Int J Clin Exp Med 2018;11(2):1105-1114
www.ijcem.com /ISSN:1940-5901/IJCEM0059464

Original Article

p38 regulates mRNA stability of cytokines stimulated by Zymosan A

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Received April 6, 2017; Accepted December 13, 2017; Epub February 15, 2018; Published February 28, 2018

Abstract: *Objective:* Zymosan A is a type of fungus polysaccharide that is derived from the cell wall of yeast and induces immune responses associated with fungal infection. In this study, our aim was to observe the expression and degradation of the following cytokines: TNF-α, CXCL1, IL-10 and IL-23a. These cytokines were induced by Zymosan A via primary peritoneal macrophages (pMΦs), and they had more AU-rich elements (ARE) within their 3'-UTR mRNA sequence. Additionally, we would like to explore the mechanism underlying the p38 regulation of the ARE-mRNA stability of the related cytokines in the MAPK signaling pathway. *Methods:* Primary peritoneal macrophages (pMΦs) were isolated and purified from C57 mice and stimulated with Zymosan A. The mRNA expressions of TNF-α, CXCL1, IL-10 and IL-23a that were induced by Zymosan A were detected by Real-time PCR, and the mRNA 3'-UTR sequences of these cytokines were analyzed using bio-informatic methods. The activation of MAPK signaling pathways and blocking experiments in pMΦs treated with Zymosan A were identified by western blot, and the change in TTP, which is a type of RNA binding protein, was observed through *in vitro* enzymatic activity experiments. *Results:* Zymosan A induces a high expression of TNF-α, CXCL1, IL-10 and IL-23a and activates the MAPK signaling pathway through the phosphorylation of p38, ERK, and JNK and the RNA binding protein TTP. Bio-informatic analysis showed that the mRNA 3'UTR region of the related cytokines contained numerous AU-rich element (ARE) sequences. In the MAPK signaling pathway activated by Zymosan A, the ERK inhibitor PD98059 and the JNK inhibitor SP600125 had no effect on the ARE-mRNA stability of these cytokines. Conversely, the p38 inhibitor SB202190 effectively inhibited the phosphorylation of the downstream MK2 and TTP within the p38 signaling pathway and quickly degraded the mRNA of the above cytokines. An *in vitro* enzymatic activity experiment also proved that the phosphorylation of TTP disappeared after the treatment of λPP, which is a type of phosphoesterase. *Conclusions:* Zymosan A induces the high expression of TNF-α, CXCL1, IL-10 and IL-23a, which contain ARE-mRNA, and effectively activates MAPK signaling pathways through the phosphorylation of p38, ERK and JNK. In MAPK signaling pathways, p38 plays an important role about the mRNA stability and can enhance the ARE-mRNA stability of the above cytokines, the mechanism of its effect may be that p38 phosphorylate the downstream MK2 and TTP, which leads to the phosphorylated TTP can't combine with the mRNA ARE sequence and degrades the mRNA effectively.

Keywords: Zymosan A, p38, signaling pathway, mRNA stability

Introduction

Fungal infection can cause a series of inflammation events. As a main fungi polysaccharide, Zymosan A can cause acute pneumonia, hepatitis and a systemic inflammatory response that has characteristics of infection [1-3] , and it is widely used in scientific research as an effective simulated model of fungal infections [4-6]. Zymosan A can combine with TLR2 and Dectin1 on the membrane of innate immune cells such as macrophages and dendritic cells, activate TLR2 and CTL signaling pathway respectively, and then induce the expression of cytokines such as TNF-α, CXCL1, IL-10, and IL-23a. The expression of cytokines is strictly regulated by organisms, including gene expression control at both the transcription and post-transcription levels; the latter level is particularly important in the regulation of mRNA stability. In recent years, research into the stability of mRNA has focused on RNA binding protein and the AU-rich element (ARE) region of the mRNA sequence. RNA binding proteins are a type of intracellular proteins that have high affinity to mRNA. ARE is a type of cis-regulatory element that is located

in the 3' end of the translation sections of mRNA (3' untranslated regions, 3'UTR) and commonly occurs in two forms, the AUUUA and UUAUUUAUU repetitive sequences [7-11]. Studies have suggested that the mRNA with rich ARE is usually unstable and that RNA binding protein combines with the ARE region of mRNA directly or indirectly to promote the degradation of mRNA [12-14]. However, in the signaling pathway induced by Zymosan A, research pertaining to mRNA stability control of related cytokines is lacking.

In cells stimulated by Zymosan A, signal transmission from upstream proteins to downstream mitogen activated protein kinase (MAPK) is activated. MAPK signaling pathways contain a series of protein kinase cascades: MAPK3K-MAPK2K-MAPK, and MAPKs are types of serine/threonine protein kinases, which also include p38, extracellular signal-regulated kinase (ERK) and c-Jun NH_2-terminal kinase (JNK). ERK plays an important role in transduction of cell division. p38 and JNK can, respectively, activate the cAMP response element binding protein (CREB) and c-Jun, which are transcription factors, initiate the transcription of IL-10 and participate in a variety of inflammatory responses. Additionally, p38 plays an important role in the stability and adjustment of mRNA [15-17]. Previous research has showed that p38-MK2 influenced the stability of mRNA to a greater extent than other molecules exerted their influence within the TLR4 signaling pathway [18-20]. However, in the signaling pathway induced by Zymosan A, the regulatory mechanism underlying the mRNA stability of related cytokines is not clear, and whether p38 participates in the mRNA stability adjustment of cytokines has not yet been reported.

In this report, we investigated the expression of cytokines induced by Zymosan A, such as TNF-α, CXCL1, IL-10 and IL-23a, whose mRNA sequences contain more AU-rich element (ARE), as well as the mechanism underlying the p38-regulated ARE-mRNA stability.

Materials and methods

Mice

Sixty male C57BL/6 mice, six to eight weeks old, were purchased from the Center of Experimental Animals, Institute Pasteur of Shanghai, Chinese Academy of Sciences (Shanghai China). All mice were maintained with standard laboratory food and water freely in animal facilities under specific pathogen-free conditions. The handing of mice and experimental procedures were approved by the Animal Welfare and Research Ethics Committee of the Institute of University of South China.

Antibodies, culture medium and reagents

The antibodies, including R-p-MK2 (S3041), R-MK2 (S3042), R-p-p38 (#9211), R-p-ERk (sc-7383), R-p-JNK (#9251), and R-p-MKK3/6 (#9-236), were purchased from Cell Signaling Technology, Inc (Boston, USA). The culture mediums and reagents such as Gibco® RPMI-1640 (Cat:22400), Gibco® DMEM (Cat:12430), Gibco® PBS (Cat:14190), Gibco® Trypsin, Gibco® P/S (Cat:15140) were purchased from Invitrogen Corp (CA, USA). The other reagents, including SYBR real-time PCR kit (Code: DRR-420A, Takara), SB202190 (Sigma, USA), PD98-059 (Cell signaling, USA), SP600125 (Invitrogen, USA), Zymosan A from *Saccharomyces cerevisiae* and LPS from *Escherichia coli* (Invitrogen, USA), and λPP (P0753, NE, USA), were purchased too.

Purification of mouse peritoneal macrophages (pMΦs)

Every male C57BL/6 mouse was injected intraperitoneally with 1 mL 3% tryptone soup, and six mice were used for each experiment. The tryptone soup was created by adding 3% tryptone to distilled water, followed by boiling under high pressure. Then, the injected mice were monitored twice every day and fed well to reduce pain and premature death. Four days after infection, the mice were sacrificed by cervical dislocation and disinfected with 75% alcohol. The abdomen of each mouse was prepared, and 6-8 ml of 1×PBS per mouse was used to wash their abdominal cavities. Then, the peritoneal macrophages as many as possible were collected in a sterile tapered tube and transferred to the cell cultivation room. Cells were purified with a 0.2 mm filter to remove fat and other impurities and centrifuged at 1000 RPM for 10 minutes, and suspended cells were combined with 10% fetal bovine serum and 1% 1640 medium. A cell concentration of 2×10^6/mL was spread onto a 6 cm cell culture plate, which was incubated at 37°C and 5% CO_2.

Bio-informatic analysis

The murine gene sequences of TNF-α, CXCL1, IL-10 and IL-23a were found in GenBank (http://

Table 1. Nucleotide sequences of primers

Primers (5'-3')	Sequence
GAPDH-F	TGGAGAAACCTGCCAAGTATGA
GAPDH-R	CTGTTGAAGTCGCAGGAGACAA
IL-10-F	ATTTGAATTCCCTGGGTGAGAAG
IL-10-R	CACAGGGGAGAAATCGATGACA
IL-23a-F	CACCAGCGGGACATATGAATCTA
IL-23a-R	CAGAACTGGCTGTTGTCCTTGA
CxCL1-F	GCTGGGATTCACCTCAAGAA
CxCL1-R	CTTGGGGACACCTTTTAGCA
TNF-α-F	GTCCCCAAAGGGATGAGAAGTT
TNF-α-R	GTTTGCTACGACGTGGGCTACA

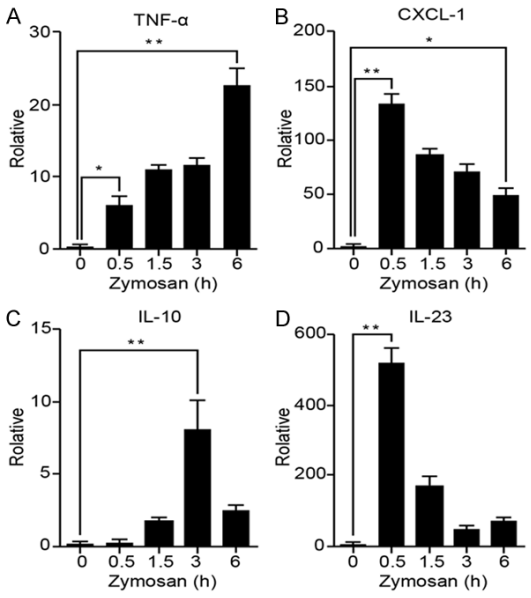

Figure 1. ARE-mRNA expression levels for TNF-α (A), CXCL-1 (B), IL-10 (C), and IL-23a (D) induced by Zymosan A. pMΦ cells were treated with Zymosan A (100 ng/mL) for 0, 0.5, 1.5, 3 and 6 hours. Total RNA were isolated, and the expression level of mRNA was determined by real-time PCR. Data were reported as the mean ± SD of triplicates. The level of mRNA at 0 hour was the control. Compared with control, *P<0.05; **P<0.01.

www.ncbi.nlm.nih.gov). The mRNA 3'UTR base compositions of TNF-α, CXCL1, IL-10 and IL-23a were analyzed using the DNAstar software (DNAstar, INC), and the database of the gene un-translated regions were searched and analyzed using the UTRblast software (http://bighost. Area. Ba. cnr.it/BIG/UTR Home/).

Fluorescent real-time quantitative polymerase chain reaction

Total RNA was extracted from pre-treated purified macrophage cells and reverse-transcribed to cDNA following the manufacturer's instructions. Real-time quantitative PCR was performed using TaqMan universal PCR master mix on an ABI Prism 7900 sequence detection system (Applied Biosystems, USA). The primers were designed as displayed in **Table 1**. The PCR program included 40 cycles of 95°C for 30 s, 95°C for 15 s and 60°C for 30 s. PCR products were determined using the comparative threshold cycle method described by Applied Biosystems.

Activation of MAPK signaling pathways and blocking experiment

In activation experiment, the pMΦs were treated with Zymosan A (100 ng/mL) and LPS (10 ng/mL) for 15, 30 and 60 min. The phosphorylation of MAPKs was checked with antibody p-MKK3/6, p-p38, p-ERK, p-JNK, p-MK2 through western blot. In the blocking experiment, the pMΦs were pre-treated for 30 min with DMSO or three types of inhibitors, *i.e.,* p38 inhibitor SB202190, ERK inhibitor PD98059, and JNK inhibitor SP600125, to block the targeted protein kinase, and then were stimulated with Zymosan A (100 ng/mL) and LPS (10 ng/mL) for 60 min. The phosphorylation of MAPK signaling pathways were detected by western blot via the relevant antibodies to identify the block effects of the inhibitors. The *in vitro* enzyme activity experiment also confirmed that the downstream TTP phosphorylation had occurred.

Western blot

The protein samples were extracted from cells that had been stimulated by Zymosan A or other factors. The protein concentration was determined with Bradford analysis, using albumin for the standard curve. Equal amounts of whole cell extracts were separated with SDS-PAGE and transferred to PVDF membrane. According to the standard protocol, after the membrane was blocked by 5% milk and washed by TBST sufficiently, the membrane was incubated with the primary antibodies and then the secondary antibodies. Finally, the ECL method was used to detect the phosphorylation of protein kinases.

Statistical analysis

Data were expressed as the mean standard deviation (SD) or number (%) and compared by Student's t-test. P<0.05 was considered statistically significant, and P<0.01 was considered

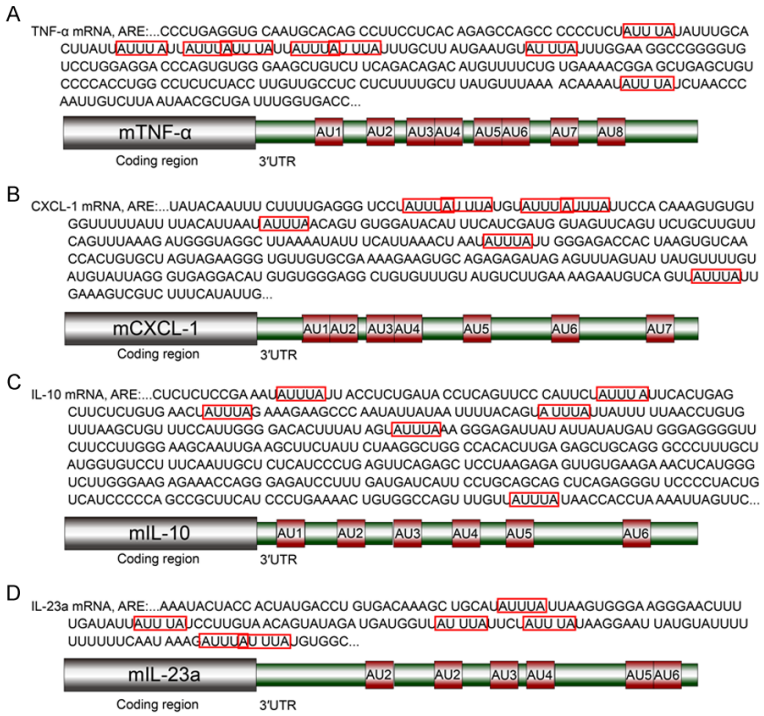

Figure 2. The mRNA 3'UTR sequences of TNF-α (A), CXCL1 (B), IL-10 (C), and IL-23a (D) contained the numbers of AU-rich element. The mRNA 3'UTR sequence of TNF-α contained eight AU-rich elements, CXCL1 contained seven AU-rich elements, and IL-10 and IL-23a contained six AU-rich elements.

very significant. Statistical analysis was performed using the Statistical Package for Social Sciences version 10.0 (SPSS Inc, Chicago, USA).

Results

Zymosan a induced high mRNA expression of TNF-α, CXCL1, IL-10 and IL-23A in pMΦs

Time course studies of cytokine production from the pMΦs treated with Zymosan A (100 μg/ml) were performed to assess their expression levels. Rat peritoneal macrophages (pMΦs) were treated with Zymosan A (100 μg/ml) for 0.5, 1.5, 3 and 6 h, followed by the termination of the stimulation. RNA was extracted from cells for real-time PCR. The results showed that Zymosan A induced the pMΦs to express high mRNA levels of TNF-α, CXCL1, IL-10 and IL-23a. At 0.5 h, the induced quantities of CXCL1 and IL-23a were approximately 1500 times and 5000 times greater, respectively, compared with the previous stimulation. TNF-α peaked at 6 h, and IL-10 peaked at 3 h, and they were approximately 200 and 80 times greater, respectively, compared with the prior stimulation (**Figure 1**).

The mRNA sequences of TNF-α, CXCL1, IL-10 and IL-23a had many AU-rich elements and were analyzed by bio-informatic method

The mRNA sequences of TNF-α, CXCL1, IL-10 and IL-23a induced by Zymosan A were analyzed through bio-informatic method. The bio-informatic analysis showed that the mRNA 3'UTR sequences of these high expression cytokines had rich AREs. TNF-α contained 8 AREs, CXCL1 contained 7 AREs, and IL-10 and IL-23a each contained 6 AREs (**Figure 2**).

The mRNA degradation of the aboved cytokines was observed under the natural state in pMΦs induced by Zymosan A

For exploring the change in mRNA stability, we used Zymosan A to stimulate the pMΦs for 3 hours, added the actinomycin D to end the transcription, and then checked the change of the mRNA quantities and observed the mRNA degradation of the cytokines under the natural state through real-time PCR. A control group was also designed without stimulation by Zymosan A. We found that the mRNA levels of these related cytokines, which have rich AREs such as TNF-α, CXCL1, IL-10 and IL-23a, degraded rapidly after ended transcription in the control group, the mRNA quantities of these cytokines reduced to approximately 10% of the original level after 4 hours. However, it degraded slowly in the group stimulated with Zymosan A. The mRNA level of CXCL1 degraded to approximately 60%, IL-10 and IL-23a degraded to approximately 50%, and TNF-α degraded to 40% of the original quantity after 4 hours (**Figure 3**).

The MAPK signaling pathway in pMΦs treated with Zymosan A or LPS was detected

To assess whether the MAPK signaling pathway was involved in the mRNA stability of the cyto-

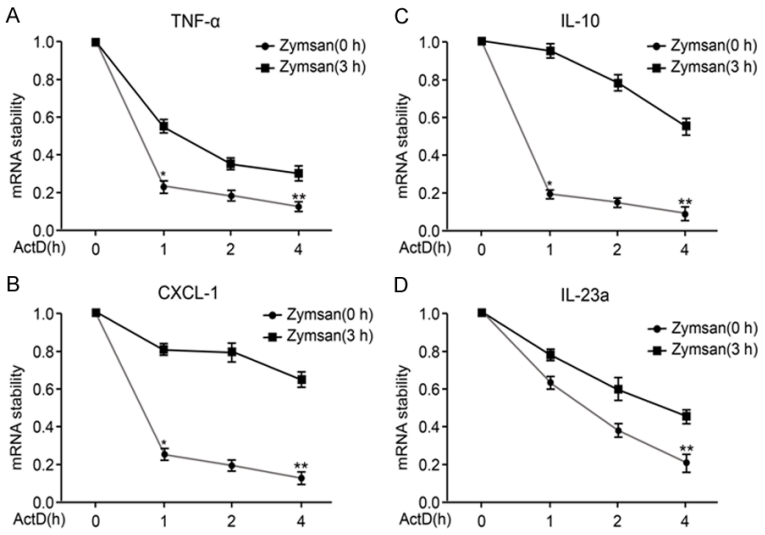

Figure 3. The ARE-mRNA degradation degree of TNF-α (A), CXCL-1 (B), IL-10 (C), and IL-23a (D) induced by Zymosan A were detected by real-time PCR. The pMΦs were treated or untreated with Zymosan A (100 ng/mL) for 3 hours. The reaction was terminated at 1, 2 and 4 hours, and Actinomycin D (5 μg/mL) was added to each sample, respectively. Total RNA was isolated, and the mRNA degradation degree was detected by real-time PCR. Data were reported as the mean ± SD of triplicates. The quantity of mRNA at 0 hour was the control. Compared with control, *P<0.05, **P<0.01.

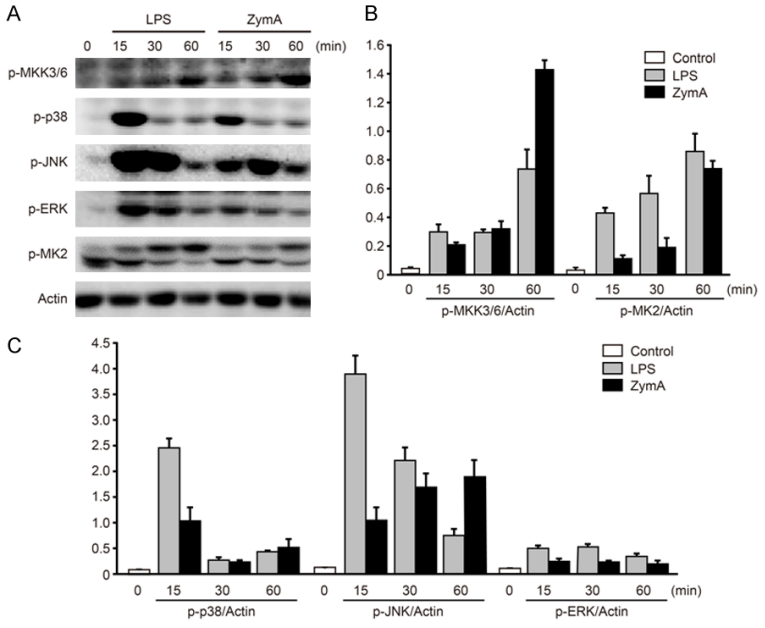

Figure 4. The phosphorylation of MAPKs induced by Zymosan A in pMΦs was detected by western blot. The pMΦs were treated with Zymosan A (100 ng/mL) and LPS (10 ng/mL) for 15, 30 and 60 min. Then, proteins were isolated by adding cell lysis buffer. A. The phosphorylation of MAPKs was checked by western blot with the antibodies p-MKK3/6, p-p38, p-ERK, p-JNK, and p-MK2. Actin served as an internal control. (p-) means phosphorylated. The experiments were repeated three times. B, C. Gray value analysis of MAPKs differentially expressed. Bars represent mean ± SD. (n = 3).

kines induced by Zymosan A, the MAPK signaling pathway was detected by western blot. The pMΦs were treated with Zymosan A (100 μg/ml) and LPS (10 μg/ml) for 15, 30 and 60 min, then the proteins were isolated by adding cell lysis buffer. We used the p-MKK3/6, p-p38, p-ERK, p-JNK, and p-MK2 antibodies to check the phosphorylation of MAPKs via western blot. The results showed that Zymosan A, which corresponded to the LPS control group, induced the phosphorylation of p38, ERK and JNK at 15 minutes and continued until 60 minutes. The upstream kinase MMK3/6 and the downstream substrate of p38 protein kinase MK2 were also phosphorylated by Zymosan A (**Figure 4**).

The influence of MAPK inhibitors on the ARE-mRNA stability of cytokines induced by Zymosan A

To further investigate the role of different MAPKs in expression of TNF-α, CXCL1, IL-10 and IL-23a, we used three inhibitors of MAPKs, *i.e.,* p38 inhibitor SB202190, ERK inhibitor PD98059 and JNK inhibitor SP600125, to detect the effect. The pMΦs were treated with Zymosan A (100 μg/mL) for 3 hours, then actinomycin D (5 μg/mL) was added to each sample to interrupt transcription. Subsequently, DMSO, p38 inhibitor SB202190, ERK inhibitor PD98059 and JNK inhibitor SP600125 were added to each sample. At 1, 2 and 4 hour time point, the stimulation was discontinued. The total RNA at each time point were isolated to check

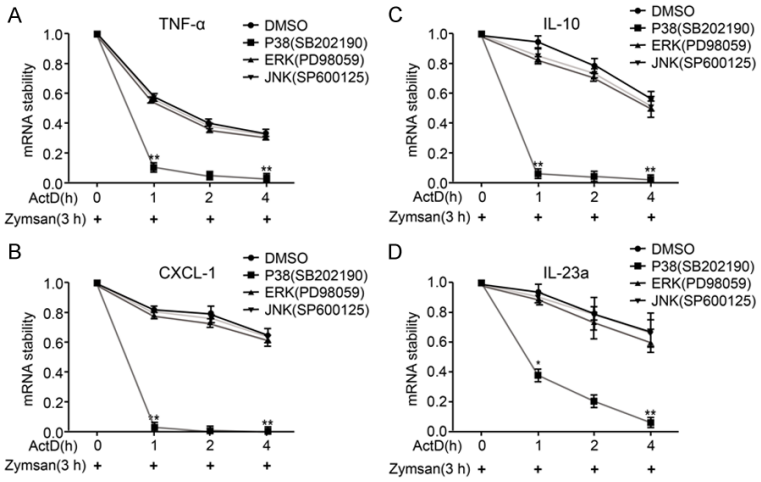

Figure 5. The MAPKs inhibitors effect on the mRNA degradation of TNF-α (A), CXCL-1 (B), IL-10 (C), and IL-23a (D) induced by Zymosan A. The pMΦs were treated with Zymosan A (100 ng/mL) for 3 hours, and Actinomycin D (5 μg/mL) was added to end the stimulation. At the same time, DMSO, p38 inhibitor SB202190, ERK inhibitor PD98059, and JNK inhibitor SP600125 were added to each sample. The reaction was ended at 1, 2 and 4 hours. Total RNA were isolated, and the mRNA degradation degree was checked by real-time PCR. Data were reported as the mean ± SD of triplicates. The level of mRNA at 0 hour was the control. Compared with control, *P<0.05; **P<0.01.

the mRNA degradation of TNF-α, CXCL1, IL-10 and IL-23a by real-time PCR. The results showed that the mRNA level of TNF-α degraded to 10% or less than the original amount after 4-hours treatment with the p38 inhibitor SB202190. However, there were no significant differences on the degree of mRNA degradation among the DMSO, ERK inhibitor PD98059 and JNK inhibitor SP600125 treatment groups. The mRNA levels of TNF-α of the three groups degraded to 40% of the original amount after 4 hours. Similarly, it was observed in CXCL1, IL-10 and IL23a (**Figure 5**). The data illustrated that p38 inhibitor SB202190 significantly influenced the ARE-mRNA stability, it also indicates that p38 plays an important regulatory role on the ARE-mRNA stability of cytokines stimulated by Zymosan A.

The underlying mechanism of the p38 regulating ARE-mRNA stability

Having discovered that p38 played an important role in regulating the ARE-mRNA stability of cytokines stimulated by Zymosan A, we wanted to investigate the underlying mechanisms in several experiments. First, the pMΦs were pretreated with DMSO and the inhibitors SB202190, PD98059 and SP600125 for 30 min. Then, they were stimulated with Zymosan A (100 μg/ml) or LPS (10 μg/ml) for 60 min. Cell lysis buffer was added to the samples to isolate the protein. The phosphorylation of these kinases was checked with antibody p-MK2, p-CREB, MK2, TTP, p-ERK and p-c-Jun via western blot, antibody β-tubulin or β-actin served as the internal control. It was found that the p38 inhibitor SB202190 inhibited the phosphorylation of CREB, which is a downstream transcription factor of the p38 signaling pathway. SP202190 also effectively inhibited the phosphorylation of MK2 and TTP (**Figure 6A** and **6B**). The ERK inhibitor PD98059 effectively suppressed the activation of ERK (**Figure 6C** and **6D**), and the JNK inhibitor SP600125 inhibited the phosphorylation of c-Jun (**Figure 6E** and **6F**), which is a downstream transcription factor of JNK. However, they could not inhibit the phosphorylation of MK2. In the *in vitro* enzyme activity experiment, the data showed that the phosphorylation of TTP disappeared after joining λPP which is a type of phosphorylated esterase. This result illustrated that TTP really was phosphorylated in the signaling pathway induced by Zymosan A (**Figure 7A** and **7B**).

Discussion

Zymosan A is a type of fungal cell wall polysaccharide, and it can be used as a pathogen associated molecular pattern (PAMP) that is recognized by membrane receptors. In the process of fungal infection, some pattern recognition receptors (PRRs) activate the MAPK and NF-κB signaling pathway, thereby inducing the production of cytokines such as TNF-α, CXCL1, IL-10 and IL-23a. The process of activation is under strict control within the transcription stage. Zymosan A combines with related receptors and can activate the transcription factors such as NF-κB and CREB, thereby starting the mRNA transcription of IL-10 and TNF-α. However, there are fewer studies concerning the regulation of mRNA stability of correlated cyto-

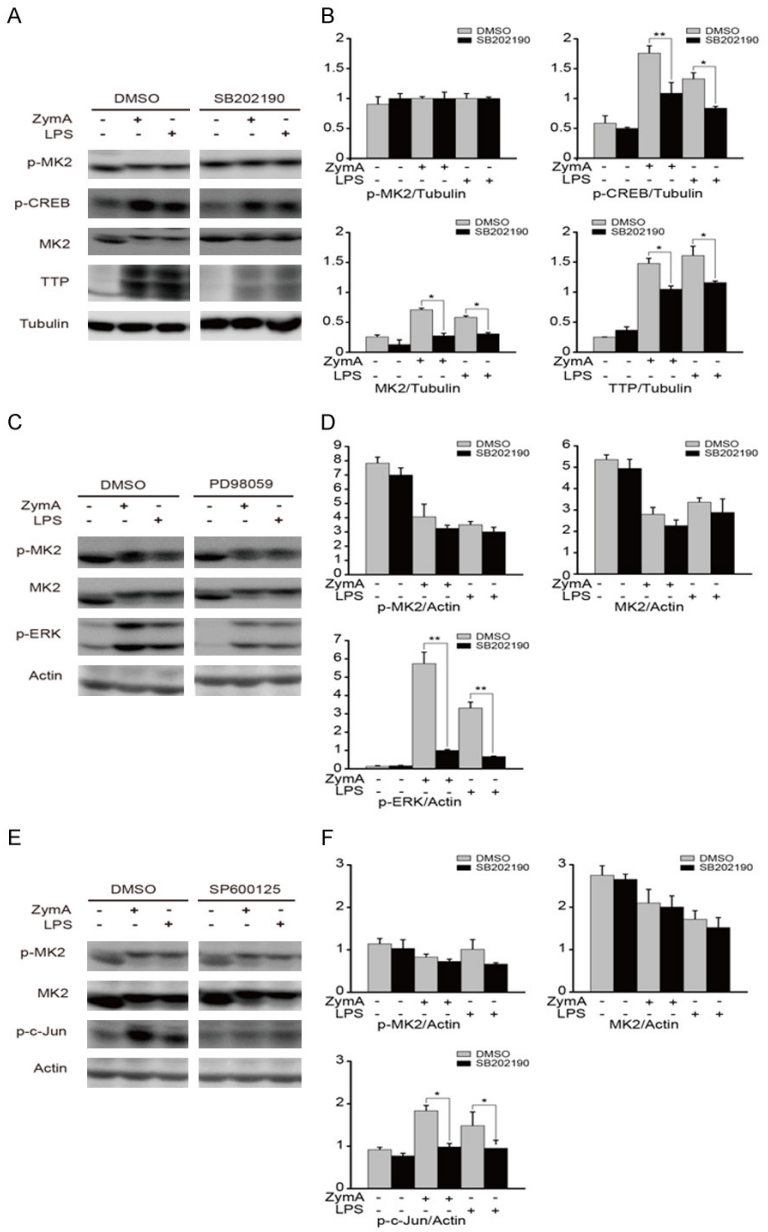

Figure 6. The effect of p38 inhibitor SB202190 (A), ERK inhibitor PD98059 (C), and JNK inhibitor SP600125 (E) in the MAPK signaling pathway were detected by western blot. The pMΦs were pretreated with DMSO or three types of inhibitors for 30 min and then stimulated with Zymosan A (100 ng/mL) or LPS (10 ng/mL) for 60 min. The proteins were isolated by adding cell lysis buffer, and the phosphorylation of the MAPK signaling pathways was determined by western blot using the relevant antibodies; tubulin or actin served as the internal control. (p-) means phosphorylated. The experiment was repeated three times. Gray value analysis of the effect of p38 inhibitor SB202190 to p38 signaling pathway (B), ERK inhibitor PD98059 to ERK signaling pathway (D) and JNK inhibitor SP600125 to JNK signaling pathway (F) expressed. Bars represent mean ± SD (n = 3). Very significantly differences between the inhibitor groups and the DMSO groups were indicated with one ($p<0.05$) or two ($P<0.05$) asterisks.

kines in the post-transcription stage. Hence, the regulatory mechanism is unclear [21-23].

In recent years, researchers have demonstrated that MAPKs adjust mRNA stability. Previous reports have focused on how p38 regulates the mRNA stability of the cytokines IL-10, TNF-α and IL-6 induced by LPS within the MAPK signaling pathway. To study whether MAPK family members participate in the regulation of ARE-mRNA stability of related cytokines, such as TNF-α, CXCL1, IL-10 and IL-23a induced by Zymosan A, we checked the expression levels of the related cytokines and their mRNA stability in the MAPK signaling pathway. The research showed that Zymosan A can effectively activate the MAPK signaling pathway by phosphorylating p38, ERK and JNK, and induces the high expression of TNF-α, CXCL1, IL-10 and IL-23a. These cytokines have same character that is their mRNA contain many AU-rich element.

We used p38 inhibitor SB202190, ERK inhibitor PD98059 and JNK inhibitor SP600125 to explore the effects of MAPKs on the ARE-mRNA stability of cytokines induced by Zymosan A [24-27]. The specific kinase block experiment revealed the mRNA quantities of TNF-α, CXCL1, IL-10 and IL-23a degraded rapidly after transcription in p38 inhibitor SB202190 group, it declined from 100% of the original level to 10% or less in 4 hours. However, in ERK inhibitor PD98059 group and JNK inhibitor SP600125 group, the degree of ARE-mRNA degradation were slow, they declined from 100% of the original level to 50% within 4 hours, the same phenomenon was observed in the untreated group. The data suggested that p38 played an important role in regulating ARE-

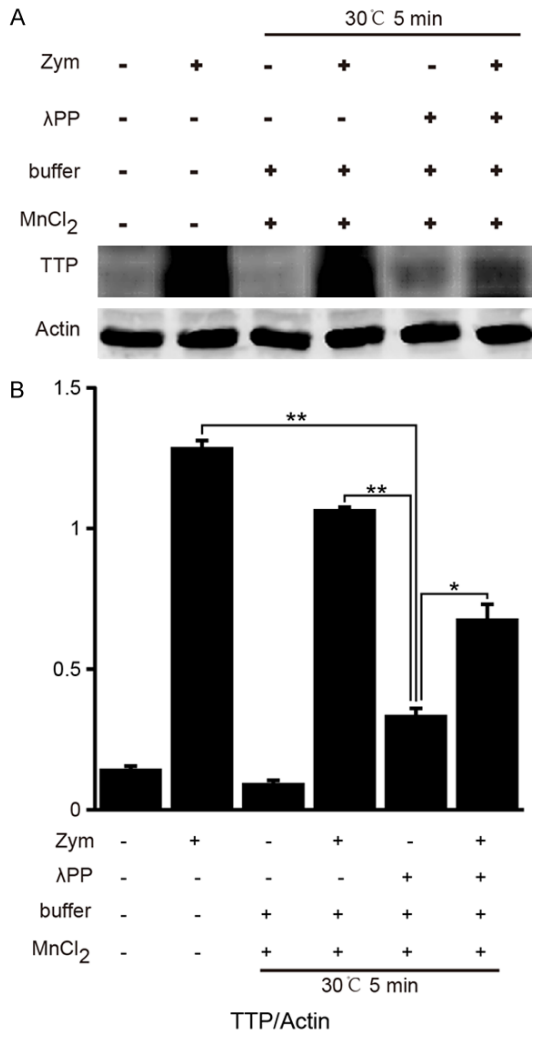

Figure 7. The dephosphorylation of TTP was checked by enzyme activity *in vitro*. The λpp was added to the protein samples, which were incubated at 30°C for 5 min; A. The dephosphorylation of TTP was determined by western blot. The experiment was repeated three times. B. Gray value analysis of the dephosphorylation of TTP expressed. Compared with the λpp group, very significantly differences were indicated with one ($p<0.05$) or two ($P<0.05$) asterisks.

mRNA stability of cytokines induced by Zymosan A and p38 could enhance the ARE-mRNA stability.

To explore the underlying mechanism of p38 regulating mRNA stability, we examined the p38 signaling pathway further. The results showed that Zymosan A effectively activated the phosphorylation of upstream MKK3/6 and downstream MK2 and RNA binding protein TTP in the p38 signaling pathway. After treatment with the p38 inhibitor SB202190, the phosphorylation of MK2 and TTP was significantly inhibited. These facts suggested that p38 regulated the mRNA stability possibly through the phosphorylation of MK2 and TTP. As an important RNA binding protein, TTP was usually combined with the ARE sequences of mRNA, thereby promoting the degradation of mRNA. So if TTP was phosphorylated by p38, it would be separated from the ARE sequences and could not degrade mRNA. Therefore, p38 could enhance the ARE-mRNA stability. The possible mechanism also explained why the ARE-mRNA stability of related cytokines was more sensitive to p38 than to ERK or JNK.

Overall, we speculated the mechanism of how p38 regulated the mRNA stability of cytokines stimulated by Zymosan A was that Zymosan A combined with cell membrane receptor and activated p38 signaling pathway, via the phosphorylation of MK2 and TTP to enhance the mRNA stability of the related cytokines.

Acknowledgements

This work was supported by the Foundation of Hunan Provincial Key Laboratory for Special Pathogens Prevention and Control Foundation (No. 2014-5), and the Hunan Province Cooperative Innovation Center for Molecular Target New Drug Study (2015-351).

Disclosure of conflict of interest

None.

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