The transcription factor cMaf is targeted by mTOR, and regulates the inflammatory response via the TLR4 signaling pathway

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Received December 19, 2016; Accepted February 12, 2018

DOI: 10.3892/ijmm.2018.3510

Abstract. cMaf is a leucine-zipper transcription factor that is involved in cell differentiation, oncogenic transformation, and human diseases; however, the functions of cMaf in inflammatory responses in macrophages are still not fully understood. Western blot analysis showed that cMaf expression was induced by lipopolysaccharide (LPS) stimulation in mouse macrophages. An enzyme-linked immunosorbent assay was performed to detect the level of expression of inflammatory cytokines after knockdown of cMaf expression in macrophages using a small interfering RNA (siRNA). Signaling pathway inhibitor analyses indicated that extracellular signal-related kinase and phosphoinositide 3-kinase contribute to mammalian target of rapamycin phosphorylation (mTOR), which controls cMaf expression at the translational level by regulating the expression of eIF4E-binding protein 1 and S6 ribosomal kinase 1 in response to Toll-like receptor 4 signaling. Histopathological findings of the lung and a survival analysis showed that mice transplanted with cMaf-knockdown macrophages were more susceptible to LPS challenge. Taken together, our study revealed that the control of cMaf expression at the translational level by mTOR regulated the expression of inflammatory genes in response to LPS challenge. Moreover, cMaf protected mice from septic shock indicating that cMaf may improve host fitness, thereby enabling the survival of certain infectious diseases.

Introduction

Host immune cells utilize Toll-like receptors (TLRs) to recognize pathogen-associated molecular patterns to transmit intracellular signals via kinase cascades, which ultimately activate transcription factors, leading to the expression of proinflammatory cytokines. However, excessive production of these cytokines disrupts immune homeostasis, and even causes tissue damage (1). To antagonize aberrant inflammatory signaling and maintain homeostasis, negative regulators are also required for inflammatory signaling. Previous studies in both humans and mutant mice with inflammatory diseases have verified some critical inhibitory pathways, including the transforming growth factor-β (TGF-β) and interleukin-10 (IL-10) pathways (2). Furthermore, the deubiquitinases A20 and CYLD, the p110d kinase, and the SHP-1/2 and MKP-1/5 phosphatases have been identified as negative regulators of inflammation in the TLR4 signaling pathway (3-7).

cMaf is a transcription factor with basic leucine zipper domains that mediate DNA binding to the cMaf recognition element (8,9). cMaf is expressed in monocyte and macrophage lineages, as well as in T cell subsets (10,11). Studies of transgenic cMaf T helper (Th) cells revealed that cMaf is important for the development of Th1 and Th17 cells (12-14). A functional analysis showed that TGF-β induces cMaf expression, which suppresses IL-22 expression in Th17 cells, enhances proinflammatory innate defense mechanisms in epithelial cells, and provides crucial protection to tissues from damage caused by inflammation and infection (15). Furthermore, cMaf regulates IL-10 expression in macrophages by directly binding to the IL-10 promoter (16).

Mammalian target of rapamycin (mTOR) can form two distinct complexes, mTOR complex 1 (mTORC1) and mTORC2, although the kinase-specific inhibitor rapamycin mainly targets mTORC1, which controls translation mainly by phosphorylating the translation initiation factor eIF4E-binding protein 1 (4EBP-1) and the S6 ribosomal kinase 1 (S6K1) (17,18). Moreover, mTOR also takes part in controlling glycolysis by promoting the expression of the transcription factors HIF-1α and c-Myc (19,20), and upregulating the expression of nutrient transporters. mTOR also participates in the control of lipid synthesis by activating the transcription factor sterol regulatory element-binding protein (SREBP), as well as in the control of autophagy (21,22). However, the precise mechanisms by which mTOR responds to TLR4 signaling in macrophages remain to be identified.

In this study, we demonstrated that cMaf expression is induced following lipopolysaccharide (LPS) challenge, which suggests that cMaf functions in the TLR4 signaling

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Key words: endotoxin shock, inflammatory response, macrophage, cMaf, mammalian target of rapamycin, Toll-like receptor

pathway. Knockdown of cMaf expression in macrophages impaired IL-10 production, but increased the expression of IL-1, IL-6, IL-12 and tumor necrosis factor-α (TNF-α). A pathway analysis using inhibitors indicated that TLR4 signaling activated extracellular signal-related kinase (ERK) and phosphoinositide 3-kinase (PI3K), which phosphorylate mTOR and control the translation, but not transcription, of cMaf via the downstream kinases S6K1 and 4EBP-1, thereby regulating inflammatory cytokine production in macrophages. *In vivo*, altered TLR4 signaling resulted in pathological symptoms of alveolar damage and led to more endotoxin-induced death of cMaf-knockdown macrophages in mice.

Materials and methods

Animals. Wild-type mice (C57/BL6) were supplied by the Shanghai Laboratory Animal Center (Shanghai, China). Animals were treated humanely, and all animal experiments conformed to the recommendations of the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication no. 85-23, revised 1996), and the study was approved by the Ethics Committee of the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. Animal survival rates were calculated at 1, 2, 3, 4, 5, 6, 7 and 8 days after LPS injection.

For the animal survival study, the animal death was used as the clinical endpoint. All mice were monitored every 6 h for 7 days. Mice were anesthetized with ketamine followed by cervical dislocation when they were found in a moribund state which was characterized by labored breathing and/or no-responsiveness to cape tapping. At the end of the study, all surviving mice were anesthetized by ketamine overdose, followed by cervical dislocation.

Reagents and cells. Roswell Park Memorial Institute (RPMI)-1640 medium, fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Invitrogen (Shanghai, China). All signaling pathway inhibitors were obtained from Calbiochem (San Diego, CA, USA). RAW264.7 macrophages were purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured in RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin, and 100 U/ml streptomycin, and maintained in a humidified incubator with 5% CO_2 at 37˚C. For preparation of peritoneal macrophages, 2 ml of 4% thioglycollate medium (R&D Systems, Minneapolis, MN, USA) was injected into the peritoneal cavity of mice. After 3 days, mice were euthanized, and the peritoneal cavity was flushed twice with 6-8 ml of phosphate-buffered saline (PBS). Collected peritoneal cells were seeded in cell culture dishes containing complete RPMI-1640 medium (10% FBS). Non-adherent cells were washed off, and adherent cells were stimulated the following day. To generate bone marrow-derived macrophages (BMDMs), bone marrow cells were cultured in RPMI-1640 medium supplemented with 30% L929-conditioned medium (containing macrophage colony-stimulating factor [M-CSF]) and 10% FBS. On day 4, non-adherent cells were removed, and fresh RPMI-1640 medium supplemented with L929-conditoned medium was added. BMDMs were used on 7-10 days.

Quantitative real-time polymerase chain reaction (qPCR). TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to isolate total RNA from the target cells, and cDNA was generated using the ReverTra Ace® qPCR RT kit (Toyobo, Osaka, Japan). The SYBR® Premix Ex Taq™ kit (Takara, Shiga, Japan) and an ABI 7500 LightCycler (Applied Biosystems, Waltham, MA, USA) were used for the qPCR analysis. The following primer sequences were used: IL-1β forward, 5'-CAACCAACAAGTGATATTCTCCATG-3' and reverse, 5'-GATCCACACTCTCCAGCTGCA-3'; IL-6 forward, 5'-AGATAAGCTGGAGTCACAGAAGGAG-3' and reverse, 5'-CGCACTAGGTTTGCCGAGTA-3'; IL-10 forward, 5'-ATTTGAATTCCCTGGGTGAGAAG-3' and reverse, 5'-CACAGGGGAGAAATCGATGACA-3'; IL-12 forward, 5'-CACCCTTGCCCTCCTAAAC-3' and reverse, 5'-CACCTGGCAGGTCCAGAG-3'; TNF-α forward, 5'-GTCCCCAAAGGGATGAGAAGTT-3' and reverse, 5'-GTTTGCTACGACGTGGGCTACA-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward, 5'-TGGAGAAACCTGCCAAGTATGA-3' and reverse, 5'-CTGTTGAAGTCGCAGGAGACAA-3'; cMaf forward, 5'-CTGAGCCAAGATTCATGTATGGG-3' and reverse, 5'-CGTTGCACCATCCAAACAGT-3'.

RNA interference and transfection. RAW264.7 cells were seeded at a density of $1x10^6$ cells/well in 6-well plates and transfected with a control small interfering RNA (siRNA) or a cMaf siRNA using the Silencer® siRNA Transfection II kit (Ambion, Austin, TX, USA) according to the manufacturer's protocol. The siRNA sequences for mouse cMaf were designed and synthesized by GenePharma Co., Ltd. (Shanghai, China). The siRNA-1 and 2 sequences were 5'-ACAGCGAGCCCAACCUUAUUG-3' and 5'-CUAAUUCUUAGAGCUUCAUAU-3', respectively, and the control siRNA sequence was 5'-AAUGCCUACGUUAAGCUAUAC-3'.

Enzyme-linked immunosorbent assay (ELISA). IL-1, IL-6, IL-10, IL-12 and TNF-α levels in the cell culture supernatants were quantified using ELISA kits (R&D Systems) according to the manufacturer's instructions.

Western blotting. To prepare whole cell lysates, cells were washed three times with PBS and incubated in lysis buffer for 30 min on ice. After boiling in sodium dodecyl sulfate (SDS) loading buffer, equal amounts of protein were subjected to SDS-polyacrylamide gel electrophoresis, and transferred to a nitrocellulose membrane. Then, the membrane was blocked in 5% nonfat dried milk containing 0.1% Tween-20 for 1 h at room temperature, and incubated at 4˚C overnight with primary antibodies against p-S6 (#4858, 1:1,000 dilution), p-p70S6K (#9234, 1:1,000 dilution), p-mTOR (#5536, 1:1,000 dilution), p-4EBP1 (#9451, 1:1,000 dilution) (Cell Signaling Technology, Danvers, MA, USA). Anti-GAPDH antibody (#AP0063, 1:2,000 dilution) was obtained from Bioworld (Minneapolis, MN, USA). Anti-cMaf antibody (sc-7866, 1:500 dilution) was from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). The membrane was washed three times with PBS and further incubated with a horseradish peroxidase-conjugated secondary antibody for 2 h at room temperature. After a final wash, immunoreactive bands were visualized using the Scientific SuperSignal West Pico Chemiluminescent Substrate reagent (Pierce, Rockford, IL, USA).

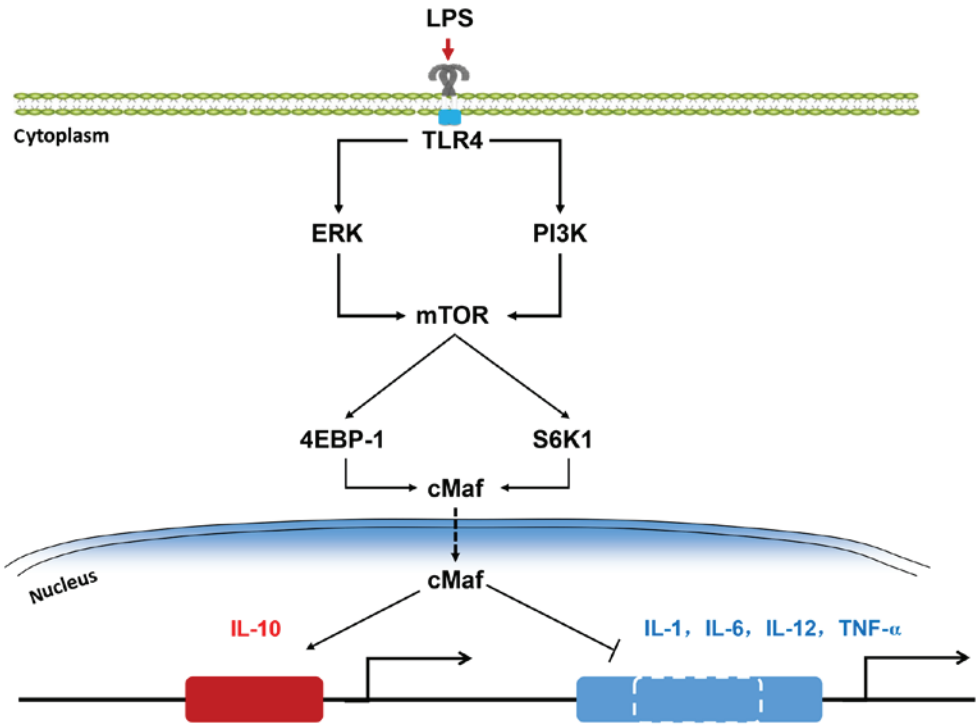

Figure 1. Diagram depicting hypothesized signaling pathways that participate in the upregulation of cMaf in lipopolysaccharide (LPS)-stimulated macrophages.

Lentivirus and infection. To stably express the cMaf siRNAs in BMDMs, pLKO.1-GFP or pLKO.1-puro plasmids expressing small hairpin RNAs (shRNAs) targeting mouse cMaf were transiently transfected into human embryonic kidney 293T cells, together with packaging plasmids (psPAX2 and pMD2G), and virus-containing media were harvested at 48 h. Supernatants containing shRNA-expressing lentiviruses were added to BMDMs at day 3. At 24 h, the medium was removed and fresh RPMI-1640 medium containing 2 μg/ml puromycin was added. After selection for 4-6 days, positive BMDMs were enriched and replated for LPS stimulation. The two shRNA targeting sequences for cMaf (cMaf-1, 5'-AGGAACTGAAGTACGTGATTC-3'; cMaf-2, 5'-GTGGTTCAGAGGATCCTTAAA-3') were from Sigma-Aldrich (St. Louis, MO, USA) TRC libraries (TRCN0000346430 and TRCN0000376317). BMDMs infected with green fluorescent protein (GFP)-expressing lentiviruses were detected by laser confocal microscope, and the knockdown efficiency was determined by immunoblotting using the anti-cMaf antibody.

In vivo knockdown and depletion of macrophages. Procedures for delivering viruses encoding shRNAs into mice and depleting cMaf in macrophages were performed as described previously (23).

Histological examinations. Tissue samples were collected 72 h after endotoxin shock and immediately fixed in 4% paraformaldehyde for 24 h. Then, tissue sections were embedded in paraffin, cut into 4-5 μm sections, and stained with hematoxylin and eosin. Two experienced pathologists who were blinded to the protocol performed the histological examinations. To grade the degree of lung injury, a scoring system was used based on the following histological features: edema, hyperemia and congestion, neutrophil margination and tissue infiltration, intra-alveolar hemorrhage and debris, and cellular hyperplasia. Each feature was graded as absent, mild, moderate, or severe, with a score of 0 to 3, respectively, and total scores were calculated for each animal.

Statistical analysis. Statistically significant differences between groups were determined by two-tailed Student's t-test and two-way analysis of variance (ANOVA). Survival was analyzed with the log-rank test. GraphPad Prism version 5.0 software was used for all analyses. $P<0.05$ was considered statistically significant and $P<0.01$ as highly significant.

Results

cMaf regulates inflammatory cytokine production in macrophages upon TLR4 activation. To understand the functions of cMaf in inflammatory responses in macrophages, we first tested whether LPS directly affects cMaf expression in three typical macrophage types (peritoneal macrophages, BMDMs, and RAW64.7 cells). Upon treatment with LPS for 0, 1, 2 and 4 h, there was a time-dependent increase of cMaf expression in the three different macrophage types, as determined by western blotting (Fig. 2A-C). Furthermore, we explored whether this increased cMaf expression accounted for the LPS-induced inflammatory response. First, we silenced endogenously expressed cMaf in RAW264.7 cells with two specific siRNAs, and western blot results verified that the expression of endogenous cMaf was obviously reduced (Fig. 2D). The supernatants of RAW264.7 cells with different treatment were collected and inflammatory cytokines were detected by ELISAs. The results showed that inflammatory cytokines, such as IL-1, IL-6, IL-10, IL-12 and TNF-α, were significantly induced by LPS. However,

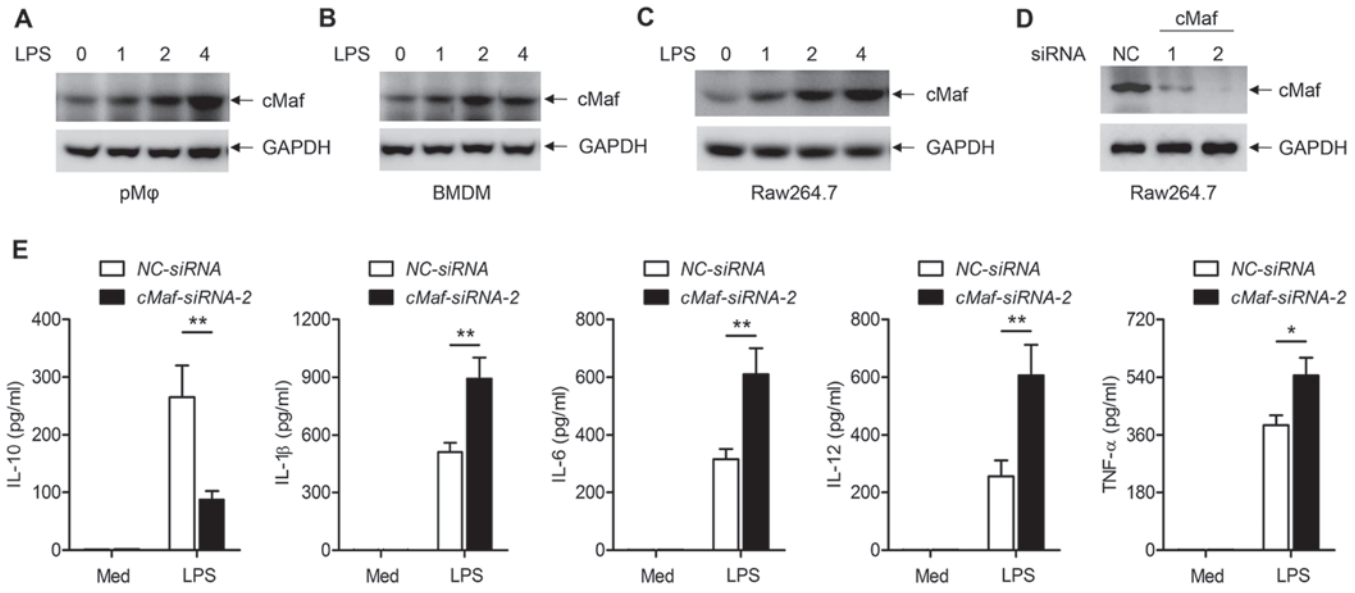

Figure 2. cMaf regulates inflammatory cytokine production in macrophages upon Toll-like receptor 4 (TLR4) activation. (A-C) Immunoblot analyses of total cMaf in the whole lysates of peritoneal macrophages (pMΦ) (A), bone marrow-derived macrophages (BMDMs) (B), and RAW264.7 cells (C) stimulated with 100 ng/ml lipopolysaccharide (LPS) for the indicated times. (D) Immunoblot analysis of total cMaf in RAW264.7 cells transfected with a negative control or two distinct cMaf small interfering RNAs (siRNAs). (E) Enzyme-linked immunosorbent assay (ELISA) analyses of interleukin-1 (IL-1), IL-6, IL-10, IL-12 and tumor necrosis factor-α (TNF-α) in the supernatants of LPS-treated RAW264.7 cells [transfected with siRNAs as in (A-D)] for 24 h. *P<0.05; **P<0.01 (two-tailed Student's t-test). Data are representative of three independent experiments with similar results (E).

knockdown of cMaf in peritoneal macrophages impaired the production of IL-10, but increased the LPS-mediated production of IL-1, IL-6, IL-12 and TNF-α (Fig. 2E). These data show that LPS-induced cMaf regulates inflammatory cytokine production in macrophages.

ERK1/2 and PI3K phosphorylate mTOR to regulate the translation of cMaf. To analyze the regulation of cMaf in TLR4-stimulated macrophages, the inhibitors SP600125, U0126, SB203580, wortmannin, PF4708671, rapamycin and SB216763 were used to determine the involvement of Jun-kinase (JNK), ERK1/2, p38 mitogen-activated protein kinase, PI3K, S6K1, mTOR and glycogen synthase kinase, respectively, in LPS-stimulated cMaf expression. Interestingly, we found that pretreatment of BMDMs with U0126, wortmannin, or rapamycin significantly reduced LPS-induced cMaf expression; however, cMaf expression remained unaltered in infected macrophages that were pretreated with the other inhibitors (Fig. 3A).

It is known that rapamycin inhibits mTOR. To further validate that the mTOR pathway is involved in LPS-induced cMaf expression, we treated BMDMs with rapamycin for 0, 1, 2 and 4 h. As shown in Fig. 3B, rapamycin strongly inhibited the phosphorylation of 4EBP1 and S6K1, which are downstream of mTOR, and western blot analyses showed that blocking the mTOR pathway significantly inhibited LPS-induced cMaf expression (Fig. 3B). However, qPCR analyses showed that cMaf mRNA remained unaltered in infected macrophages that were pretreated with rapamycin as well as U0126 or wortmannin (Fig. 3C).

Furthermore, the results showed that U0126 and wortmannin also reduced LPS-mediated cMaf expression (Fig. 3A). To investigate the roles of the PI3K and ERK pathways in LPS-induced mTOR activation, wortmannin and U0126 were used to inhibit the activation of PI3K and ERK, respectively. As shown in Fig. 2D and E, LPS-mediated mTOR phosphorylation was significantly inhibited by both U026 and wortmannin. These data suggest that ERK1/2 and PI3K phosphorylate mTOR to regulate cMaf expression at the translational level.

mTOR regulates inflammatory gene expression in LPS-stimulated macrophages. Our prior results indicated that the activation of mTOR is involved in LPS-induced cMaf expression. Next, we assessed whether mTOR regulates inflammatory gene expression in LPS-induced macrophages. A qPCR analysis showed that the inhibition of mTOR by rapamycin impaired the production of IL-10 in LPS-treated BMDMs, but increased the production of IL-1, IL-6, IL-12 and TNF-α (Fig. 4).

mTOR regulates inflammatory cytokine production via cMaf in LPS-stimulated macrophages. To further verify the importance of cMaf in the mTOR-mediated regulation of inflammatory cytokines in LPS-stimulated macrophages, BMDMs were stably transfected with recombinant lentiviruses expressing a control GFP shRNA or two distinct cMaf shRNA. The lentiviral transfection and protein knockdown efficiency in BMDMs stably expressing the control or cMaf shRNAs was verified by laser confocal microscope (Fig. 5A) and western blotting in Fig. 5B. Next, we determined whether depleting cMaf could neutralize the effect of rapamycin on the TLR4 signaling pathway. ELISA analyses showed that the production of these cytokines was mostly unaffected when cMaf expression was knocked down in BMDMs (Fig. 5C-G). Together, these results demonstrate that mTOR inhibits the production of inflammatory cytokines and increases IL-10 production via cMaf.

cMaf-knockdown mice are more susceptible to endotoxin shock. To verify our mechanistic understanding of cMaf

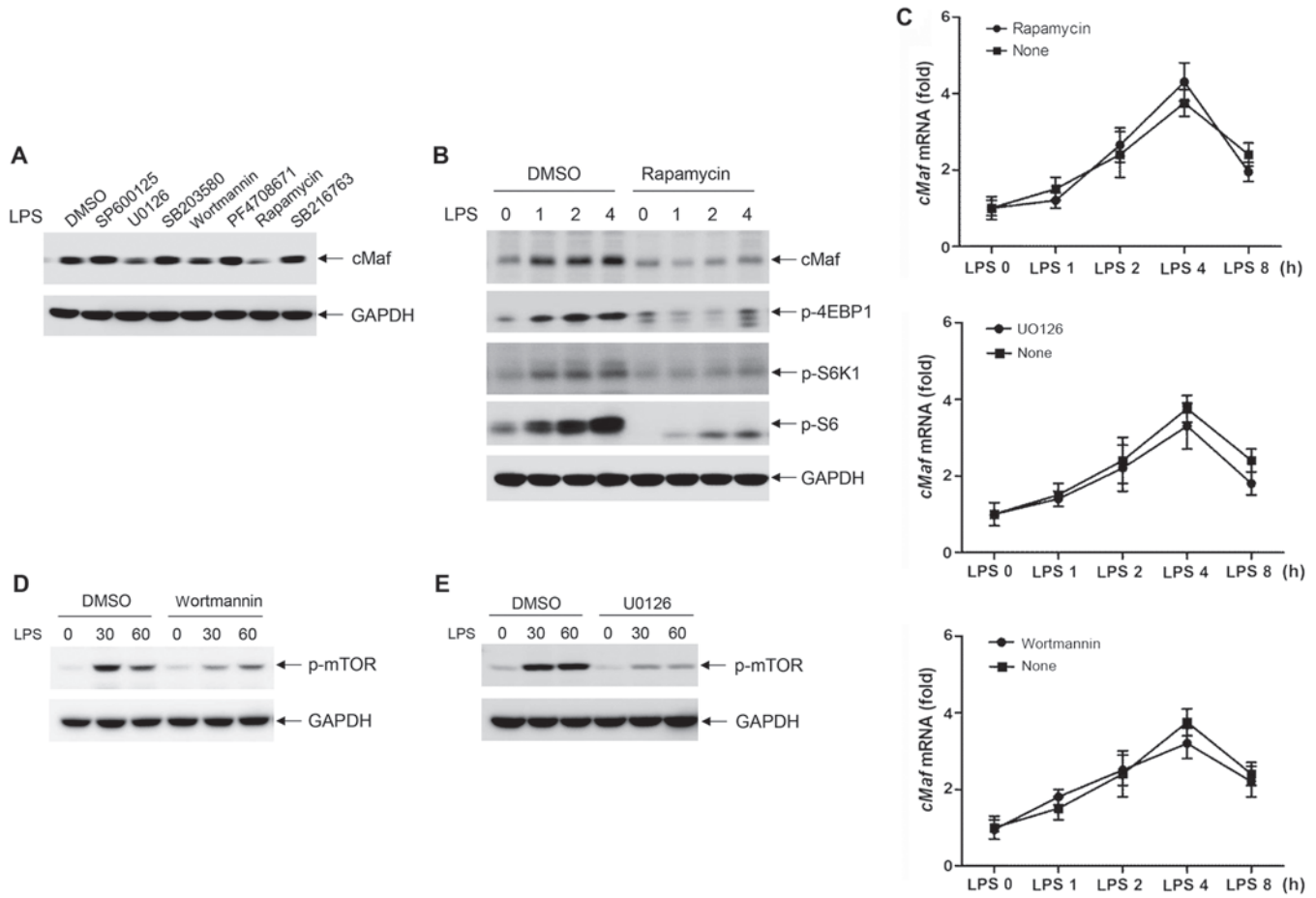

Figure 3. Extracellular signal-related kinase 1/2 (ERK1/2) and PI3K phosphorylate mTOR and regulate cMaf translation. (A) Immunoblot analysis of total cMaf in the whole lysates of bone marrow-derived macrophages (BMDMs) that were pretreated with various inhibitors (20 μM SP600125, 5 μM U0126, 20 μM SB203580, 200 nM wortmanin, 200 nM RF4708671, 100 nM rapamycin, 20 μM SB216763) for 30 min, followed by treatment for 1 h with 100 ng/ml lipopolysaccharide (LPS). (B) Immunoblot analyses of total or phosphorylated signaling proteins in the whole lysates of BMDMs that were pretreated with dimethyl sulfoxide or 100 nM rapamycin inhibitors for 30 min, followed by treatment with 100 ng/ml LPS for the indicated times. (C) qPCR analysis of cMaf mRNA in BMDMs that were treated as in (B). (D-E) Immunoblot analysis of phosphorylated mTOR in the whole lysates of peritoneal macrophages (pMΦ) that were pretreated with dimethyl sulfoxide and 200 nM wortmannin (D) or 5 μM U0126 (E) for 30 min, followed by treatment with 100 ng/ml LPS for the indicated times.

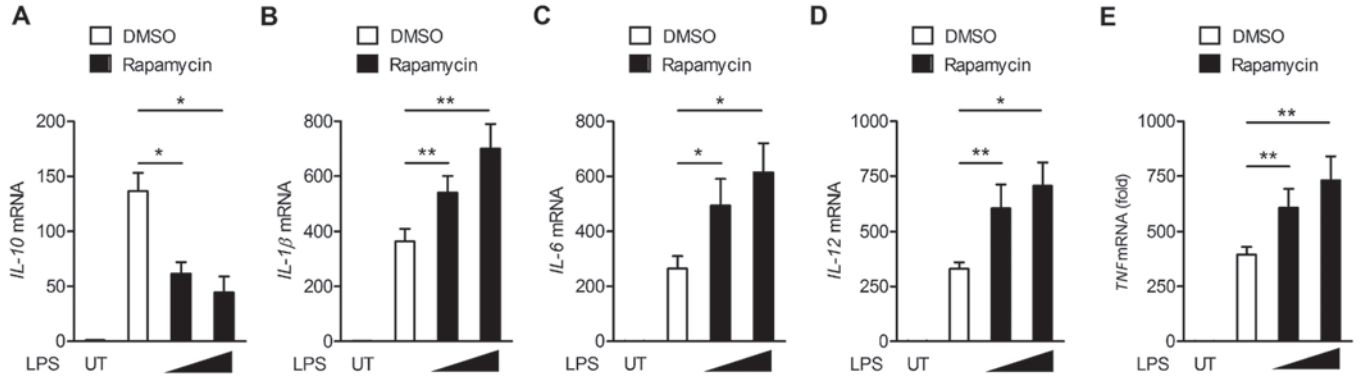

Figure 4. mTOR regulates inflammatory gene expression in lipopolysaccharide (LPS)-induced macrophages. (A-E) Enzyme-linked immunosorbent assay (ELISA) analyses of interleukin-1 (IL-1), IL-6, IL-10, IL-12 and tumor necrosis factor-α (TNF-α) in the supernatants of LPS-stimulated bone marrow-derived macrophages (BMDMs) that were pretreated with rapamycin for 30 min, followed by treatment (or not) with 100 ng/ml LPS for 24 h. *P<0.05; **P<0.01 (two-tailed Student's t-test). Data are representative of three independent experiments with similar results (A-E).

in vivo, we knocked down cMaf expression in mice by injecting a cMaf-specific shRNA, and we subjected the mice to septic shock. Mice in which cMaf expression was knocked down (cMaf-KD mice) displayed more severe lung injuries than did mice that underwent a mock knockdown of cMaf (mock mice) 3 days after acute challenge with LPS (Fig. 6A). Accordingly, the survival rate of LPS-challenged cMaf-KD mice was significantly lower than that of their LPS-treated mock

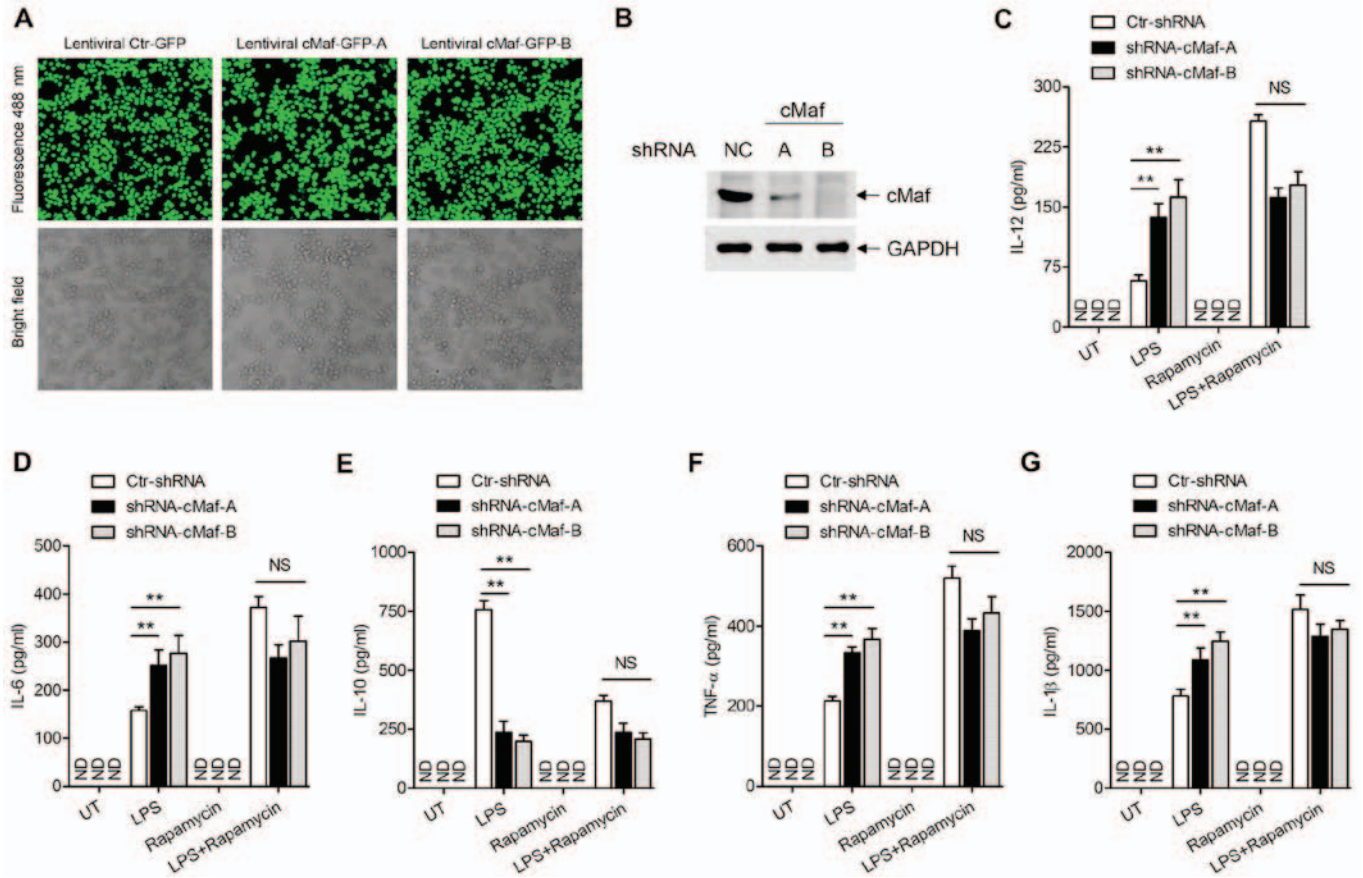

Figure 5. mTOR regulates inflammatory cytokine production via cMaf in lipopolysaccharide (LPS)-induced macrophages. (A) Laser confocal microscope at 488 nm or bright-field microscopy of bone marrow-derived macrophages (BMDMs) that were stably transfected with a control small hairpin RNA (shRNA) targeting GFP or two distinct cMaf shRNAs. (B) Immunoblot analysis of total cMaf in BMDMs that were stably transfected with the control or cMaf shRNAs. (C-G) Enzyme-linked immunosorbent assay (ELISA) analyses of interleukin-1 (IL-1), IL-6, IL-10, IL-12 and tumor necrosis factor-α (TNF-α) in the supernatants of BMDMs that were stably transduced with control or two distinct cMaf shRNAs, pretreated with dimethyl sulfoxide or 100 nM rapamycin, followed by treatment with 100 ng/ml LPS for 24 h. *P<0.05; **P<0.01; NS, not significant (two-tailed Student's t-test). Data are representative of three independent experiments with similar results (C-G).

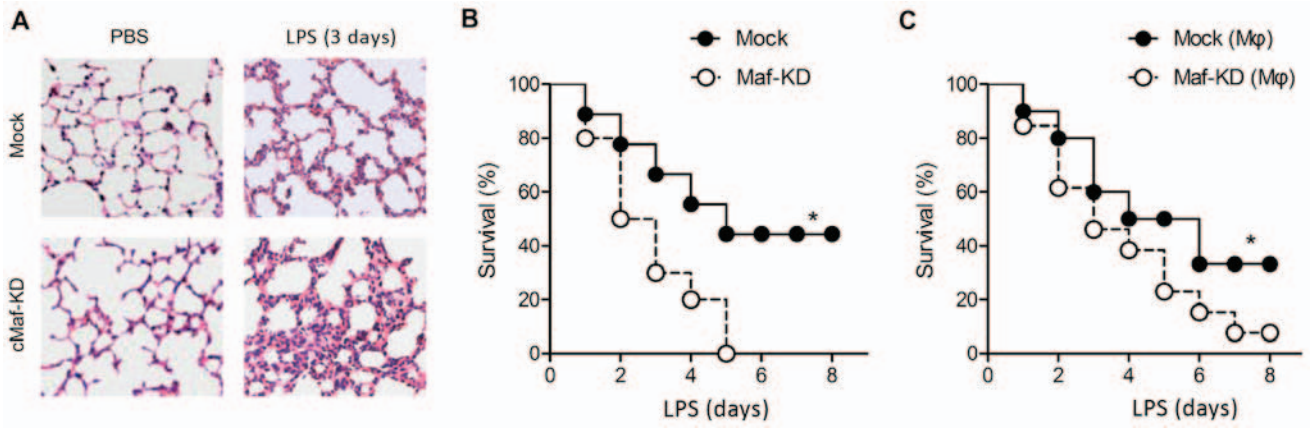

Figure 6. cMaf-knockdown mice are more susceptible to endotoxin shock. (A) Hematoxylin and eosin staining of sections of lungs from mock and cMaf-KD mice (n=5/group) that were treated with saline or stimulated for 4 days with lipopolysaccharide (LPS). Scale bars, 20 μm. (B) Survival of mock and cMaf-KD mice (n=15/group) that were treated for various times (horizontal axis) with LPS. P=0.0192 (log-rank test). (C) Survival of mice (n=20/group) that were given an adoptive transfer of macrophages that were treated with a control small hairpin RNA (shRNA) [Mock (MΦ)] or a cMaf-specific shRNA [cMaf-KD (MΦ)] and challenged with LPS as in (B). P=0.0235 (log-rank test). Data are representative of at least two experiments.

counterparts. Administration of LPS caused a 50% mortality rate within 8 days in the mock mice, whereas cMaf-KD mice were more susceptible to endotoxin shock, as evidenced by the almost 100% mortality rate within 8 days (Fig. 6B). These results are consistent with the cytokine production results in cMaf-KD macrophages (Fig. 2E). To further investigate the importance of cMaf in macrophages, we performed an adoptive cell transfer in an experimental model in which cMaf

expression was knocked down in the donor cells through the use of a cMaf shRNA. Upon LPS challenge, the survival rate of mice that received macrophages that were treated with a control shRNA with a scrambled sequence was 36%, while that of mice that received macrophages that were treated with cMaf-specific shRNA was only 5% (Fig. 6C). These results indicate that the effect of cMaf on the inflammatory response is largely mediated via macrophages.

Discussion

Aberrant inflammatory responses can cause unexpected damage to a host. mTOR functions as a key signal transducer for TLR-mediated inflammation. Subtle regulation of mTOR activity is crucial for maintaining immunological homeostasis. Here, our results show that cMaf, a leucine-zipper transcription factor, is targeted by mTOR in TLR4 signaling, and that cMaf primes TLR4-related inflammatory responses in macrophages.

In the present study, we observed the inducible expression of cMaf in different macrophages (peritoneal macrophages, BMDMs and RAW294.7 cells) following short-term LPS stimulation. As reported previously, cMaf expression also could be induced by cytokines such as IL-10, M-CSF, or TGF-β, which are expressed in T cells and macrophages (24).

Although PI3K and ERK have been implicated in IL-10 gene expression (25), the underlying mechanisms are not fully understood. Here, we first showed that the expression of the transcription factor cMaf was controlled by mTOR at the translational level, and that cMaf converged with upstream ERK and PI3K signaling pathways in response to TLR4 stimulation. cMaf is reported to bind to the IL-10 gene promoter, which induces IL-10 expression in macrophages (16). Our data showed that blocking ERK, PI3K and mTOR with inhibitors dramatically downregulated IL-10 expression both at the mRNA and protein levels. This confirmed the phenotypes of cMaf-KD macrophage upon LPS stimulation, which is consistent with previously reported results. Interestingly, cMaf-KD macrophages also exhibited upregulated IL-1, IL-6, IL-12 and TNF-α expression, which is consistent with the inhibition of mTOR. The most probable reason for this may be the inhibition of IL-10 signaling. IL-10 utilizes the IL-10 receptor, and the adaptor Janus kinase phoshorylates signal transducer and activator of transcription 3 (STAT3) at tyrosine 705. STAT3 functions as a repressor that limits inflammatory gene expression via feedback regulation, as was reported previously (25-28). Further studies are required to measure the expression of inflammatory genes in IL-10 knockout macrophages. Alternatively, cMaf functions as a dual regulator by assembling into complexes with different partners.

Together, our study showed that cMaf expression was induced in macrophages in response to LPS challenge, and the fact that cMaf protected mice from septic shock indicated that cMaf may improve host fitness, thereby enabling the survival of certain infectious diseases. Nevertheless, an analysis of the expression of inflammatory cytokines in cMaf-KD macrophages indicated that cMaf-mediated inhibition of inflammatory damage mostly depended on macrophages. A signaling pathway analysis indicated the existence of an ERK- or PI3K-driven mTOR-cMaf regulatory axis (Fig. 1). Previous studies showed that the Ras-ERK and PI3K pathways are related with cellular growth or migration, and that macrophages have been linked to tumor genesis as a result of altered immune system function (23). Therefore, we speculate that cMaf may also function in regulating tumor-related inflammatory responses via macrophages. Overall, the study of the combined functions of cMaf and mTOR in macrophage-related inflammation provide new insights into the search of new drug targets for acute inflammatory response.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and material

All the data and material in the manuscript are fully available without restriction.

Authors' contributions

CS conceived and designed the experiment. YW and CL performed the experiment. GZ analyzed the data. All authors read and approved the manuscript.

Ethics approval and consent to participate

Approved by Ethics Committee of the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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