway, anti-IRF5 (1:1000, Cat# BS60674, Bioworld, USA), anti-IRF4 (1:1000, Cat# BS2659, Bioworld), and anti-FPR2 (1:1000, Cat# DF2719, Affinity, USA) antibodies were utilized. The bands were subjected to quantification via identifying the signal intensity through Image-Pro Plus 6.0 and standardizing them to the signal for GAPDH (1:5000, Cat# AP0063, Bioworld).

Immunofluorescent staining

Macrophages were cultivated with or without treatment in 6-well dishes with glass slides. The cells were washed with PBS and fixed with 4% PFA (Sigma-Aldrich, St. Louis, MO, USA) at 4 °C for 30 min. The samples were then permeabilized with 0.1% Triton X-100 for 10 min, washed with PBS, and then blocked with 10% FBS. Immunofluorescent staining was completed for p-NF-κB p65 (1:1600), IRF4 (1:100), IRF5 (1:100), FPR2 (1:200), iNOS (1:500, Protein-tech), CD206 (1:200), and F4/80 (1:200, Abcam). The samples were then incubated with the DyLight 488/594 labeled secondary antibody ((Beyotime Biotechnology, Jiangsu, China). Immunocytochemistry specimens were evaluated in a semi-quantitative or quantitative manner by two separate researchers in a blinded way.

Statistics

The data are presented as the mean ± SD. All statistics were analyzed with Statistical Package for Social Sciences (version 16.0, SPSS Inc., Chicago, USA). A two-sided Student’s t test was employed to study the diversity between two groups. One-way ANOVA and Bonferroni’s post-test were employed for analyses involving more than two groups. P < 0.05 was set as the threshold of significance.

Results

Roles of LXA4 in the activation of RAW264.7 cells

Figure 1A shows the molecular structural formula of LXA4. To determine if LXA4 altered the activity of RAW264.7

| Gene  | Sense (5’-3)          | Anti-sense (5’-3)               |
|-------|-----------------------|---------------------------------|
| IL-1β | TTTGAAAGTGGAGCGAGACCCCA | CACAGCTTCTCCACAGGACCCACA       |
| IL-4  | ATCGGAGTGGCGGAGGAGGCCTACAA | CGAGACCGCCTTGGGAAGGCTCCTACAA |
| IL-6  | TTCTGAGCAGTGGACGAGTCGTT | CACACTTGTCTTCTTCATTCCACGA      |
| IL-10 | TTACGGTGGAGGAAGTGAGTGCCC | GACACCTGAGTGTGTGAGCCTTGA      |
| Arg1  | AGAAGAACGCTGAGTCGAGGAA | AGATGCTCCAACTGGCAGAC           |
| iNOS  | GGCGCTGACCGAGAGATCATATG | GCCGGTGACTTCTGAGTGATCA      |
| Actin | CTGAGAGGGAAATCGTGCCTGA | CCAAGGATTTCCATACCCAGGA       |
cells, a CCK-8 analysis was performed after treatment with various concentrations of LXA4 (10–500 nM) for 24 h. We found that LXA4 did not have any cytotoxic effects on RAW264.7 cells (Fig. 1B). Therefore, in the subsequent experiments, RAW264.7 cells were pretreated with LXA4 (10 and 500 nM) for 30 min prior to stimulation with LPS (1 μg/ml) for 24 h. We found that the inhibitory roles of LPS in RAW264.7 cells were alleviated to the greatest extent when the concentration of LXA4 was 200 nM. We found similar results with IL-4 incubation (20 ng/ml). To study the role of LXA4 in macrophage stimulation, RAW264.7 cells were changed with and without LXA4 (200 nM) treatment, and the morphological changes of the cells were observed. As shown in Fig. 1C, no remarkable variation in cell morphology was observed in the two groups. Thus, we selected LXA4 at 200 nM concentration for further experiments.

To explore the roles of LXA4 in LPS-induced M1 macrophage polarization in vitro and IL-4-induced M2 macrophage polarization in vitro, we observed the morphological changes of RAW264.7 macrophages after LXA4 for

![Molecular structural formula of LXA4](image)

**Fig. 1** Effect of LXA4 on the activation of RAW264.7 macrophages stimulated by LPS or IL-4. (A) Molecular structural formula of LXA4. (B) Effect of different concentrations of LXA4 on cell viability. (C) Light microscopy observation of cell morphology. (D) RAW264.7 macrophages were pretreated with different concentrations of LXA4 for 30 min, incubated with or without LPS (1 μg/ml) for 24 h, and then, cell survival was determined by the CCK-8 method. (E) Cell viability was determined by pretreatment of RAW264.7 macrophages with different concentrations of LXA4 for 30 min, incubated with or without IL-4 (20 ng/ml) for 48 h, and then, cell survival was determined by the CCK-8 method. (F) Cell morphology was observed by light microscopy under different modes of intervention. Data are presented as the mean ± SEM from three independent experiments performed in triplicate. *P < 0.05 as compared with the control group; #P < 0.05 as compared with the LPS-treated group.
30 min followed by LPS for 24 h. Light microscopy and immunofluorescence analyses (Fig. 1F) showed that naïve RAW264.7 macrophages are round or oval, densely packed, well adhered to the wall, and have few protrusions. LXA4 for 30 min followed by LPS for 24 h. In contrast, the morphology of IL-4-induced RAW264.7 cells was more round after LXA4 treatment. These results suggested that 200 nM LXA4 can inhibit LPS-induced morphological changes but promote IL-4-induced morphological changes in macrophages.

**Effect of LXA4 on LPS-induced cytokine production in RAW264.7 cells**

RAW264.7 macrophages were pretreated with LXA4 for 30 min and then stimulated with LPS for 24 h. In order to determine the roles of LXA4 in the production of LPS-induced inflammatory mediators, the effect of LXA4 on inflammatory cytokine expression was assessed via ELISA and qPCR. The mRNA expression of IL-1β, IL-6, TNF-α, IL-4, and IL-10 induced by LXA4 was analyzed by qPCR. The results revealed that the transcript levels for M1 pro-inflammatory cytokines were remarkably elevated after LPS exposure, as evidenced by the generation of IL-1β and TNF-α (Fig. 2A–C), whereas the mRNA levels of anti-inflammatory cytokines (IL-10 and IL-4) were remarkably reduced (Fig. 2D, E). Consistent with the qPCR results, the ELISA results revealed that the levels of pro-inflammatory cytokines (IL-6 and TNF-α) were significantly elevated, while the levels of anti-inflammatory cytokines (IL-10) were significantly reduced in LPS-induced cells (Fig. 2F–H). The results presented in this section show that the mRNA (qPCR) and protein (ELISA) levels of pro-inflammatory cytokines are increased in RAW264.7 cells after exposure to LPS, while the mRNA and protein levels of anti-inflammatory cytokines are reduced.

**LXA4 inhibited M1 macrophage polarization in vitro**

CD86 is an M1 subtype macrophage-specific membrane surface protein that serves as a marker of M1 polarization. To investigate the effect of LXA4 on RAW246.7 macrophage polarization, we detected the expression of CD86 by flow cytometry. CD86 protein expression was significantly upregulated after LPS stimulation, while CD86 is downregulated after LXA4 pretreatment in LPS-stimulated cell (Fig. 3E). To further clarify whether LXA4 could inhibit the shift of RAW264.7 cells to the M1 phenotype, we examined the expression of iNOS by ELISA and qPCR. We found that iNOS was significantly increased in the LPS-treated group, whereas the expression of iNOS was inhibited after LXA4 administration (Fig. 3A, B). Western blot results showed that after LPS stimulation, the expression of iNOS and CD86 was remarkably elevated, while the expression of iNOS and CD86 was reduced in the cells pretreated with LXA4 (Fig. 3C). We then detected iNOS expression using immunofluorescence. Compared with the control group, iNOS immunoreactivity was enhanced after LPS administration, while iNOS immunoreactivity was reduced in cells pretreated with LXA4 (Fig. 3D). The above results suggested that LXA4 can inhibit the LPS-induced polarization of RAW264.7 macrophages to the M1 phenotype.

**LXA4 downregulated the activity of NF-κB 65 and IRF5 in vitro**

We then investigated the mechanism underlying the LXA4-dependent suppression of macrophage polarization to the M1 phenotype in vitro. In LPS-exposed macrophages, western blot and immunocytochemistry revealed that LXA4 increased the expression of FPR2 and decreased the expression of IRF5 (Fig. 4A–C), indicating that IRF5 was involved in the LXA4-mediated suppression of M1 macrophage polarization. In addition, LPS treatment increased P-P65 expression, as evidenced by western blot analysis (Fig. 4D). Immunocytochemistry revealed that the expression of p-NF-κB p65 was remarkable within macrophage nuclei increased relative to control group (Fig. 4E). After LXA4 treatment, p-NF-κB p65 expression was decreased, and intranuclear localization was decreased. Thus, LXA4 inhibits M1 macrophage polarization via targeting the typical NF-κB signal. Collectively, these discoveries revealed that LXA4 suppresses the polarization effects of p-NF-κB p65 and IRF5 on M1 macrophages in vitro.

**LXA4 promote the polarization of M2 macrophage in vitro**

As mentioned earlier, macrophages can be classified into two phenotypes: the inflammatory/damaging (M1 type) and healing/repair (M2 type). We found that LXA4 inhibited the polarization of macrophages toward M1 and, thus, reduced the release of inflammatory factors. To explore whether LXA4 could affect the polarization of M2 macrophages and, thus, have a protective effect, we induced M2-type macrophages with IL-4 for 48 h. The expression of specific phenotypic markers, Arg-1 and CD206, in M2-type macrophages was then detected. As shown in Fig. 5A, flow cytometry results showed that the levels of CD206 protein in the IL-4 group were increased as compared with the control group. The mRNA expression of Arg-1 was detected by qPCR and ELISA. The results showed that the protein level of Arg-1 in the IL-4 group was increased as compared with the control group (Fig. 5B, C). The above experiments indicated that IL-4 can successfully polarize macrophages into M2 macrophages. After pretreatment with LXA4, the expression levels of CD206 and Arg-1 were significantly increased.
higher than those of the IL-4 group (Fig. 5A–C). Additionally, immunofluorescence and western blot results also verified the above conclusion (Fig. 5D, E) indicating that LXA4 promotes macrophage polarization into M2 macrophages.

To further elucidate the mechanism underlying LXA4-induced polarization of M2 macrophages, we explored the activity of FPR2 and IRF4, which were found to be involved in the modulation of M2 macrophages (Satoh et al. 2010). We found that the expression of FPR2 was increased in IL-4-exposed macrophages, as was the expression of IRF4 (Fig. 6A–C). Thus, FPR2 and IRF4 might be essential in the LXA4-mediated polarization of M2 macrophages.

Specifically, LXA4 might promote the polarization of M2 macrophages by inducing IRF4 activity via FPR2.

Discussion

Macrophages, as essential innate immune cells, play an important role in the initiation and progression of inflammatory responses (Daskalaki et al. 2018; Funes et al. 2018). Macrophages can secrete cytokines, present antigens, and phagocytose pathogens—all of which are closely related to the physiological and pathological processes of the
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Body, such as defense, metabolism, and inflammatory repair (Cavaillon 1994; Kim and Nair 2019). As a highly heterogeneous and plastic immune cell, macrophages can change according to the microenvironment in vivo and adapt to the body's environment. When macrophages are exposed to microbial products (LPSs), they are polarized into M1 macrophages, while IL-4-stimulated macrophages are polarized into M2 macrophages (Ricardo et al. 2008; Mily et al. 2020). In our study, iNOS and CD86 were highly expressed in M1 macrophages, while Arg-1 and CD206 were highly expressed in M2 macrophages. The M1 and M2 activation states were confirmed using the markers above. It has been found that LPSs induce nuclear NF-κB and AP-1 aggregation and significantly reduce the release of inflammatory cytokines (Yacoubian and Serhan 2007). Our results were consistent with this, suggesting that LXA4 can inhibit the LPS-induced polarization of RAW264.7 macrophages into M1-type macrophages. Furthermore, LXA4 promoted the IL-4-dependent polarization of activated macrophages into M2 macrophages. However, the underlying mechanism of how LXA4 influences the polarization of macrophages remains to be further studied.

Studies have recently revealed that NF-κB is a pivotal modulator of the initiation and resolution of inflammatory...
Fig. 4 LXA4 downregulated the activity of IRF5 and NF-κB 65 in vitro. (A) Cells were pretreated with LXA4 for 30 min with or without LPS (1 μg/ml) for 24 h. Cultured cells were collected and assessed by western blot for the expression of FPR2 and IRF5. (B) Immunofluorescence staining showed upregulation of macrophage FPR2 expression after LPS treatment. LXA4 promoted LPS-mediated induction; scale bar = 50 μm. (C) LXA4 decreased LPS-induced IRF5 overexpression in macrophages; scale bar = 50 μm. (D) Western blot results showed that LXA4 inhibited the LPS-induced increase in p-NF-κB p65 expression level in macrophages without a significant change in p65 levels. (E) Immunofluorescence staining showed upregulation of p-NF-κB p65 expression in macrophages after LPS treatment; scale bar = 50 μm. Data are presented as the mean ± SEM of three independent experiments. *P < 0.05 as compared with the control group; #P < 0.05 as compared with the LPS-treated group.
events via modulating the polarization of macrophages (Zheng et al. 2020). NF-κB is a pleiotropic TF composed of five sub-groups, including RelA (p65), NF-κB1 (p105/p50), NF-κB2 (p100/p52), c-Rel, and RelB (Holdsworth and Neale 1984). Research has revealed that p-NF-κB p65 participates in the polarization of M1 macrophages (Lowe et al. 2014). Our results also indicated that p-NF-κB p65 is involved in the polarization of M1 macrophages, which is related to the inhibition of LXA4-induced M1 macrophage polarization.

Apart from NF-κB signal transmission, IRF5 has been found to be related to M1 macrophage polarization (Krausgruber et al. 2011). IRF5, a component of the IFN-regulation factor family, can trigger stimulation genes that encode type I IFN and inflammatory cell factors, such as TNF-α, IL-6, and IL-12 (Krausgruber et al. 2011). These cytokines participate in the modulation of immune cells and are responsible for the mediation of inflammatory damage in various tissues, such as renal tissues (Awad et al. 2015).
our study, elevated IRF5 expression in LPS-exposed macrophages revealed an underlying effect on the polarization of M1 macrophages. After LXA4 exposure, IRF5 activity was downregulated and hence suppressed the polarization of M1 macrophages and decreased inflammatory damage. For that reason, our results demonstrated that IRF5 might be a pivotal modulator of the polarization of M1 macrophages and might be involved in the anti-inflammatory roles of LXA4 in inflammation.

Interestingly, unlike the polarization of M1 macrophages, LXA4 promotes M2 macrophage polarization, as evidenced by an increase in the proportion of M2 cells and Arg-1 levels in vitro. In addition, upregulating the expression of IRF4 might be one of the causal links whereby LXA4 promotes M2 macrophage polarization. Previous studies found that IRF4 induced the polarization of M2 macrophages with JMJD3 treatment (Gaikwad and Heneka 2013). Our results also supported the involvement of IRF4 in the polarization of M2 macrophages and indicated that LXA4-mediated induction of M2 macrophage polarization might be achieved via upregulation of IRF4 activity.

A previous study demonstrated that the biological effects of LXA4 in vivo are mediated through its receptor FPR2, a G-protein-coupled membrane receptor that is highly expressed in macrophages and can bind to LXA4, its ligand, through a lipid-binding domain and then transfers
extracellular signals into effector cells via a protein-binding domain, thereby eliciting the corresponding biological effects (Pirault and Bäck 2018). It was shown that LXA4 mediates the M2-type macrophage transformation and promotes connective tissue repair through FPR2 (Dakin et al. 2012). We showed that LXA4 may be regulated through the FPR2 pathway to inhibit M1-type macrophage polarization and reduce inflammatory factor synthesis and release while enhancing M2 macrophage polarization and inducing anti-inflammatory effects.

In conclusion, the present study is the first to show that LXA4 may be mediated via FPR2 to inhibit IRF5 activity, promote IRF4 activation, and downregulate p-NF-κB p65 activity, thereby suppressing the polarization of M1 macrophages and further facilitating the polarization of M2 macrophages. This may, to some extent, elucidate the cellular and molecular mechanisms of inflammatory injury and repair. Our results also offer a novel strategy for the prevention and therapy of inflammatory injury in the future.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by JY, FL and LC. The first draft of the manuscript was written by JY and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability All data are available from the corresponding author upon request.

Declarations

Competing interests The authors declare that there is no conflict of interest regarding the publication of this paper.

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