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Transmembrane TNF-α Reverse Signaling Alleviates Lipopolysaccharide-induced Inflammation by Regulating the MCPIP1/SIRT1/NF-κB Pathway

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ABSTRACT

Background: Studies have demonstrated that transmembrane tumor necrosis factor-α (tmTNF-α) plays an anti-inflammatory role. tmTNF-α has a dual function, acting as both a signaling ligand and a receptor that transmits reverse signaling to cells expressing tmTNF-α. However, the role and mechanisms of tmTNF-α reverse signaling in sepsis are not fully understood.

Objective: To explore the potential role and mechanisms of tmTNF-α reverse signaling in lipopolysaccharide (LPS)-induced inflammation.

Methods: The expression levels of tmTNF-α and TNF-α mRNA were evaluated using flow cytometry and real-time PCR, respectively. We employed the anti-TNF-α drug infliximab to stimulate tmTNF-α reverse signaling and measured interleukin-6 (IL-6) and monocyte chemoattractant protein (MCP)-1 production through real-time PCR and ELISA in THP-1-derived macrophages. The location of p65 was determined through immunofluorescence assay. The phosphorylation and acetylation of p65, as well as the expression levels of MCP-induced protein 1 (MCPIP1) and Sirtuin 1 (SIRT1), were evaluated using western blotting.

Results: Our findings revealed that tmTNF-α reverse signaling reduced the expression of IL-6 and MCP-1 triggered by LPS. tmTNF-α reverse signaling inhibited the nuclear translocation of p65, suppressed p65 phosphorylation and acetylation, and upregulated the expression of negative regulatory molecules MCPIP1 and SIRT1 in the LPS/ toll-like receptor 4 (TLR4) signaling pathway .

Conclusion: This study demonstrates that tmTNF-α reverse signaling plays a negative regulatory role in inflammation triggered by LPS by inhibiting the TLR4/ nuclear factor kappa B (NF-κB) signaling pathway. This study helps to further understand the function of tmTNF-α reverse signaling and offers new therapeutic possibilities for sepsis and other inflammatory diseasesconditions.

Keywords: Transmembrane TNF-α, Reverse signaling, LPS/TLR4 signaling pathway, MCPIP1, SIRT1

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INTRODUCTION

Sepsis is a worldwide health concern that results in life-threatening organ failure due to the body's chaotic response to infection (1). Despite advances in understanding the pathophysiology and developing therapeutic approaches, sepsis still carries a high mortality rate. According to the latest Global Burden of Diseases, Injuries, and Risk Factors Study, there were nearly 50 million sepsis cases and 11 million deaths globally in 2017, accounting for almost 20% of global fatalities (2). In the initial stage of sepsis, infection triggers he release of inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α into the systemic circulation from immune cells (e.g., macrophages) by activating toll-like receptors and nuclear factor kappa B (NF-κB) signaling (3). A moderately regulated immunoinflammatory response helps the body effectively defend against pathogenic microorganisms, while an overactive inflammatory response typically leads to tissue injury, organ dysfunction, and even death (4, 5). Therefore, inhibiting inflammatory cytokine storms helps alleviate the excessive inflammatory response in sepsis.

TNF-α is a pleiotropic cytokine initially synthesized as a transmembrane protein. It can function through two types: a transmembrane form TNF-α (tmTNF-α) and a soluble form TNF-α (sTNF-α). The latter is released from the enzymatic cleavage of tmTNF-α through TNFα-converting enzyme (TACE) (6, 7). TNF-α mediates its versatile biological activities through binding to one of two distinct receptors: TNF receptor (TNFR) 1 and TNFR2 (8). It is commonly recognized that sTNF-α is a pro-inflammatory cytokine that exerts an important pathogenic effect in sepsis, and its production usually precedes that of other cytokines and promotes a cascade of inflammatory mediators (9, 10). tmTNF-α, as a transmembrane protein, functions as a ligand by binding to TNF receptors to transmit signals and also acts as a receptor that transmits signals from outside to inside (reverse) back to tmTNF-α-bearing cells (11). Recent evidence suggests that tmTNF-αhas anti-inflammatory properties, unlike the pathogenic effect of sTNF-α in sepsis. Uncleavable tmTNF-α-transgenic mice exhibit a resistance to lipopolysaccharide (LPS)-induced septic shock (12). tmTNF-α can significantly reduce LPS-triggered secretion of IL-1β and IL-6 in macrophages by suppressing TLR4 signaling pathways through TNFR2 signaling (13). Additionally, tmTNF-α inhibits the formation of inflammatory mediators in monocytes and macrophages triggered by LPS through its reverse signaling (14, 15). The anti-TNF-α agent infliximab (IFX) can alleviate inflammation by inducing apoptosis through tmTNF-α reverse signaling. This process leads to the exhaustion of inflammatory cells in inflammatory diseases (16, 17). However, the role and molecular mechanisms of tmTNF-α reverse signaling in sepsis are still largely unknown.

Toll-like receptor 4 (TLR4) is responsible for triggering the proinflammatory cytokine cascade response, and its canonical agonist is LPS, a significant contributor to gram-negative sepsis (18). Upon stimulation by LPS, TLR4 interacts with myeloid differentiation factor 88 (MyD88) and TIR domain-containing adaptor inducing interferon-beta (TRIF) to induce activation of MyD88-dependent and -independent signaling pathways. The former signaling pathway is essential for the production of pro-inflammatory cytokines, which triggers NF-κB translocation to the cell nucleus, leading to the release of multiple cytokines (19, 20). TLR4 signaling is crucial for defending against infectious pathogens. However, excessive activation of TLR4 can disrupt immune homeostasis, leading to harmful and excessive inflammatory responses, and various diseases, including sepsis (21, 22). TLR4 signaling is tightly regulated by multiple negative regulatory factors to prevent inappropriate inflammatory responses and maintain immune homeostasis (23). Our previous study showed that

increasing tmTNF-α levels with a specific tmTNF-α Ab inhibits LPS/TLR4 signaling by promoting the production of negative regulators of the pathway (13). Further research is needed to fully understand the role of tmTNF-α reverse signaling in the LPS/TLR4 signaling pathway.

Here, we activated tmTNF-α reverse signaling using IFX and investigated the potential effects of tmTNF-α reverse signaling on LPS-triggered inflammatory responses in vitro, as well as the molecular mechanisms involved. Our findings demonstrate that tmTNF-α reverse signaling negatively regulates LPS-triggered inflammation byinhibiting the TLR4/NF-κB signaling pathway. This reverse signaling pathway inhibited the phosphorylation and acetylation of NF-κB p65 by upregulating the negative regulatory molecules MCPIP1 and Sirtuin 1 (SIRT1) in the LPS/TLR4 signaling pathway. Our study contribute to a better understanding of the function of tmTNF-α reverse signaling and is expected to provide evidence for its future applicationin treating sepsis and other inflammatory diseases.

MATERIALS AND METHODS

Antibodies and Reagents

Infliximab (IFX) was obtained from MedChem Express (NJ, USA). Phorbol 12-myristate 13-acetate (PMA) and *Escherichia coli* O111: B4-derived LPS were obtained from Sigma-Aldrich (MO, USA). The TNF-α antibody (#sc-52746) was obtained from Santa Cruz Biotechnology (CA, USA). The phospho-NF-κB p65 (Ser536) (#3033), acetyl-NF-κB p65 (Lys310) (#3045) and IκB**α** (#4814) antibodies were obtained from Cell Signaling Technology (MA, USA). The NF-κB p65 polyclonal antibody (#14-6731-81) was obtained from Invitrogen (CA, USA). The MCPIP1 antibody (#25009-1-AP) was obtained from Proteintech (Wuhan, China). The SIRT1 (#ab32441) and β-actin (#ab8227) antibodies were obtained from Abcam (MA, USA).

Cell Culture

The THP-1 cell line (a human monocyte cell line) was obtained from the American Type Culture Collection and maintained at 37°C in 5% CO_2 using RPMI-1640 medium (HyClone, UT, USA) containing 10% heat-inactivated fetal bovine serum (Gibco, MA, USA), 100 **μg**/mL streptomycin and 100 U/mL penicillin. To induce differentiation into macrophages, THP-1 cells were treated with 100 ng/ml PMA for three days.

Flow Cytometry

THP-1-derived macrophages were incubated with a primary anti-TNF-α antibody at 4 °C for one hour, then stained with a FITC-conjugated goat anti-mouse secondary antibody (AS001, ABclonal Technology, Wuhan, China). Finally, the cells were analyzed using an LSR II flow cytometer (Becton Dickinson, CA, USA).

Quantitative Real-time Polymerase Chain Reaction (PCR)

Total RNA was extracted from THP-1-derived macrophages using TriPure Isolation Reagent (Roche, Basel, Switzerland). Subsequently, following the manufacturer's instructions, 1 **μg** of RNA was reverse-transcribed into cDNA using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, MA, USA). The primers were obtained from Sangon Biotech (Shanghai, China), and their sequences can be found in Table 1. Relative mRNA levels for *TNF-α*, *IL-6*, *MCP-1*, *MCPIP1*, and *SIRT1* were quantified through real-time PCR using SYBR Green qPCR Master (Roche) on a CFX96 real-time PCR instrument (Bio-rad, CA, USA). The data was then analyzed using the $2^{-\triangle\triangle Ct}$ method and normalized to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*).

Enzyme-linked Immunosorbent Assay (ELISA)

IL-6 levels were measured using a commercial ELISA kit following the

Table 1. The sequences of primers for real-time PCR

Gene	Forward (5′-3′)	Reverse (5′-3′)
TNF-α	TCAGCCTCTTCTCCTTCCTG	GGCTACAGGCTTGTCACTCG
IL-6	CCTTCCAAAGATGGCTGAAA	CAGGGGTGGTTATTGCATCT
MCP-1	CCCCAGTCACCTGCTGTTAT	TGGAATCCTGAACCCACTTC
MCPIP1	TTCCTGCGTAAGAAGCCACT	AGAGAGCTGGACTGGGATGA
SIRT1	GCAGATTAGTAGGCGGCTTG	TCTGGCATGTCCCACTATCA
GAPDH	CAAGGTCATCCATGACAACTTTG	GTCCACCACCCTGTTGCTGTAG

manufacturer's guidelines and detected with a microplate reader (Tecan, Shanghai, China). The IL-6 ELISA kit was purchased from ABclonal Technology (Wuhan, China).

Immunofluorescence Assay

THP-1-derived macrophages adhered on coverslips were first fixed with 4 % paraformaldehyde. They were then permeabilized with 0.5% Triton-X100, followed by a 1-hour block with 5% bovine serum albumin (BSA, Sigma-Aldrich). Immunostaining was carried out by incubating with an anti-p65 antibody at 4 °C overnight, followed by a 2-hour incubation with an Alexa Flour 488 chicken anti-rabbit secondary antibody (#A-21441, Invitrogen). The nuclei were stained with 4,6-diamidino-2-phenyl indole (DAPI). Cell images were captured using a Nikon ECLIPSE Ti-S microscope (Nikon, Tokyo, Japan).

Western Blot Analysis

To isolate total protein, cells were lysed on ice using RIPA buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors 0.5 mM PMSF, 5 **μ**g/ml aprotinin, and 5 **μ**g/ml leupeptin for 30 minutes. After centrifugation at 15,000 ×g for 15 minutes at 4 °C, the total protein was collected. Subsequently, 50 **μ**g of protein was separated by 12% SDS-polyacrylamide gels and transferred onto PVDF membranes (Millipore, MA, USA). After blocking to prevent nonspecific binding, the membranes were incubated at 4 °C with antibodies against IκB**α,** p65, phosphorylated p65, acetylated p65, MCPIP1, SIRT1 and β-actin overnight. Next, the membranes were incubated with horseradish peroxidase (HRP) conjugated secondary antibodies (Abcam, MA, USA), and the bands were detected using an ECL western blotting reagent (Pierce, IL, USA) and visualized using a gel imaging system (Shanghai Peiqing Technology Co., Ltd., Shanghai, China).

Statistical Analysis

All experimental data are presented as the mean±standard error of the mean (SEM) from at least three independent experiments. Data were statistically analyzed using GraphPad Prism V9 software (California, USA) with either one-way or two-way analysis of variance (ANOVA) and Tukey's post hoc test. A p-value less than 0.05 was considered statistically significant.

RESULTS

The Expression of tmTNF-α in THP-1-derived Macrophages Treated with LPS

The results of the dynamic changes in the expression of tmTNF-α and TNF-α mRNA in THP-1-derived macrophages stimulated with LPS showed that, tmTNF-α expression levels in THP-1-derived macrophages were relatively high in the absence of LPS stimulation. Following LPS treatment, the level of tmTNF-α declined at the 1-hour time-point. However, tmTNF-α returned to the initial level at the 2-hour time-point after LPS stimulation and remained relatively stable at 4 and 6 hours after LPS treatment (Fig. 1A). Meanwhile, LPS rapidly increased

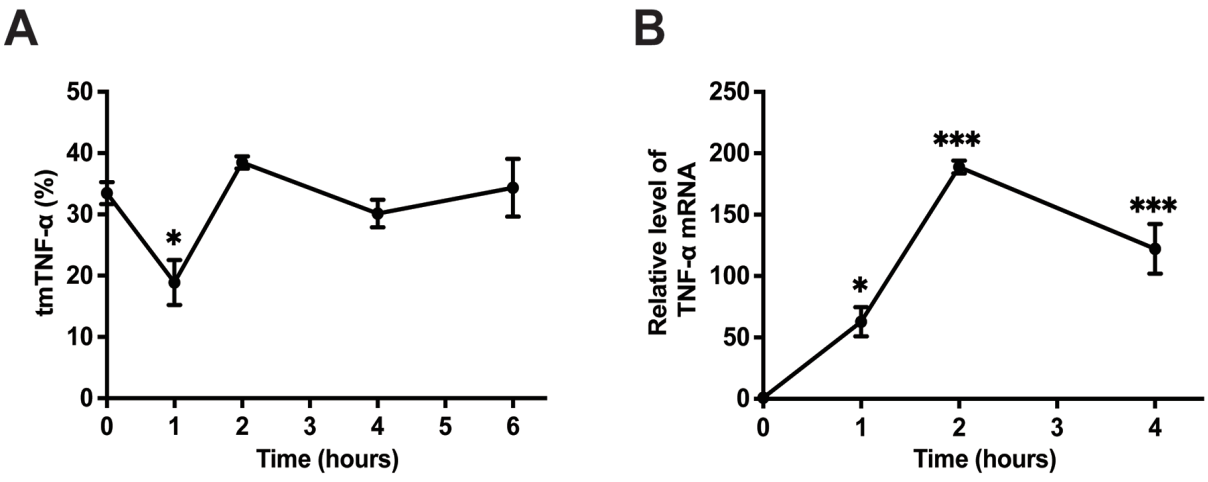

Fig. 1. The impact of LPS on the expression of tmTNF-α and TNF-α mRNA in THP-1-derived macrophages. THP-1-derived macrophages were treated with 100 ng/ml LPS for different time points. (A) tmTNF-α on the cell surface was detected using flow cytometry. (B) The relative mRNA level of TNF-α was determined through quantitative real-time PCR and normalized to GAPDH. The results are presented as means±SEM from a minimum of three independent experiments. *p<0.05, ***p<0.001.

the expression of TNF-α mRNA in THP-1-derived macrophages (Fig. 1B).

tmTNF-α Reverse Signaling Alleviates LPS-triggered Inflammatory Mediators' Expression in Macrophages

Previous studies have shown that usingthe chimeric anti-TNF antibody IFX to activate tmTNF-α reverse signaling can be effective (24-26). In our study, we used IFX to treat THP-1-derived macrophages to investigate the regulatory role of tmTNF-α reverse signaling on LPS-triggered inflammatory responses in macrophages. As demonstrated in Fig. 2, stimulation of THP-1-derived macrophages with LPS, led to an increase in the expression of inflammatory mediators IL-6 and MCP-1. However, treatment with IFX significantly reduced the production of pro-inflammatory mediators IL-6 (Fig. 2A) and MCP-1 (Fig. 2B) mRNA in LPS-activated THP-1 macrophages. Furthermore, IFX effectively decreased the secretion of IL-6 LPS-stimulated cells (Fig. 2C). Interestingly, IFX alone did not impact the levels of IL-6 and MCP-1 compared to controls (Fig. 2). These results suggest that tmTNF-α reverse signaling can effectively alleviate the expression of LPS-triggered inflammatory mediators in macrophages.

tmTNF-α Reverse Signaling Suppresses the Translocation of NF-κB p65 to the Cell Nucleus

Initiation of the NF-κB pathway by LPS/TLR4 leads to the degradation of the inhibitor of NF-κB (IκB**α)** with the release of NF-κB p65, which then translocates to the cell nucleus, triggering the transcription of proinflammatory genes (19). Therefore, we investigated the impact of tmTNF-α reverse signaling on the NF-κB pathway. Our findings revealed that, in THP-1-derived macrophages, tmTNF-α reverse signaling significantly inhibited the degradation of IκB**α** following LPS stimulation (Fig. 3A). Additionally, the immunofluorescence results showed that tmTNF-α reverse signaling inhibited the LPS-induced nuclear translocation of p65 in macrophages (Fig. 3B).

tmTNF-α Reverse Signaling Inhibits the Phosphorylation and Acetylation of NF-κB p65

After the p50–p65 subunit of NF-κB translocates to the nucleus, p65 undergoes a series of post-translational modifications, including phosphorylation and acetylation (27). Therefore, we investigated the effects of tmTNF-α reverse signaling on the phosphorylation and acetylation of NF-κB

p65. As shown in Fig. 4, tmTNF-α reverse signaling significantly inhibited the phosphorylation of NF-κB p65 induced by LPS at the 0.5-hour mark, as well as the acetylation of NF-κB p65 induced by LPS at both the 0.5-hour and 1-hour points in THP-1-derived macrophages. These findings isuggest that tmTNF-α reverse signaling can reduce the LPS-triggered inflammation by inhibiting of the TLR4/NF-κB signaling pathway.

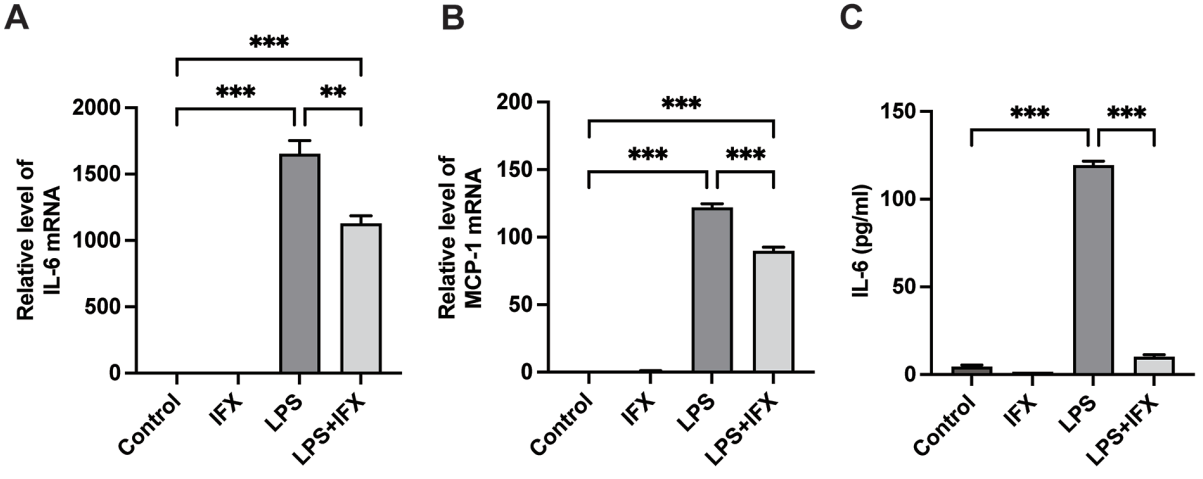

Fig. 2. tmTNF-α reverse signaling alleviates LPS-induced production of inflammatory mediators in macrophages. (A–B) THP-1-derived macrophages were treated with 100 ng/ml LPS with or without 10 μg/ml IFX for 4 h. Relative mRNA levels of IL-6 (A) and MCP-1 (B) were assessed by quantitative real-time PCR and normalized to GAPDH. (C) THP-1-derived macrophages were treated with 100 ng/ml LPS with or without 10 μg/ml IFX for 12 h. IL-6 production was detected by ELISA. Results are presented as means±SEM of at least three independent experiments. ***p*<0.01, ****p*<0.001.

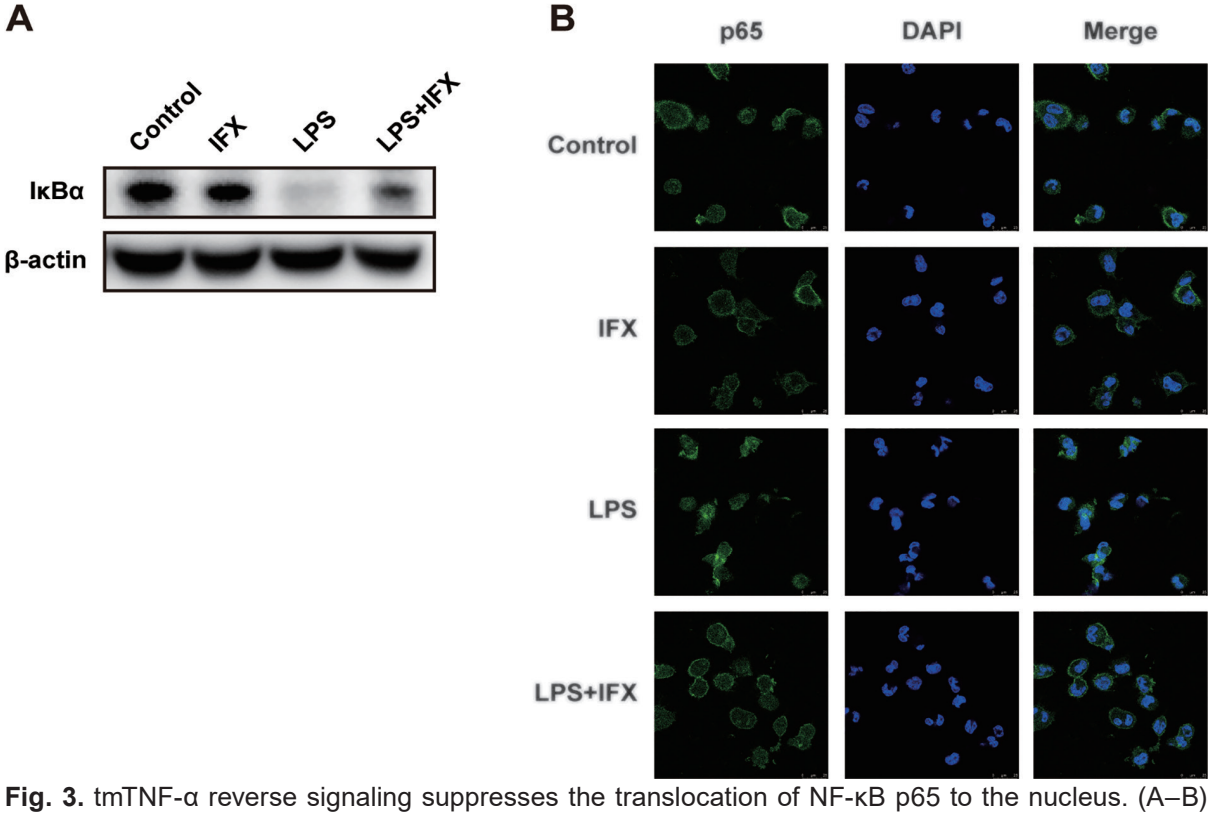

Fig. 3. tmTNF-α reverse signaling suppresses the translocation of NF-κB p65 to the nucleus. (A–B) THP-1-derived macrophages were treated with 100 ng/ml LPS with or without 10 μg/ml IFX for 1 h. (A) Representative images of western blot analysis of the IκBα level in total protein. (B) Representative images of NF-κB p65 nuclear translocation analyzed by immunofluorescence. NF-κB p65 (green), DAPI (blue). Representative images from at least three independent experiments are shown.

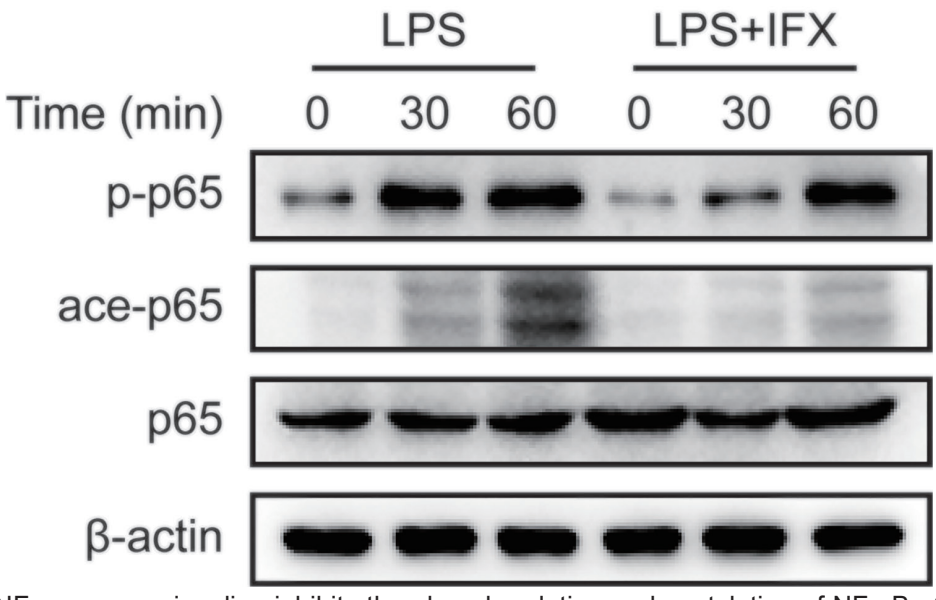

Fig. 4. tmTNF-α reverse signaling inhibits the phosphorylation and acetylation of NF-κB p65. THP-1-derived macrophages were treated with 100 ng/ml LPS with or without 10 μg/ml IFX for 30 min and 1 h. Representative images of western blot analysis showing the phosphorylation and acetylation of NF-κB p65 in THP-1-derived macrophages. Images from at least three independent experiments are presented. P-p65, phosphorylated p65; ace-p65, acetylated p65.

tmTNF-α Reverse Signaling Increases the Expression of Negative Regulators of the LPS/TLR4 Signaling Pathway, MCPIP1 and SIRT1

To further understand how tmTNF-α reverse signaling suppresses the activation of the LPS-triggered TLR4 signaling pathway, we investigated its effect on the negative regulators of TLR4/NF-κB pathway. Real-time PCR data revealed increased mRNA transcription of MCPIP1 (Fig. 5A) and SIRT1 (Fig. 5B) in THP-1-derived macrophages in LPS- and IFX-treated compared to those treated with LPS-alone. Western blot results showed exposure to LPS and IFX also enhanced the expression of MCPIP1 and SIRT1 at the protein level compared to the LPS-alone group (Fig. 5C). These findings indicate that tmTNF-α reverse signaling can upregulate the production of negative regulators of the LPS/TLR4 signaling pathway, MCPIP1 and SIRT1.

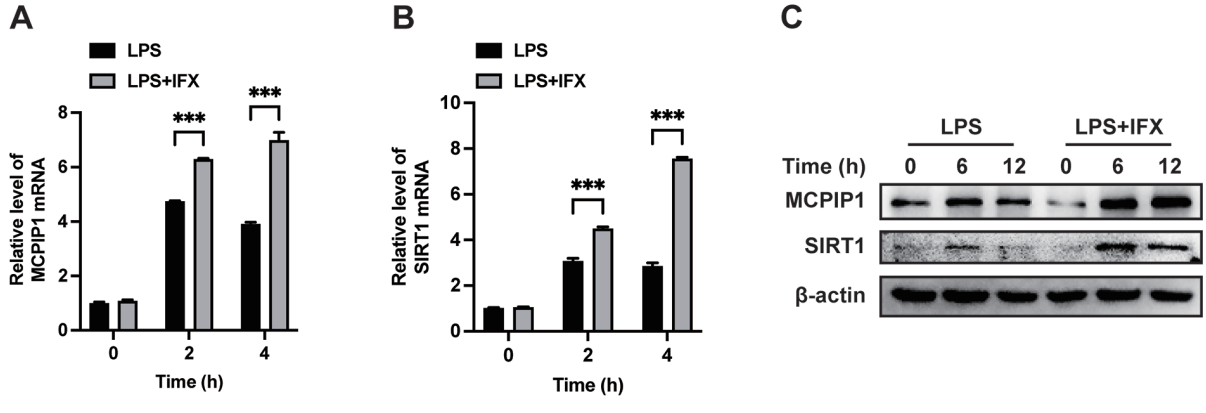

Fig. 5. tmTNF-α reverse signaling increases the expression of the negative regulators of the LPS/TLR4 signaling pathway MCPIP1 and SIRT1. (A–B) THP-1-derived macrophages were treated with 100 ng/ml LPS with or without 10 μg/ml IFX for 2 and 4 h. The relative mRNA levels of MCPIP1 (A) and SIRT1 (B) were assessed using quantitative real-time PCR and normalized to that of GAPDH. (C) THP-1-derived macrophages were treated with 100 ng/ml LPS with or without 10 μg/ml IFX for 6 and 12 h. Western blots from three independent experiments show the levels of MCPIP1 and SIRT1 in total protein. Results are presented as means±SEM of at least three independent experiments. ***p<0.001.

DISCUSSION

Pathogenic infections result in the rapid and massive release of various cytokines, including TNF-α, MCP-1, IL-1, IL-6, and IL-8. This cytokines initiate excessive or persistent inflammation, leading to multi-organ dysfunction and death in sepsis. Treatments that can alleviate inflammatory cytokine storms are considered potential therapeutic targets in sepsis (28). In this study, we investigated the potential role and molecular mechanisms of tmTNF-α reverse signaling in the context of LPS-triggered inflammatory responses. Our findings suggest that tmTNF-α reverse signaling negatively regulates TLR4/NF-κB signaling pathways by upregulating the expression of negative regulators MCPIP1 and SIRT1, thereby reducing the inflammatory response induced by LPS.

tmTNF-α, as the precursor of sTNF-α, exists as a membrane-bound protein on the surface of activated macrophages, lymphocytes, and various other immune cells. It has been reported to exhibit distinct roles from sTNF-α under physiological and pathological conditions (11, 29). sTNF-α is a potent inflammatory cytokine essential for the development of sepsis (9). Rats infused with sTNF-α alone can exhibit clinical symptoms of sepsis, such as hypotension, metabolic acidosis, hemoconcentration, and even death (30). sTNF-α also amplifies the inflammatory cascade by activating monocytes/macrophages to secrete other inflammatory mediators and induces the production of prostaglandin E2 and nitric oxide to participate in the pathological development of sepsis (31-33). Antagonistic TNF-α activity has been shown to enhance the survival rate of sepsis animal models; however, in clinical trials of sepsis, anti-TNF-α treatment did not significantly improve survival as expected (34). This may be attributed to the unselective neutralization of both types of TNF-α, which can lead to defects in their mediated immune functions. Studies have shown that unselective blockade of tmTNF-α and sTNF-α when using anti-TNF-α therapies for autoimmune diseases can increase the risk of infections, cancers, and new autoimmune diseases (35). Despite these risks, all existing anti-TNF-α agents are non-selective, and there is limited clinical evidence available regarding the selective blockade of TNFR1 or TNFR2 signaling pathways (36). Growing evidence suggests that tmTNF-α plays a protective role in chronic inflammation, intracellular bacterial infections, and autoimmune diseases (37). tmTNF-α performs its biological function through cell-to-cell contact. It not only acts as a ligand by binding to TNFR, but also serves as a receptor that transmits reverse signals to tmTNF-α-expressing cells.

The ability of tmTNF-α to act as a receptor and transmit "reverse signals" into cells expressing tmTNF-α has been confirmed in monocytes, macrophages, T lymphocytes, B lymphocytes, and NK lymphocytes. Its intracellular signaling is primarily regulated through the phosphorylation and dephosphorylation of a casein kinase I (CKI) sequence found in the intracellular domain (38). As a ligand, tmTNF-α plays a protective and anti-inflammatory role in sepsis through TNFR2 signaling (13). IFX can form stable complexes with tmTNF-α, and binding of the IFX to tmTNF-α can trigger reverse signaling (39). Therefore, as shown in previous studies utilizing IFX to activate tmTNF-α reverse signaling (24-26), it was selected for this study. Our results indicate that while the cleaving of tmTNF-α induced by LPS results in decrease of tmTNF-α at 1 h, LPS-stimulated macrophage activation also prompts the cells to produce a significant amount of TNF-α. The newly synthesized TNF-α is initially expressed as a transmembrane protein before being converted into the soluble form (6), allowing tmTNF-α expression to return to its initial level and remain at a consistent level, facilitating effective reverse signaling. We observed that, as a receptor, tmTNF-α can also reduce LPS-induced inflammatory

mediators through its reverse signaling. Our data align with previouse published results, demonstrating that tmTNF-α reverse signaling contributes to resistance against LPS. Silvia Kirchner et al. have found that tmTNF-α reverse signaling can inhibit LPS-mediated phosphorylation of the mitogen-activated protein kinase/extracellular signal-regulated kinase in monocytes/macrophages (40). Anna Pallai et al. reported that tmTNF-α reverse signaling suppresses LPS-triggered formation of pro-inflammatory cytokines in macrophages by upregulating TGF-β (14). However, further research is needed to explore how tmTNF-α reverse signaling influences the LPS/TLR4 signaling pathway.

Here, our results shown that tmTNF-α reverse signaling suppresses LPS- triggered nuclear translocation of p65, as well as the phosphorylation and acetylation of p65. These data suggest that tmTNF-α reverse signaling can inhibit the LPS/TLR4/NF-κB signaling pathway. TLR4 belongs to the TLR family which can be activated by pathogen-associated molecular patterns (PAMPs) to trigger nonspecific immune and inflammatory responses (41). Sepsis is the most severe form of the PAMP/TLR4 diseases and modulators of the TLR4 signaling pathway can improve the outcomes of sepsis animal models (42). Beyond recognizing PAMPs, TLR4 also responds to damage-associated molecular patterns (DAMPs) that emerge from injured or dead tissues (42). When LPS activates TLR4, it sets off a cascade where IκB kinase (IkkB) is activated, leading to the phosphorylation and degradation of IκBα. Subsequently, NF-κB undergoes nuclear translocation and activates pro-inflammatory gene expression (43). Therefore, tmTNF-α reverse signaling can alleviate inflammation by inhibiting the LPS/TLR4/NF-κB signaling pathway.

Further investigation into the potential molecular mechanisms by which tmTNF-α reverse signaling regulates TLR4 signaling, revealed that tmTNF-α reverse signaling upregulates the negative regulatory molecules MCPIP1 and SIRT1in the LPS/TLR4/NF-κB signaling pathway. MCPIP1, also known as ZC3H12A and Regnase-1, belongs to the CCCH zinc finger protein family. MCPIP1 contains a ubiquitin-associated domain and a PilT N-terminus domain-like RNase domain at its N-terminus, enabling it to function as both a deubiquitinase and an RNase. This dual role positions MCPIP1 as a key player in modulating the inflammatory response (44, 45). MCPIP1 can be induced by LPS, and then negatively regulates LPS-induced inflammatory signaling protecting mice against septic shock induced by LPS (46). As a deubiquitinase, MCPIP1 negatively regulates NF-κB signaling by detaching ubiquitin moieties from TNF receptor-associated factors (TRAFs) (47). As an RNase, MCPIP1 can inhibit the production of inflammatory cytokines (e.g., IL-6) by binding to their 3′-untranslated regions for mRNA degradation (48). SIRT1 is a highly conservative NAD^+-dependent deacetylase that plays multiple biological functions by deacetylating a variety of proteins (49). SIRT1 plays a critical role in inhibiting NF-κB activation by deacetylating the NF-κB p65 subunit at lysine 310, inducing its anti-inflammatory effects (50). SIRT1 can also directly inhibit the transcription of genes responsible for pro-inflammatory cytokines by mediating the deacetylation of histones in the promoter region of target genes (49). Therefore, it can be hypothesized that tmTNF-α reverse signaling suppresses the LPS/TLR4/NF-κB signaling pathway through up-regulating MCPIP1 and SIRT1 expression.

CONCLUSION

In conclusion, our investigation demonstrates that tmTNF-α reverse signaling has a negative regulatory effect in LPS-induced inflammation by increasing MCPIP1 and SIRT1 expression to suppress the TLR4/NF-κB signaling pathways. Further research into the functions of tmTNF-α reverse signaling functions could offer new therapeutic approaches for sepsis and other inflammatory diseases.

AUTHORS' CONTRIBUTION

Huiming Ye provided the overall principles and direction of the study. Chenxi Li, Mingxin Lin, Xiujuan Li, Lijin Chen, and Yubin Lin conducted all experiments. Chenxi Li and Mingxin Lin analyzed the data and wrote the manuscript. All authors have read and approved the final version of the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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