Am J Clin Exp Immunol 2025;14(2):86-95
www.ajcei.us /ISSN:2164-7712/AJCEI0160236

Original Article

BMAL1 deficiency in macrophages exacerbates sepsis-induced inflammatory response and organ damage by regulating PGC-1α

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Received August 31, 2024; Accepted April 5, 2025; April 15, 2025; Published April 30, 2025

Abstract: BMAL1 is a core gene involved in the regulation of circadian rhythm; however, its role in sepsis remains incompletely understood. In this study, we investigated the molecular mechanisms by which BMAL1 influences sepsis. Sepsis models were established both in vivo using C57BL/6J mice and in vitro using THP-1-derived macrophages. We observed a significant downregulation of BMAL1 expression in peritoneal macrophages and hepatic Kupffer cells during sepsis. Overexpression of BMAL1 in macrophages via plasmid transfection suppressed LPS-induced inflammatory responses and promoted M2 macrophage polarization. Conversely, administration of STL1267, a BMAL1 inhibitor, reduced BMAL1 expression in mice and further exacerbated systemic inflammation and multi-organ injury. Moreover, we identified PGC-1α as a key downstream effector of BMAL1. Knockdown of PGC-1α using short hairpin RNA (shRNA) abrogated BMAL1-mediated anti-inflammatory effects. Collectively, these findings uncover a novel mechanism by which BMAL1 regulates acute inflammatory responses and organ damage in sepsis, highlighting its potential as a therapeutic target.

Keywords: BMAL1, PGC-1α, macrophages, sepsis

Introduction

Sepsis remains a major global public health challenge [1], with an estimated 50 million new cases annually and approximately 10 million sepsis-related deaths, accounting for nearly 20% of all global mortality [2]. Understanding the mechanisms underlying the development of sepsis is therefore critical for improving its prevention and treatment. Macrophages play a central role in orchestrating the inflammatory response during sepsis [3]. Our previous research demonstrated that macrophage immune tolerance can effectively mitigate sepsis progression. For instance, TEPP-46-induced tetramerization of pyruvate kinase M2 (PKM2) promotes mitochondrial biogenesis, inducing endotoxin tolerance in macrophages and thereby attenuating sepsis development [4]. Similarly, signal transducer and activator of transcription 5a (STAT5a) has been shown to suppress macrophage pyroptosis by negatively regulating nuclear factor-κB (NF-κB) signaling, promoting immune tolerance and inhibiting LPS-induced inflammation [5].

Emerging evidence indicates that circadian rhythm disruptions in leukocyte and cytokine levels are common in patients with severe trauma, and early circadian rhythm disturbances are closely associated with the onset and severity of sepsis [6]. Although a relationship between circadian rhythm and sepsis has been suggested, the underlying mechanisms remain unclear [7]. The circadian rhythm, maintained by an autoregulatory transcription-translation feedback loop, relies on the rhythmic expression of core circadian clock proteins [8]. Among them, BMAL1 (Brain and Muscle ARNT-Like 1) is a pivotal component, and its deletion results in a complete loss of circadian rhythmicity [9]. BMAL1 forms a heterodimer with CLOCK, which binds to E-box elements across the genome to regulate the expression of

https://doi.org/10.62347/QCMB2857

numerous clock-controlled genes (CCGs) [10]. Beyond circadian regulation, BMAL1 also contributes to key physiological and pathological processes, including tumor biology, inflammation, and aging [11, 12]. For example, BMAL1 and CLOCK cooperate to facilitate DNA double-strand break repair and enhance resistance to chemotherapy in tumors [13]. In the central nervous system, myeloid-specific BMAL1 deficiency disrupts microglial synaptic pruning and accelerates cognitive aging [14].

However, whether BMAL1 plays a regulatory role in macrophage-mediated inflammatory responses during sepsis has not been clearly defined. In this study, we explored the functional role and underlying mechanisms of BMAL1 in sepsis by conducting both in vitro and in vivo experiments. Our findings provide novel theoretical insights into the role of BMAL1 in regulating acute inflammatory responses and may contribute to the development of new therapeutic strategies for severe infectious diseases such as sepsis.

Methods and materials

Reagents

LPS (L9641) was purchased from Sigma-Aldrich. Mouse TNF-α (EK0527) and IL-6 (EK-0411) enzyme-linked immunosorbent assay (ELISA) kits were obtained from Boster Biological Technology. Plasmids and lentiviruses carrying shRNAs were procured from GenePharma (Shanghai) and GeneChem (Shanghai), respectively. Anti-PGC-1α (#2178, 1:1,000) and anti-β-actin (#4967, 1:1,000) antibodies were purchased from Cell Signaling Technology, while the anti-BMAL1 antibody (ab230822, 1:1,000) was obtained from Abcam. Goat anti-Mouse IgG (A21010, 1:10,000) and Goat anti-Rabbit IgG (A21120, 1:10,000) secondary antibodies were acquired from Abbkine. The BMAL1 inhibitor STL1267 (HY-148711) was purchased from MedChemExpress. Primers used for RT-qPCR were synthesized by Sangon Biotech (Shanghai).

Cell culture and transfection

The THP-1 human monocytic cell line was obtained from the American Type Culture Collection (ATCC). Peritoneal macrophages (PMs) and Kupffer cells (KCs) were isolated from C57BL/6J mice as previously described [4]. All cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco Life Technologies), supplemented with 10% fetal bovine serum (FBS) (PAN-Biotechnology) and 1% penicillin-streptomycin (Beyotime Biotechnology), and maintained at 37°C in a humidified incubator with 5% CO_2. BMAL1-overexpressing plasmids were constructed by GenePharma (Shanghai). Plasmid transfection into THP-1 cells was carried out following our previously published protocol [15]. Briefly, plasmids were transformed into *Escherichia coli* and amplified through large-scale bacterial culture. Plasmid DNA was then extracted and purified, and subsequently transfected into THP-1 cells. Lentiviral particles carrying shRNAs targeting PGC-1α or control sequences were generated by GeneChem (Shanghai). Lentiviral transduction was performed according to the manufacturer's instructions. For shRNA delivery, 2×10^5 THP-1 cells were seeded in 2 mL of culture medium containing 10 μg/mL polybrene (GeneChem) and infected with 2 μL of lentiviral solution (MOI = 30). After 72 hours, transduction efficiency was assessed using western blotting and RT-qPCR.

Acute septic animal model and macrophage inflammation model

To establish an acute sepsis animal model, male C57BL/6J mice (6-8 weeks old, 22-25 g) were intraperitoneally injected with lipopolysaccharide (LPS, 4 mg/kg body weight) or an equal volume of saline as a control. Peritoneal macrophages (PMs), Kupffer cells (KCs), serum, and tissues including the liver, spleen, and lungs were collected 6 hours after LPS administration. Survival was monitored hourly for 24 hours following LPS injection. All animals were obtained from the Experimental Animal Center of Chongqing Medical University. Mice were housed in groups of five per cage under standard laboratory conditions (12-hour light/dark cycle, temperature-controlled environment, 50% humidity) with ad libitum access to standard rodent chow and water. All animal procedures were conducted in accordance with ethical standards and approved by the Ethics Committee of Chongqing University Three Gorges Hospital. For the in vitro macrophage inflammation model, THP-1 cells were

stimulated with 150 ng/mL LPS to induce an inflammatory response.

Western blotting

Western blotting was performed as previously described [4]. Briefly, cells were harvested and lysed using a whole protein extraction kit (KeyGen Biotech). Protein concentrations were determined with a BCA Protein Assay Kit (Beyotime Biotechnology) according to the manufacturer's instructions. Protein samples were mixed with loading buffer and denatured by boiling at 100°C for 10 minutes. Equal amounts of protein (10-20 μg) were separated by SDS-PAGE on 10% polyacrylamide gels (Beyotime Biotechnology) and then transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore).

Membranes were blocked with 5% bovine serum albumin (BSA) for 1 hour at room temperature, followed by overnight incubation at 4°C with primary antibodies against BMAL1 and PGC-1α. After washing, membranes were incubated with appropriate HRP-conjugated secondary antibodies at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection reagent (MedChemExpress), with exposure times of 3-5 seconds. Band intensities were quantified using Quantity One software (Bio-Rad).

RT-qPCR

Real-time reverse transcription polymerase chain reaction (RT-qPCR) was performed to evaluate gene expression levels in macrophages, following procedures described previously [4]. Briefly, total RNA was extracted from macrophages using TRIzol® reagent (Invitrogen), and reverse transcription was carried out using the PrimeScript™ RT Reagent Kit (TaKaRa Biotechnology) to synthesize complementary DNA (cDNA). Gene-specific primers were synthesized by Sangon Biotech (Shanghai). GAPDH was used as an internal control for normalization. Relative gene expression was calculated using the 2^-ΔΔCq method.

Primer sequences were as follows: Bmal1 Forward: 5'-TGACCCTCATGGAAGGTTAGAA-3', Reverse: 5'-GGACATTGCATTGCATGTTGG-3'; Pgc-1α Forward: 5'-TATGGAGTGACATAGAGTGT-GCT-3', Reverse: 5'-CCACTTCAATCCACCCAGAA-AG-3'; Tnf-α Forward: 5'-CCCTCACACTCAGAT-CATCTTCT-3', Reverse: 5'-GCTACGACGTGGGC-TACAG-3'; Arg1 Forward: 5'-CTCCAAGCCAAA-GTCCTTAGAG-3', Reverse: 5'-AGGAGCTGTCATT-AGGGACATC-3'; CD206 Forward: 5'-CTCTGTT-CAGCTATTGGACGC-3', Reverse: 5'-CGGAATTT-CTGGGATTCAGCTTC-3'; GAPDH Forward: 5'-AGGTCGGTGTGAACGGATTTG-3', Reverse: 5'-TGTAGACCATGTAGTTGAGGTCA-3'.

Hematoxylin-eosin (HE) staining

HE staining of the mouse liver, spleen, and lung tissues was performed by the Pathology Department of Chongqing Medical University.

Statistical analysis

All statistical analyses for RT-qPCR and ELISA data were performed using GraphPad Prism 5 software. Data are presented as the mean ± standard deviation (SD) from three or more independent biological replicates. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for comparisons among multiple groups. For comparisons between two groups, the Student's *t*-test was applied. A P value of < 0.05 was considered statistically significant.

Results

Decreased BMAL1 expression in septic mice

BMAL1 is a key gene involved in the regulation of the circadian rhythm [16]. The BMAL1 protein plays a crucial role in maintaining the body's circadian rhythm; however, its abnormal expression has been closely linked to tumorigenesis, aging, immune regulation, and inflammation [11]. To investigate whether BMAL1 expression is modulated by LPS exposure during the early stages of sepsis, we treated THP-1 macrophages with 150 ng/mL LPS (LPS group) or an equivalent volume of PBS (control group) for 24 hours. As expected, LPS treatment significantly reduced both the protein and mRNA expression levels of BMAL1 in THP-1 cells (**Figure 1A**, **1B**).

To determine whether similar effects occur in vivo, we established a mouse model of sepsis via intraperitoneal injection of LPS (4 mg/kg) for 4 hours or an equal volume of saline (control

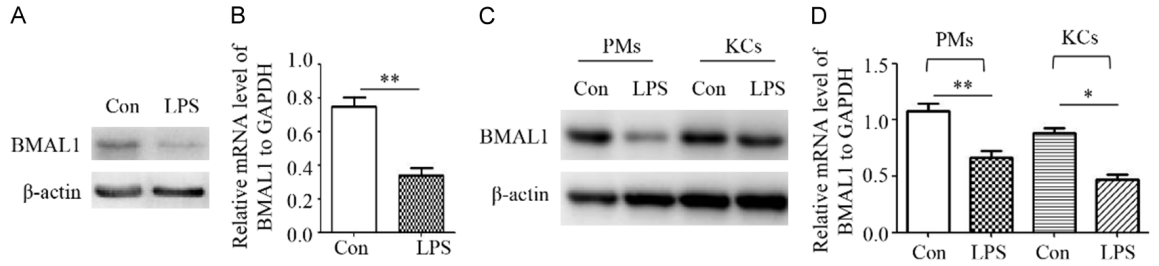

Figure 1. Expression of BMAL1 in macrophages. A. THP-1 macrophages were treated with 150 ng/ml of LPS for 24 h. The expression of the BMAL1 protein was assessed via western blotting. B. THP-1 macrophages were treated with 150 ng/ml of LPS for 24 h. The mRNA expression of BMAL1 was assessed via RT-qPCR. C and D. A mouse model of sepsis was established via intraperitoneal injection of LPS (4 mg/kg) for 4 h. PMs and KCs were isolated. The protein and mRNA expression of BMAL1 was detected via western blotting and RT-qPCR, respectively. Representative data from three separate experiments are shown. $*P < 0.05$, $**P < 0.01$.

group), followed by the isolation of peritoneal macrophages (PMs) and liver Kupffer cells (KCs). Consistent with the in vitro findings, BMAL1 expression at both the protein and mRNA levels was significantly reduced in PMs and KCs from the LPS-treated mice compared to controls (**Figure 1C**, **1D**). Collectively, these results confirm that BMAL1 expression in macrophages is markedly suppressed in both in vitro and in vivo models of sepsis.

Overexpression of BMAL1 inhibited the inflammatory response and promoted M2 polarization

To further investigate the regulatory effect of BMAL1 on the LPS-mediated inflammatory response in macrophages, we overexpressed BMAL1 in THP-1 cells via plasmid transfection. As shown in **Figure 2A** and **2B**, BMAL1 expression was significantly upregulated at both the mRNA and protein levels 72 hours post-transfection. Notably, overexpression of BMAL1 markedly suppressed the secretion of the proinflammatory cytokines IL-6 and TNF-α in LPS-stimulated THP-1 cells (**Figure 2C**), suggesting that BMAL1 plays a key inhibitory role in the inflammatory response triggered by LPS.

Given that macrophage polarization is closely associated with their inflammatory profile, we further examined the effect of BMAL1 overexpression on polarization markers. Consistent with our previous findings [17], LPS stimulation promoted M1-type polarization, as evidenced by increased TNF-α expression and reduced levels of M2-type markers (CD206, Arg-1). However, BMAL1 overexpression significantly reduced TNF-α expression while upregulating CD206 and Arg-1 levels compared to the LPS-only group (**Figure 2D**). These results indicate that BMAL1 not only suppresses the LPS-mediated inflammatory response but also promotes a shift from M1- to M2-type polarization in macrophages.

It is well established that the inflammatory response of macrophages is closely associated with their polarization status. In our previous study, we demonstrated that macrophages undergo M1-type polarization following LPS stimulation [17]. To further investigate the role of BMAL1 in macrophage polarization, we evaluated the expression levels of M1-type (TNF-α) and M2-type (CD206, Arg-1) polarization markers in THP-1 cells using ELISA. As expected, compared with the control group, LPS stimulation induced a shift toward M1-type polarization in THP-1 cells, accompanied by a decrease in M2-type marker expression.

However, in the BMAL1 overexpression group, TNF-α expression was significantly reduced, while the levels of CD206 and Arg-1 were markedly increased compared with the LPS group (**Figure 2D**). These findings suggest that BMAL1 overexpression not only inhibits the LPS-induced proinflammatory response but also promotes a shift toward M2-type polarization in macrophages.

BMAL1 deficiency aggravated multiple organ damage in septic mice

The results described above demonstrate that BMAL1 possesses anti-inflammatory properties. To further investigate the regulatory role of BMAL1 in septic mice, we utilized STL1267, a

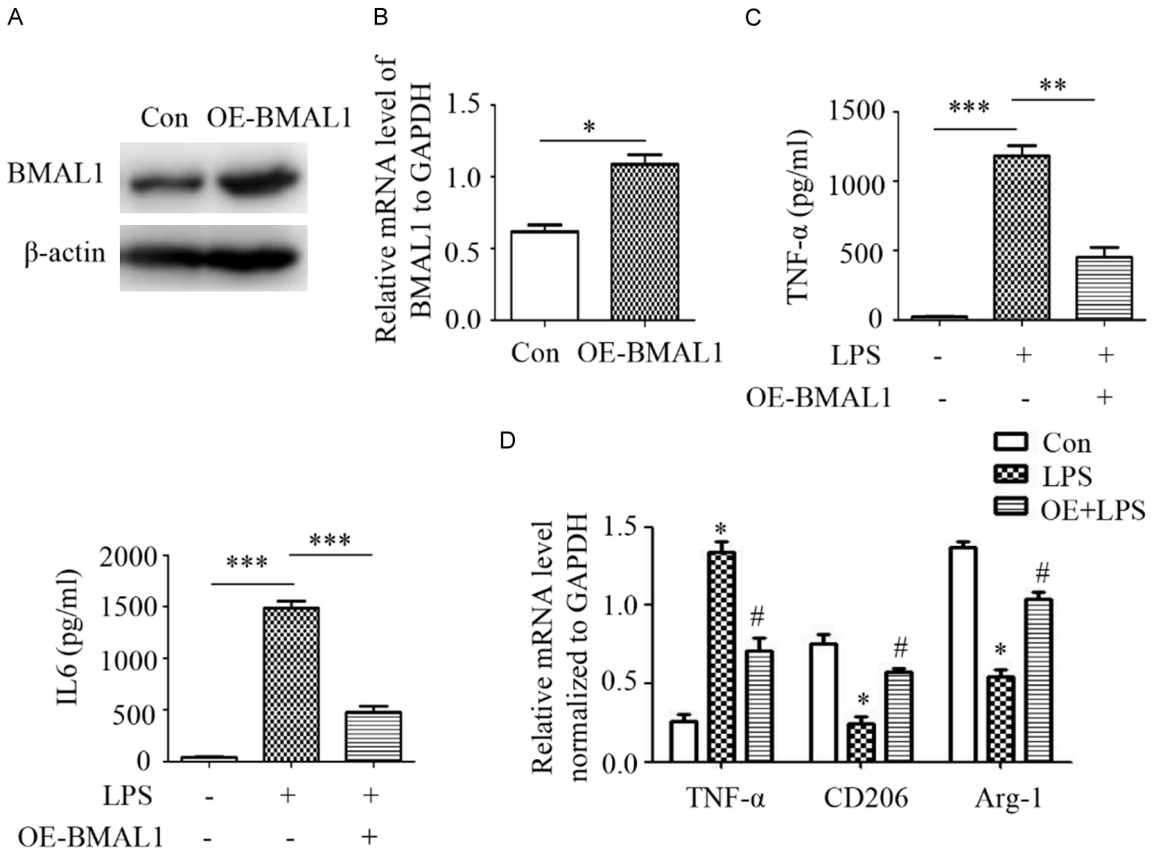

Figure 2. Overexpression of BMAL1 inhibited the inflammatory response *in vitro*. A and B. THP-1 cells were transfected with plasmid for 72 h. The protein and mRNA expression of BMAL1 was assessed via western blotting and RT-qPCR, respectively. C. After overexpressing BMAL1, cells were treated with 150 ng/ml of LPS for 24 h. The levels of the proinflammatory factors IL-6 and TNF-α in the supernatant were detected using ELISA. D. The polarization level of cells was detected via RT-qPCR. Representative data from three separate experiments are shown. *P < 0.05, **P < 0.01, ***P < 0.001.

selective inhibitor that suppresses BMAL1 expression in macrophages [18]. In this study, mice were administered STL1267 at a dose of 50 mg/kg via tail vein injection, while the control group received an equivalent volume of saline. As shown in **Figure 3A**, STL1267 treatment significantly reduced BMAL1 expression in both peritoneal macrophages (PMs) and Kupffer cells (KCs) compared with the control group, indicating effective silencing of BMAL1 in macrophages.

Moreover, inhibition of BMAL1 by STL1267 led to elevated levels of the proinflammatory cytokines IL-6 and TNF-α in the serum of septic mice (**Figure 3B**). Histological analysis using H&E staining further revealed that STL1267 treatment aggravated inflammatory injury in the liver, spleen, and lungs (**Figure 3C**). Additionally, STL1267 administration significantly reduced the survival rate of septic mice (**Figure 3D**).

PGC-1α is the key factor for the BMAL1-mediated regulation of sepsis

Although we demonstrated that BMAL1 exerts an anti-inflammatory effect and that BMAL1 deficiency exacerbates the inflammatory response and multiple organ damage in septic mice, the specific regulatory mechanism remains unclear. The peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1) family, particularly PGC-1α, consists of transcriptional coactivators initially identified through their interaction with nuclear receptor PPARγ in response to cold exposure [19]. Recent studies have reported that PGC-1α is involved in several inflammatory and metabolic diseases and plays a critical role in regulating

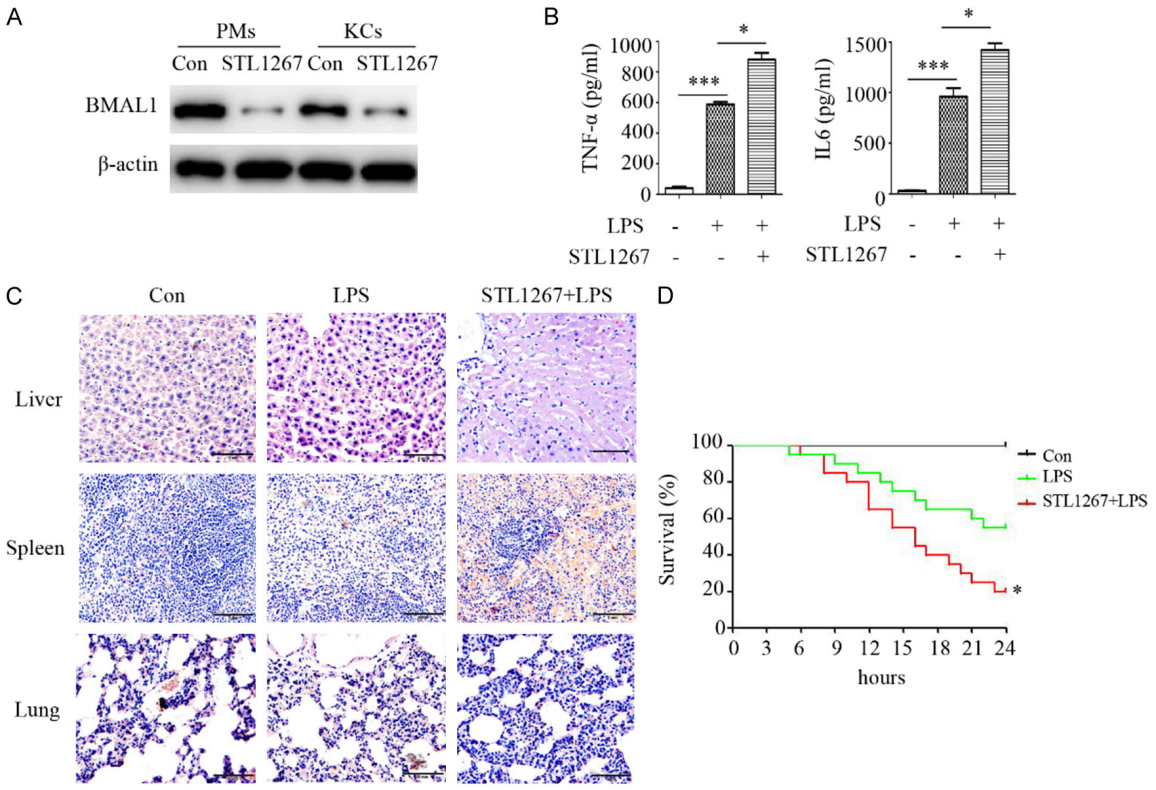

Figure 3. Inhibition of BMAL1 aggravated multiple organ damage in septic mice. A. STL1267 (50 mg/kg) was injected into mice via the tail vein. The protein expression of BMAL1 in PMs and KCs was assessed via western blotting. B. Mice were pretreated with STL1267, followed by the establishment of the sepsis model. IL-6 and TNF-α in the serum were detected using ELISA. C. HE staining was used to visualize pathological changes in the liver, spleen, and lung. D. Twenty-four-hour survival rate of mice. Representative data from three separate experiments are shown. $*P < 0.05$, $***P < 0.001$.

mitochondrial function, oxidative stress, and metabolic pathways across various tissues [20]. Furthermore, PGC-1α has been identified as a key downstream target of BMAL1 [21]. Based on this, we further investigated whether BMAL1 modulates the inflammatory response through the regulation of PGC-1α.

As shown in **Figure 4A**, PGC-1α expression in THP-1 cells was significantly downregulated upon LPS stimulation. However, overexpression of BMAL1 markedly attenuated the LPS-induced suppression of PGC-1α expression, suggesting that BMAL1 positively regulates PGC-1α and that PGC-1α functions downstream of BMAL1. Consistently, overexpression of BMAL1 significantly upregulated PGC-1α expression in THP-1 cells (**Figure 4B**).

To further examine the regulatory role of PGC-1α, we used shRNA to knock down its expression in THP-1 cells (**Figure 4C**, **4D**). Subsequently, BMAL1 was overexpressed, and a cellular sepsis model was established. We found that the anti-inflammatory effect of BMAL1 on LPS-induced macrophage activation was significantly diminished following PGC-1α knockdown (**Figure 4E**). These findings indicate that BMAL1 regulates the inflammatory response of macrophages during sepsis, at least in part, through PGC-1α.

Discussion

Sepsis is a common acute and severe infectious disease, and one of the leading causes of death among patients admitted to intensive care units (ICUs) [1]. It is characterized by an excessive inflammatory response mediated by immune cells during the early stages, followed by immune cell “paralysis” in the later stages, ultimately leading to life-threatening multiple organ failure [22]. Monocytes and macrophages are the primary immune cells involved in

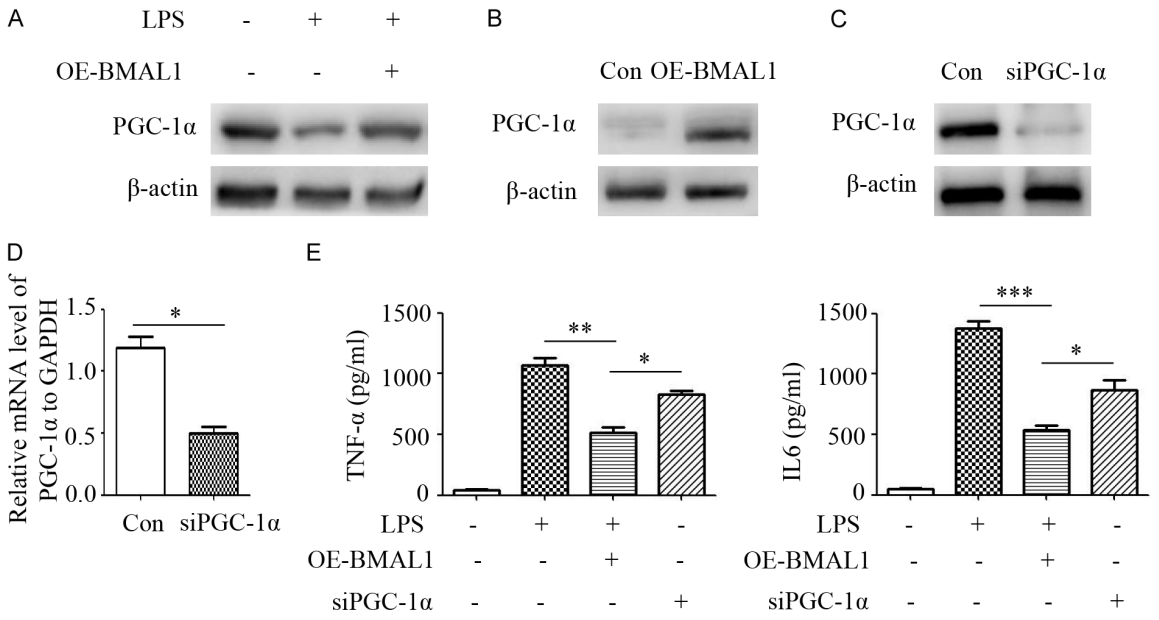

Figure 4. PGC-1α is the key regulator of BMAL1. A. THP-1 cells were overexpressed with BMAL1 and then treated with 150 ng/ml of LPS for 24 h. The protein expression of PGC-1α was assessed via western blotting. B. THP-1 cells were overexpressed with BMAL1. The protein expression of PGC-1α was assessed via western blotting. C and D. THP-1 cells were transfected with shPGC-1α for 72 h. The protein and mRNA expression of PGC-1α was assessed via western blotting and RT-qPCR, respectively. E. The levels of IL-6 and TNF-α in the supernatant were detected using ELISA. Representative data from three separate experiments are shown. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

mediating the inflammatory response in sepsis [23]. Recent studies have shown that the progression of sepsis is closely associated with autophagy, apoptosis, and mitochondrial biogenesis in monocytes and macrophages [24]. In this study, we focused primarily on the THP-1 macrophage cell line, as well as mouse primary peritoneal macrophages (PMs) and Kupffer cells (KCs).

The circadian rhythm is primarily regulated by core clock proteins such as BMAL1, CLOCK, CRY, PER, and REV-ERBα/β [25]. BMAL1 and CLOCK form heterodimers that positively regulate the transcription of *Per* and *Cry* genes [26]. The accumulated PER and CRY proteins, in turn, inhibit the binding of BMAL1/CLOCK heterodimers to E-box elements via negative feedback, thereby suppressing BMAL1/CLOCK-mediated transcription [25]. This negative feedback loop drives the rhythmic oscillation characteristic of the biological clock [25]. Clock proteins are involved in various physiological processes, including circadian rhythm regulation, tissue development, and organismal longevity [27, 28]. Disruption of these proteins has been closely linked to pathological conditions such as aging and tumorigenesis [11, 12].

Recent studies have confirmed that the circadian rhythm regulated by clock genes also plays a role in modulating inflammatory responses. For example, activation of the clock gene REV-ERBα in lung epithelial cells and fibroblasts has been shown to alleviate smoking-induced lung inflammation and epithelial-mesenchymal transition [29]. Conversely, the circadian rhythm of white blood cells and cytokines is often disrupted in patients with severe trauma; indeed, early circadian rhythm disturbances are closely associated with the onset and severity of sepsis [6]. BMAL1 is considered the most critical clock protein in circadian rhythm regulation. However, whether BMAL1 is involved in the pathogenesis of sepsis and the mechanisms underlying its potential regulatory role remain to be further explored.

In the present study, we found that both the protein and mRNA expression levels of BMAL1 in macrophages were significantly decreased in sepsis models. Overexpression of BMAL1 via plasmid transfection inhibited the LPS-

mediated inflammatory response and promoted M2-type polarization of macrophages. STL1267, a selective inhibitor of BMAL1, was used to investigate the in vivo effects of BMAL1 suppression. Tail vein injection of STL1267 to inhibit BMAL1 in macrophages exacerbated the inflammatory response and organ damage in septic mice. Overall, these findings suggest that BMAL1 exerts an anti-inflammatory effect in the context of sepsis.

PGC-1α is a member of the PGC-1 coactivator family and is currently recognized as being closely associated with cellular energy metabolism and the inflammatory response [19]. Upon stimulation by low-density lipoprotein (LDL), intracellular methyltransferase 3 (METTL3) promotes m6A methylation of PGC-1α by cooperating with the RNA methylation reader YTHDF2. This process enhances the degradation of PGC-1α, thereby triggering an inflammatory response [30]. Moreover, our previous research demonstrated that PGC-1α is a key regulator of endotoxin tolerance in Kupffer cells (KCs), as suppression of PGC-1α expression disrupts this tolerance and promotes inflammation [4].

Previous studies have confirmed that BMAL1 regulates mitochondrial homeostasis in renal ischemia-reperfusion injury through the SIRT1/PGC-1α axis, suggesting that PGC-1α acts as a key downstream effector of BMAL1 [31]. Based on this, we further investigated whether BMAL1 modulates the inflammatory response by regulating PGC-1α. In the LPS-induced sepsis model, we observed a reduction in PGC-1α expression in macrophages. Notably, silencing PGC-1α in macrophages using a lentiviral approach significantly attenuated the anti-inflammatory effect of BMAL1 overexpression. These findings suggest that the circadian clock protein BMAL1 regulates the inflammatory response and sepsis in macrophages, at least in part, via PGC-1α.

Our previous research has shown that PGC-1α plays a crucial role in regulating mitochondrial biogenesis [4]. Therefore, we further explored whether the BMAL1/PGC-1α axis mediates the macrophage inflammatory response and sepsis by influencing mitochondrial function. However, in the current study, we did not observe a clear regulatory effect of the BMAL1/PGC-1α axis on mitochondrial function in macrophages. The precise mechanism by which the BMAL1/PGC-1α axis modulates the inflammatory response in macrophages thus remains to be elucidated

Acknowledgements

This study was supported by the Natural Science Foundation of Chongqing (CSTB2022-NSCQ-BHX0711), Wanzhou District "Doctor Through Train" Project (wzstc-20220126), The National Natural Science Foundation of China (82302430) and Basic Key Projects of Chongqing University Three Gorges Hospital (2022YJKYXM-002).

Disclosure of conflict of interest

None.

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References

[1] Liu D, Huang SY, Sun JH, Zhang HC, Cai QL, Gao C, Li L, Cao J, Xu F, Zhou Y, Guan CX, Jin SW, Deng J, Fang XM, Jiang JX and Zeng L. Sepsis-induced immunosuppression: mechanisms, diagnosis and current treatment options. Mil Med Res 2022; 9: 56.

[2] Binnie A, Tsang JLY, Hu P, Carrasqueiro G, Castelo-Branco P and Dos Santos CC. Epigenetics of sepsis. Crit Care Med 2020; 48: 745-756.

[3] Venet F and Monneret G. Advances in the understanding and treatment of sepsis-induced immunosuppression. Nat Rev Nephrol 2018; 14: 121-137.

[4] Yi Z, Wu Y, Zhang W, Wang T, Gong J, Cheng Y and Miao C. Activator-mediated pyruvate kinase M2 activation contributes to endotoxin tolerance by promoting mitochondrial biogenesis. Front Immunol 2021; 11: 595316.

[5] Wang T, Zhong H, Zhang W, Wen J, Yi Z, Li P and Gong J. STAT5a induces endotoxin tolerance by alleviating pyroptosis in kupffer cells. Mol Immunol 2020; 122: 28-37.

[6] Coiffard B, Diallo AB, Culver A, Mezouar S, Hammad E, Vigne C, Nicolino-Brunet C, Dignat-George F, Baumstarck K, Boucekine M, Leone M and Mege JL. Circadian rhythm disruption and sepsis in severe trauma patients. Shock 2019; 52: 29-36.

[7] Mul Fedele ML, Senna CA, Aiello I, Golombek DA and Paladino N. Circadian rhythms in bacterial sepsis pathology: what we know and

what we should know. Front Cell Infect Microbiol 2021; 11: 773181.
[8] Vasey C, McBride J and Penta K. Circadian rhythm dysregulation and restoration: the role of melatonin. Nutrients 2021; 13: 3480.
[9] Xie M, Tang Q, Nie J, Zhang C, Zhou X, Yu S, Sun J, Cheng X, Dong N, Hu Y and Chen L. BMAL1-downregulation aggravates porphyromonas gingivalis-induced atherosclerosis by encouraging oxidative stress. Circ Res 2020; 126: e15-e29.
[10] Zheng Y, Pan L, Wang F, Yan J, Wang T, Xia Y, Yao L, Deng K, Zheng Y, Xia X, Su Z, Chen H, Lin J, Ding Z, Zhang K, Zhang M and Chen Y. Neural function of Bmal1: an overview. Cell Biosci 2023; 13: 1.
[11] Fan R, Peng X, Xie L, Dong K, Ma D, Xu W, Shi X, Zhang S, Chen J, Yu X and Yang Y. Importance of Bmal1 in Alzheimer's disease and associated aging-related diseases: mechanisms and interventions. Aging Cell 2022; 21: e13704.
[12] Qu M, Zhang G, Qu H, Vu A, Wu R, Tsukamoto H, Jia Z, Huang W, Lenz HJ, Rich JN and Kay SA. Circadian regulator BMAL1::CLOCK promotes cell proliferation in hepatocellular carcinoma by controlling apoptosis and cell cycle. Proc Natl Acad Sci U S A 2023; 120: e2214829120.
[13] Zhang C, Chen L, Sun L, Jin H, Ren K, Liu S, Qian Y, Li S, Li F, Zhu C, Zhao Y, Liu H and Liu Y. BMAL1 collaborates with CLOCK to directly promote DNA double-strand break repair and tumor chemoresistance. Oncogene 2023; 42: 967-979.
[14] Iweka CA, Seigneur E, Hernandez AL, Paredes SH, Cabrera M, Blacher E, Pasternak CT, Longo FM, de Lecea L and Andreasson KI. Myeloid deficiency of the intrinsic clock protein BMAL1 accelerates cognitive aging by disrupting microglial synaptic pruning. J Neuroinflammation 2023; 20: 48.
[15] Ou Z, Zhong H, Zhang L, Deng M, Zhang W, Wang J, Feng H, Gong J, Miao C and Yi Z. Macrophage membrane-coated nanoparticles alleviate hepatic ischemia-reperfusion injury caused by orthotopic liver transplantation by neutralizing endotoxin. Int J Nanomedicine 2020; 15: 4125-4138.
[16] Schibler U. BMAL1 dephosphorylation determines the pace of the circadian clock. Genes Dev 2021; 35: 1076-1078.
[17] Shapouri-Moghaddam A, Mohammadian S, Vazini H, Taghadosi M, Esmaeili SA, Mardani F, Seifi B, Mohammadi A, Afshari JT and Sahebkar A. Macrophage plasticity, polarization, and function in health and disease. J Cell Physiol 2018; 233: 6425-6440.
[18] Murray MH, Valfort AC, Koelblen T, Ronin C, Ciesielski F, Chatterjee A, Veerakanellore GB, Elgendy B, Walker JK, Hegazy L and Burris TP. Structural basis of synthetic agonist activation of the nuclear receptor REV-ERB. Nat Commun 2022; 13: 7131.
[19] Haemmerle G, Moustafa T, Woelkart G, Büttner S, Schmidt A, van de Weijer T, Hesselink M, Jaeger D, Kienesberger PC, Zierler K, Schreiber R, Eichmann T, Kolb D, Kotzbeck P, Schweiger M, Kumari M, Eder S, Schoiswohl G, Wongsiriroj N, Pollak NM, Radner FP, Preiss-Landl K, Kolbe T, Rülicke T, Pieske B, Trauner M, Lass A, Zimmermann R, Hoefler G, Cinti S, Kershaw EE, Schrauwen P, Madeo F, Mayer B and Zechner R. ATGL-mediated fat catabolism regulates cardiac mitochondrial function via PPAR-α and PGC-1. Nat Med 2011; 17: 1076-1085.
[20] Rius-Pérez S, Torres-Cuevas I, Millán I, Ortega ÁL and Pérez S. PGC-1α, inflammation, and oxidative stress: an integrative view in metabolism. Oxid Med Cell Longev 2020; 2020: 1452696.
[21] Liu C, Li S, Liu T, Borjigin J and Lin JD. Transcriptional coactivator PGC-1alpha integrates the mammalian clock and energy metabolism. Nature 2007; 447: 477-481.
[22] Nakamori Y, Park EJ and Shimaoka M. Immune deregulation in sepsis and septic shock: reversing immune paralysis by targeting PD-1/PD-L1 pathway. Front Immunol 2021; 11: 624279.
[23] Rimmelé T, Payen D, Cantaluppi V, Marshall J, Gomez H, Gomez A, Murray P and Kellum JA; ADQI XIV Workgroup. Immune cell phenotype and function in sepsis. Shock 2016; 45: 282-291.
[24] Wen X, Xie B, Yuan S and Zhang J. The "Self-Sacrifice" of ImmuneCells in sepsis. Front Immunol 2022; 13: 833479.
[25] Curtis AM, Bellet MM, Sassone-Corsi P and O'Neill LA. Circadian clock proteins and immunity. Immunity 2014; 40: 178-186.
[26] An Y, Yuan B, Xie P, Gu Y, Liu Z, Wang T, Li Z, Xu Y and Liu Y. Decoupling PER phosphorylation, stability and rhythmic expression from circadian clock function by abolishing PER-CK1 interaction. Nat Commun 2022; 13: 3991.
[27] Pan X, Mota S and Zhang B. Circadian clock regulation on lipid metabolism and metabolic diseases. Adv Exp Med Biol 2020; 1276: 53-66.
[28] Papagiannakopoulos T, Bauer MR, Davidson SM, Heimann M, Subbaraj L, Bhutkar A, Bartlebaugh J, Vander Heiden MG and Jacks T. Circadian rhythm disruption promotes lung tumorigenesis. Cell Metab 2016; 24: 324-331.
[29] Wang Q, Sundar IK, Lucas JH, Muthumalage T and Rahman I. Molecular clock REV-ERBα regulates cigarette smoke-induced pulmonary in-

flammation and epithelial-mesenchymal transition. JCI Insight 2021; 6: e145200.
[30] Zhang X, Li X, Jia H, An G and Ni J. The m(6)A methyltransferase METTL3 modifies PGC-1α mRNA promoting mitochondrial dysfunction and oxLDL-induced inflammation in monocytes. J Biol Chem 2021; 297: 101058.
[31] Ye P, Li W, Huang X, Zhao S, Chen W, Xia Y, Yu W, Rao T, Ning J, Zhou X, Ruan Y and Cheng F. BMAL1 regulates mitochondrial homeostasis in renal ischaemia-reperfusion injury by mediating the SIRT1/PGC-1α axis. J Cell Mol Med 2022; 26: 1994-2009.