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Efficacy of caerulomycin A in modulating macrophage polarization and cytokine response in a murine model of lipopolysaccharide-induced sepsis

Jun Zhang[1*] and Shiyue Tang[1]

Abstract

Background Sepsis is characterized by an excessive immune response. Modulation of the immune response, particularly macrophage polarization, may provide therapeutic benefit. The effects of Caerulomycin A (caeA), a known STAT1 phosphorylation inhibitor, on macrophage polarization and inflammatory markers were explored using a lipopolysaccharide (LPS)-induced sepsis mouse model.

Methods A sepsis model was established in C57BL/6 mice induced by intraperitoneal injection of LPS, and the survival rate of mice was observed after treatment with different doses of caeA to determine the optimal therapeutic dose. For in-vitro assays using the RAW264.7 macrophage cell line, the concentration of caeA that was non-toxic to cell survival was screened using the MTT assay, followed by the analyses by qRT-PCR, ELISA, Western blot and flow cytometry for M1/M2 type macrophage markers (CD86, NOS2, CD206, ARG1) and inflammatory factors (IL-1β, IL-6, TNF-α, IL-4, and IL-10) expression. In addition, the phosphorylation levels of STAT1 and STAT6 in the JAK–STAT signaling pathway were detected.

Results The results of in-vivo experiments showed that caeA treatment (20 mg/kg) significantly increased the survival of LPS-induced septic mice and decreased the expression of M1-type macrophage markers (CD86 and NOS2) and pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) while increasing the expression of M2-type markers (CD206 and ARG1) and anti-inflammatory cytokines (IL-4 and IL-10) expression. In in-vitro experiments, 20 μM caeA effectively inhibited LPS-induced polarization of M1-type macrophages without affecting the activity of RAW264.7 cells, and caeA significantly inhibited the phosphorylation of STAT1 yet enhanced the phosphorylation level of STAT6, as detected by Western blot.

Conclusions CaeA effectively modulates macrophage polarization and attenuates the inflammatory response in septic mice, possibly by affecting the JAK–STAT signaling pathway. These findings support further exploration of the potential of caeA as a therapeutic agent for sepsis.

Keywords Caerulomycin A, Macrophage polarization, Sepsis therapy, Cytokine modulation, Immunomodulation

Introduction

Sepsis arises from dysregulation in immune responses to infection, which leads to widespread inflammation and organ dysfunction. Due to its high morbidity and mortality, it is a major healthcare challenge that affects millions

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of patients annually all over the world. Sepsis affects around 1.7 million adult patients each year and causes hospitalized deaths in the US. Study estimates that sepsis will account for nearly 20% of global deaths in recent years, underscoring its significant public health impact [1–3]. The condition begins with an infection that triggers an immune response in the host. This response can become "dysregulated", meaning that it is excessive and uncontrolled. This leads to the release of inflammatory molecules, resulting in extensive tissue damage, leading to organ dysfunction and failure. The pathophysiology of sepsis involves complex interactions between various immune cells, inflammatory cytokines, and the coagulation system, which can lead to severe immune dysfunction and catabolic states [4–6]. In addition, the development of sepsis is primarily defined by the interaction of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) with pattern recognition receptors (PRRs) found on specific immune cells, such as macrophages [7]. As the central components of the innate immune system, macrophages are essential not only for triggering the natural immune response by recognizing risk factors within the cellular microenvironment but also for modulating the host's immune response through various polarization states [8]. The polarization of macrophages into M1 or M2 phenotypes is essential for the advancement of septic shock. Lipopolysaccharide (LPS), located on the surface membrane of Gram-negative bacteria, interacts with Toll-like receptor 4 (TLR4), initiating a series of pro-inflammatory processes [9]. This immunological reaction results in an excessive release of cytokines that significantly contribute to both the onset and progression of sepsis [10]. Currently, management of sepsis includes prompt recognition and treatment of underlying infections, support of organ function, and modulation of host responses to reduce the risk of further damage. Treatment strategies include rapid administration of antibiotics, aggressive fluid resuscitation, and supportive care measures such as organ support in the intensive care unit [11–13]. Despite improvements in the recognition and early treatment of sepsis, it remains challenging due to its complex pathophysiology and the variability of individual responses to infection.

Caerulomycin A (caeA) was initially recognized as an antibiotic that exhibited effective antimicrobial properties against a wide range of pathogens [14]. It has received widespread attention for its immunosuppressive effects, particularly its ability to inhibit the differentiation of Th17 cells, a subset of pro-inflammatory T cells implicated in autoimmune diseases. The compound acts by modulating key signaling pathways, particularly the JAK–STAT pathway, affecting immune responses and cell proliferation [15, 16]. Recent studies have explored the versatility of caeA in various fields of medicine. Its potential role in treating autoimmune diseases by reducing excessive immune responses without compromising overall immune function has been investigated. Its effect on macrophage polarization has also been a focus of attention, with findings suggesting that caeA can shift the macrophage state from pro-inflammatory (M1) to anti-inflammatory (M2), which facilitates the control of chronic inflammatory conditions [14]. Despite its potential, the clinical application of caeA is still in its infancy. Future research should focus on comprehensive clinical trials to assess its safety and efficacy in humans, particularly in the treatment of autoimmune diseases.

This study concluded that caeA has potent anti-inflammatory effects on sepsis, primarily through modulation of macrophage polarization and cytokine production. These findings indicate that caerulomycin A was an effective therapeutic agent for sepsis and that further clinical studies are needed to explore its efficacy and safety in human subjects. This comprehensive approach combining molecular techniques with animal models provides important insights into the potential of caeA as a modulator of immune responses in sepsis.

Materials and methods

Animals and experimental procedures

Animal-involved experiments were approved by the Animal Experimental Ethical Committee of Jinhua Central Hospital, adhering strictly to the guidelines for humane care outlined by the National Institutes of Health. Male C57BL/6 mice were obtained from SLAC Laboratory Animal Co., Ltd. (aged 8–10 weeks, Shanghai, China) and were kept in controlled room temperature (22 ± 2 ℃) and humidity (60–80%) under a 12 h/12 h light/dark cycle and housed with food and water available ad libitum. Animals were received an intraperitoneal injection of 10 mg/kg lipopolysaccharide (LPS) from Escherichia coli O55:B5 and were observed for 12 h. The mice were divided into two groups for the study: a control group (Con, $n = 3$) and a sepsis group (Sepsis, $n = 3$). At the end of the observation period, mice were euthanized with excess carbon dioxide, then, blood samples as well as lung and heart tissues were collected for further analysis. caeA was sourced from MCE (Catalog no. HY-114495, Monmouth Junction, NJ, USA).

Cell culture methodology

DMEM (Catalog no. A1896701, GIBCO, Waltham, MA, USA) containing 1% penicillin–streptomycin (PS) and 10% fetal bovine serum (FBS) was prepared to culture RAW264.7 cells obtained from the American Type Culture Collection (ATCC, TIB-71, Manassas, VA, USA) in

an incubator. The culture condition was set at the temperature of 37 °C with 5% CO_2.

Cell viability assay

Each well containing cells was supplemented with 10 µL MTT solution (5 mg/mL MTT in medium, catalog no. M6494, Invitrogen, Carlsbad, CA, USA) for 4-h incubation at 37 °C. Next, 100 µL of dimethyl sulfoxide (DMSO, catalog no. BP231-100, Thermo Fisher Scientific, Waltham, MA, USA) was used to solubilize the formazan crystals formed in each well. The absorbance was subsequently measured using a Fluostar Omega microplate reader (BMG Labtech, Cary, NC, USA).

ELISA

ELISA was employed to quantify the cytokine levels in LPS-induced RAW264.7 cells treated or untreated with Caerulomycin [17]. Following treatment, cell culture supernatants were collected, and the concentration of cytokines was detected using ELISA kits specific for IL-1β, IL-6, TNF-α, IL-4, and IL-10 (Catalog no. ILB00C, D6050, MTA00B, M4000B, D1000B, R&D Systems, Minneapolis, MN, USA). The absorbance was measured spectrophotometrically at a wavelength of 450 nm using a microplate reader (SpectraMax M5, Molecular Devices, San Jose, CA, USA) [18].

Flow cytometry analysis

Flow cytometry was performed to analyze the blood samples. Initially, the blood sample was subjected to centrifugation using 30% Percoll to separate cells. Erythrocytes were lysed using a red blood cell lysis buffer (Catalog no. C3702, Beyotime, Shanghai, China). Subsequently, the remaining cells were resuspended in 1% bovine serum albumin for antibody staining. After staining, the cells were washed twice using FACS buffer and then resuspended in FACS buffer (300 µL). Analysis was conducted using a FACSAria flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA), with data acquisition and analysis performed using FACSDiva software (version 9.0, BD Biosciences, USA) [19].

RNA extraction and qRT-PCR analysis

The total RNA was extracted from both blood and cell samples via Trizol reagent (Catalog no. 15596026, Invitrogen, USA). The PrimeScript™ RT Reagent Kit with gDNA Eraser (Perfect Real Time, Catalog No. RR047A, Takara, Tokyo, Japan) was applied in the reverse transcription to synthesize complementary DNA (cDNA). Q-PCR was subsequently conducted on a CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). A total of 25 µL qRT-PCR reaction volume consisted of 2 µL of cDNA, appropriate primers, 8.5 µL of nuclease-free water, and 12.5 µL TB Green® Premix Ex Taq™ II (Tli RNaseH Plus, Catalog No. RR820A, Takara, Japan). Thermal cycling conditions were as follows: an initial denaturation at 95 °C for 30 s, followed by 36 cycles of 5 s at 95 °C and 30 s at the annealing temperature, with a final extension at 60 °C for 30 s [20]. The $2^{-\Delta\Delta CT}$ method was employed to calculating the relative mRNA expressions, which were normalized to that of the housekeeping gene Actb. Table 1 provided the information for the primer sequences for all genes.

Western blotting analysis

Protein samples were lysed using RIPA buffer (Catalog no. 89901, Thermo Fisher Scientific, USA), and protein concentration was quantified employing a BCA protein assay kit (Catalog no. P0011, Beyotime, China). 10% SDS–PAGE was used to separate 30 µg of protein per sample and the proteins were subsequently moved onto PVDF membranes (Catalog no. IPVH00010, Millipore Corporation, Billerica, MA, USA). The membranes were sealed with 5% non-fat dry milk for 1 h at room temperature. Following the blocking, primary antibodies targeting STAT1 (10144-2-AP, Proteintech, Wuhan, China), phosphorylated STAT1 (P-STAT1, 28977-1-AP, Proteintech, China), STAT6 (51073-1-AP, Proteintech, China), phosphorylated STAT6 (P-STAT6, ab263947, Abcam, Cambridge, UK), and the loading control GAPDH (60004-1-Ig, Proteintech, China) were incubated with the membranes at 4 °C together overnight. Following the incubation with the primary antibodies, TBST was employed for washing the membranes, which were further incubated with the appropriate secondary antibodies at room temperature for 1 h. The enhanced chemiluminescent detection kit (Catalog no.WBKLS0500, Millipore Corporation, USA) was used for detecting the protein bands. Band density was quantified using Scion Image software (version 4.0.2, Informer Technologies, Inc., USA) [21]. The intensities of the Western blot bands

Table 1 Primer sequences for all genes

Gene	Forward primer sequence (5–3)	Reverse primer sequence (5–3)
Cd86	ACGTATTGGAAGGAGATTACAGCT	TCTGTCAGCGTTACTATCCCGC
Cd206	GTTCACCTGGAGTGATGGTTCTC	AGGACATGCCAGGGTCACCTTT
Nos2	GAGACAGGGAAGTCTGAAGCAC	CCAGCAGTAGTTGCTCCTCTTC
Arg1	CATTGGCTTGCGAGACGTAGAC	GCTGAAGGTCTCTTCCATCACC
Actb	CATTGCTGACAGGATGCAGAAGG	TGCTGGAAGGTGGACAGTGAGG

were quantified using ImageJ software (NIH, Bethesda, MD, USA) with background subtraction. The relative protein expression levels were normalized to GAPDH as a loading control.

Statistical analysis

All analyses and graphical representations in this study were conducted using GraphPad Prism software (version 8, GraphPad, Inc., La Jolla, CA, USA). The log-rank tests were applied to compare the differences in survival rates of different groups of mice. One-way ANOVA and *t* tests were used to compare differences in cytokine concentrations and protein expression between multiple groups. Three technical replications were performed for each experiment. The results are expressed as the mean ± standard deviation (SD), including a 95% confidence interval (CI), and statistical significance was established at $p < 0.05$.

Results

Elevated inflammatory markers in sepsis mice

In the mouse sepsis model induced using LPS, we first monitored the survival rates of septic mice and normal mouse groups. The results showed that the mortality rate of septic mice was significantly increased (Fig. 1A). At the same time, we observed a significant increase in the mRNA levels of the inflammatory markers CD86 and NOS2 in the peripheral blood, which are usually associated with inflammatory responses (Fig. 1B, C). Furthermore, we analyzed the concentration of inflammatory factors in the peripheral blood by ELISA. The results showed significantly increased concentrations of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6

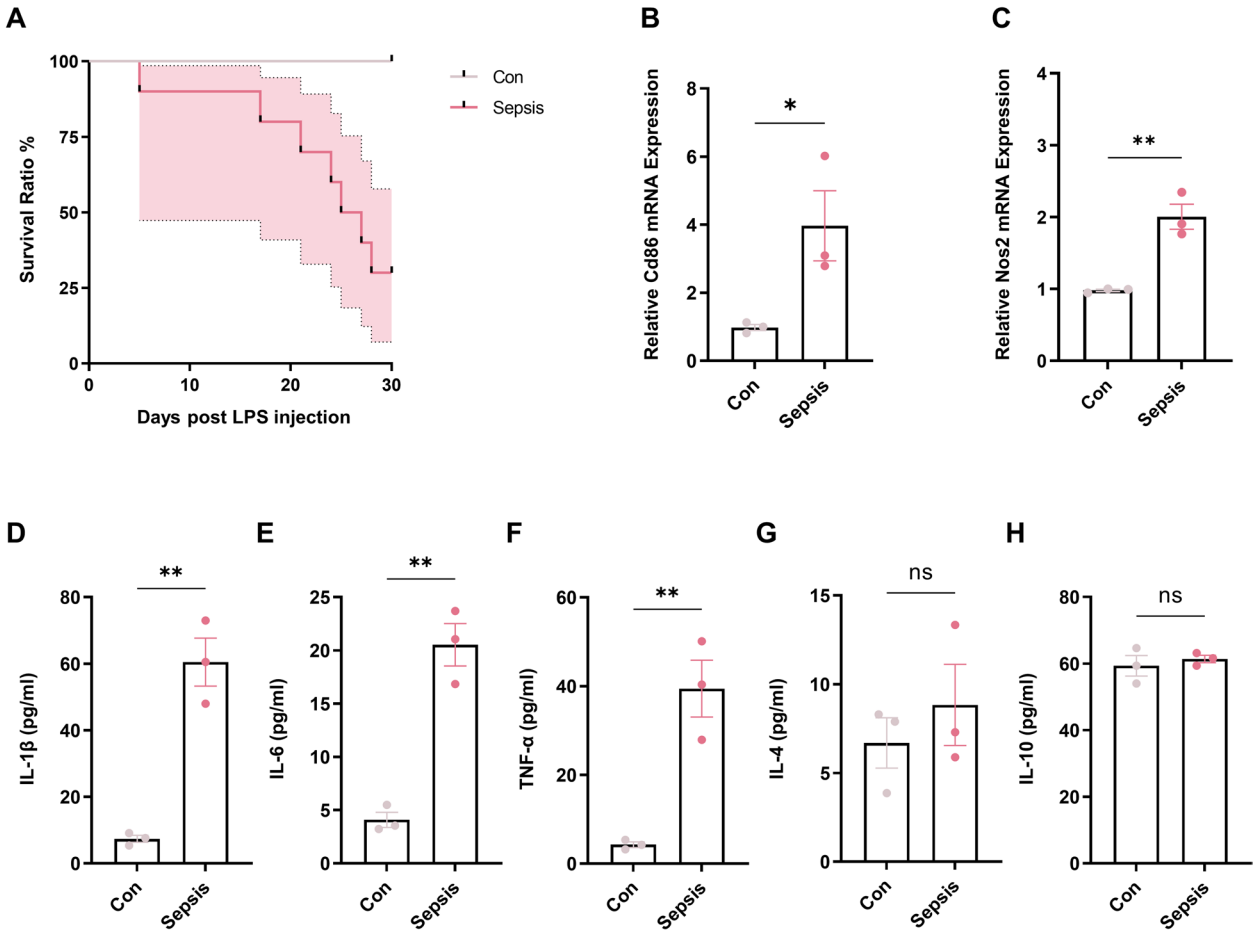

Fig. 1 Inflammatory indicators and expression levels of anti-inflammatory factors in Sepsis. **A** Survival curves of sepsis mice and normal mice. **B** Relative CD86 mRNA expression levels in sepsis and normal mice. **C** Relative mRNA expression levels of NOS2 in sepsis mice and normal mice. **D** The level of IL-1β in sepsis mice and normal mice. **E** The levels of IL-6 in sepsis mice and normal mice. **F** The levels of TNF-α in sepsis mice and normal mice. **G** The level of IL-4 protein in sepsis mice and normal mice. **H** The level of IL-10 protein in sepsis mice and normal mice. Con refers to C57BL/6 mice untreated for use as controls, and Sepsis group refers to mice induced into a sepsis model by intraperitoneal injection of 10 mg/kg LPS. All data of three independent trials were expressed as mean ± standard deviation

(Fig. 1D–F) in the peripheral blood samples of sepsis-modeled mice, whereas the concentrations of inflammation-suppressive cytokines IL-4 and IL-10 did not change significantly (Fig. 1G, H). These results indicate that septic mice develop a typical inflammatory response, further validating our ability to establish a mouse model that can be used in subsequent studies.

Macrophages in sepsis show a trend of M1 polarization

Considering the significant changes of inflammation-related cytokines in the peripheral blood of mice, we further examined the immune cell types in the peripheral blood using flow cytometry. As shown in Fig. 2A, 16.0% of the target cells were screened by FSC/SSC gating, and further screening yielded 95.9% of the live cells; among the live cells, 47.3% of the myeloid cell population was identified by CD11B and CD45 labeling, whereas macrophages with high expression of F4/80 and low expression of LY6G accounted for 7.05% of the myeloid cells. Subsequently, we determined the phenotype of these macrophages by qRT-PCR. The experimental data showed that in mice of LPS-induced sepsis model, the expression of the markers CD86 and NOS2 was significantly increased in M1-type macrophages (Fig. 2B, C), whereas the expression of the markers CD206 and ARG1 was significantly decreased in M2-type macrophages (Fig. 2D, E). The same experiment was performed using an in-vitro model to detect the same markers after LPS treatment of the mouse monocyte macrophage leukemia cell line RAW264.7 so as to validate this result (Fig. 3A). And consistent results were observed. In detail, the expression of CD86 (M1-type macrophage marker) was significantly upregulated in response to LPS stimulation, whereas that of CD206 (M2-type marker) was significantly downregulated (Fig. 3B, C). In addition, the concentrations of pro-inflammatory cytokines TNF-α and IL-1β were significantly promoted in the cell culture medium of LPS-induced RAW264.7 cells (Fig. 3D, E), while no significant changes on the concentrations of inflammation-suppressing cytokines IL-4 and IL-10 (Fig. 3F, G). The JAK–STAT signaling pathway is closely related to macrophage phenotypic changes, and Western Blotting analysis showed that after LPS stimulation, the phosphorylation of STAT1 was significantly enhanced in the RAW264.7 cells, while

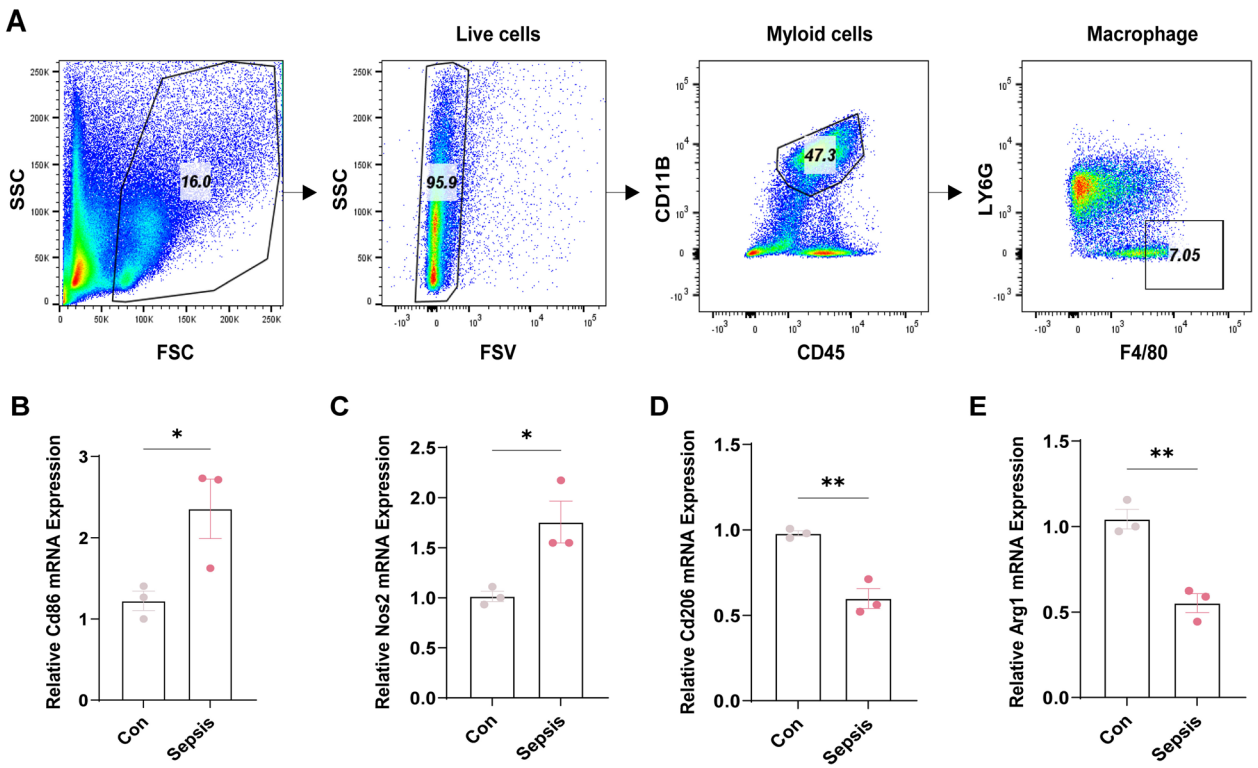

Fig. 2 Macrophages undergo M1 polarization in a mouse model of sepsis. **A** Cell types in the peripheral blood of sepsis mice. **B** Expression level of CD86 mRNA in macrophages. **C** Expression level of NOS2 mRNA in macrophages. **D** Expression level of CD206 mRNA in macrophages. **E** Expression level of ARG1 mRNA in macrophages. Con refers to C57BL/6 mice untreated for use as controls, and Sepsis group refers to mice induced into a sepsis model by intraperitoneal injection of 10 mg/kg LPS. All data of three independent trials were expressed as mean ± standard deviation

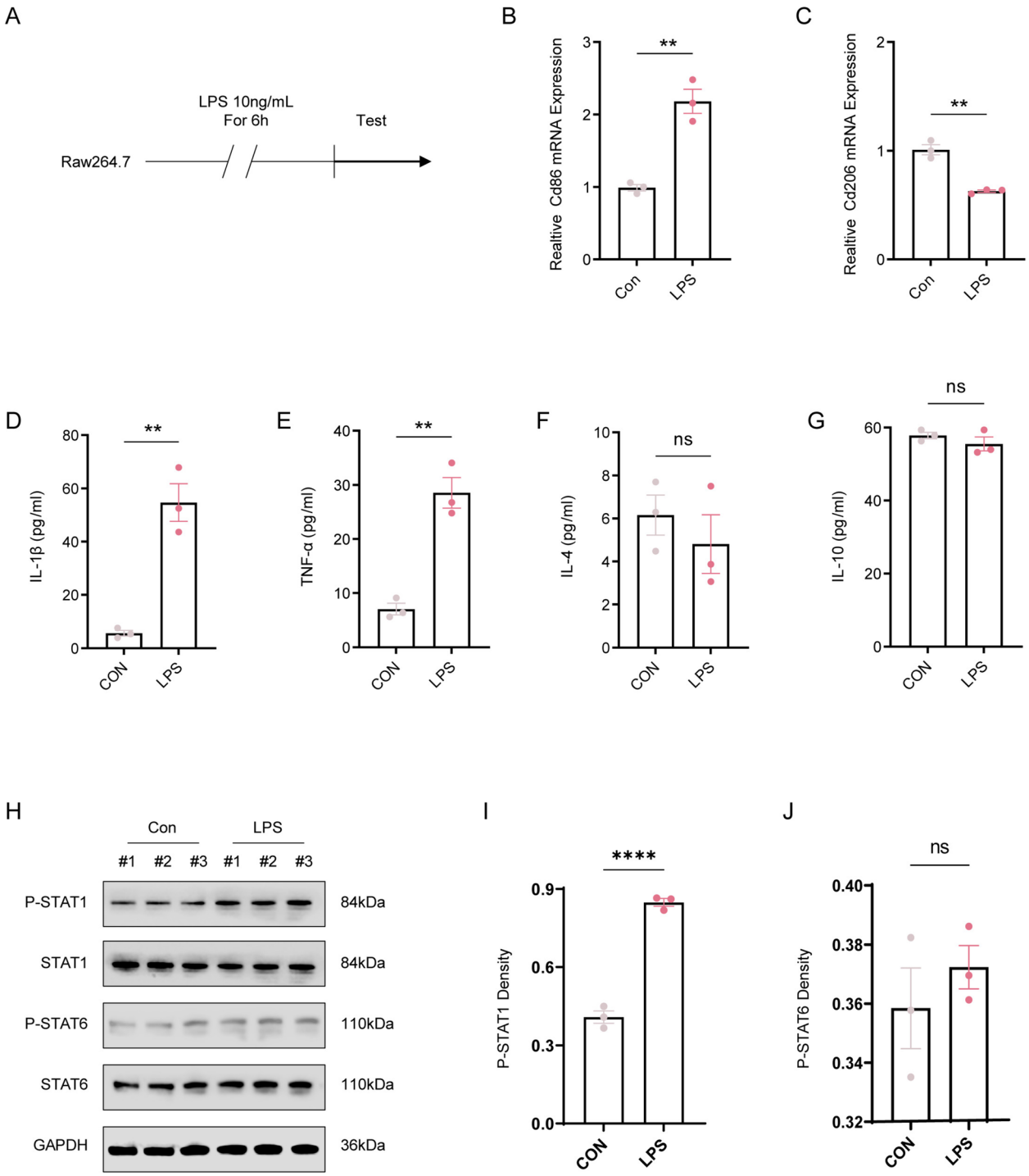

Fig. 3 In-vitro cellular assay confirming that macrophages in sepsis tend to be M1 polarized. **A** Flowchart of LPS-stimulated mouse monocyte macrophage leukemia cell line RAW264.7. **B** CD86 mRNA expression level in RAW264.7 cells. **C** Expression level of CD206 mRNA in RAW264.7 cells. **D** The level of IL-1β in RAW264.7 cells. **E** The level of TNF-α in RAW264.7 cells. **F** Expression level of IL-4 protein in RAW264.7 cells. **G** The level of IL-10 in RAW264.7 cells. **H** Expression level of JAK-STAT signaling pathway proteins in RAW264.7 cells by WB. **I** Phosphorylation level of STAT1 in RAW264.7 cells. **J** Phosphorylation level of STAT6 in RAW264.7 cells. Con refers to untreated RAW264.7 cells to be used as a control group, and LPS group refers to RAW264.7 cells treated in 10 ng/mL of LPS for 6 h. All data of three independent trials were expressed as mean ± standard deviation

the phosphorylation of STAT6 was not significantly changed, further demonstrating the occurrence of M1-type macrophage polarization (Fig. 3H–J).

Caerulomycin A inhibits M1 polarization and pro-inflammatory factor expression

Although caeA has been reported as an inhibitor of STAT1, its effect on macrophage regulation remains unclear. Therefore, we first determined the safe dose of caeA in in-vitro mimetic therapy by CCK-8 experiments. The experimental results showed that high concentrations of caeA exerted an inhibitory effect on macrophage activity, whereas there was no significant effect on cell activity at low concentrations (Fig. 4A). On this basis, we determined 20 M as the optimal therapeutic concentration of caeA in an in vitro macrophage model of LPS-induced inflammation (Fig. 4B). In further experiments, we divided macrophages into three groups: a control group, an LPS-stimulated group, and an LPS-stimulated plus caeA treatment group (Fig. 5A). We found that caeA treatment significantly reversed LPS-induced M1-type polarization (Fig. 5B, C). Meanwhile, the treatment of caeA effectively suppressed the concentration of pro-inflammatory cytokines in the culture medium and promoted the secretion of anti-inflammatory cytokines by macrophages (Fig. 5D–G).

Caerulomycin A inhibits STAT1 phosphorylation and thus macrophage M1 polarization

We further explored the effect of caeA on protein phosphorylation levels in the JAK–STAT signaling pathway. Macrophages in the control group (LPS-treated) showed significant STAT1 and STAT6 phosphorylation, and in the caeA-treated group (LPS + caeA), the phosphorylation level of STAT1 was decreased and that of STAT6 was increased (Fig. 6A–C). This finding supports the hypothesis that caeA may inhibit M1-type macrophage polarization and pro-inflammatory responses by modulating the STAT signaling pathway.

Mouse experiments further confirm Caerulomycin A inhibits macrophage M1 polarization

Finally, we constructed a mouse model of sepsis to further confirm the function of caeA. Compared to mice with sepsis, caeA treatment greatly alleviated the damage caused by sepsis, and survival was mitigated (Fig. 7A). We observed a decrease in the mRNA expression levels of CD86 and NOS2 in the peripheral blood of caeA-treated septic mice, while the level of CD206 was significantly increased (Fig. 7B–E). In addition, the levels of IL-1β, IL-6, and TNF-α in the peripheral blood of caeA-treated septic mice were significantly decreased, whereas the level of IL-4 was increased (Fig. 7F–J). Mouse assays further confirmed that Caerulomycin A inhibited macrophage M1 polarization.

Discussion

The present study explored the potential role of caeA in sepsis treatment. Based on the experiments with the LPS-induced mouse sepsis model and the RAW264.7 macrophage cell line, it was found that caeA significantly reversed the M1-type polarization trend of macrophages and downregulated pro-inflammatory factors such as IL-6, IL-1β, and TNF-α while elevating the expression of the anti-inflammatory factors IL-4 and IL-10. These effects were accompanied by inhibition of STAT1 phosphorylation and changes in STAT6 phosphorylation. In the animal model, caeA treatment improved the survival

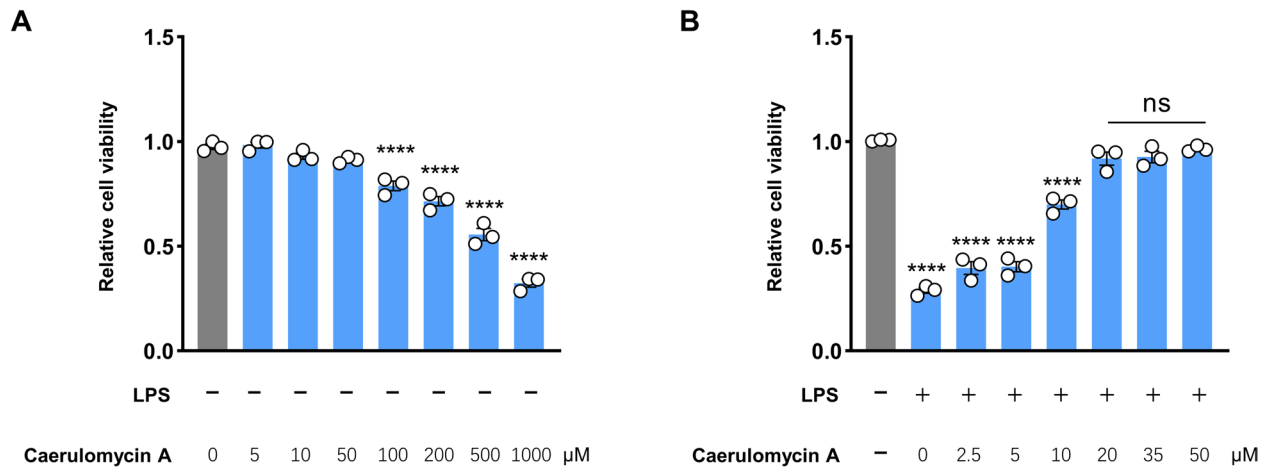

Fig. 4 caeA can inhibit macrophage activity. **A** CCK-8 assay confirms that caeA can inhibit macrophage activity. **B** Optimal therapeutic concentration of caeA was determined

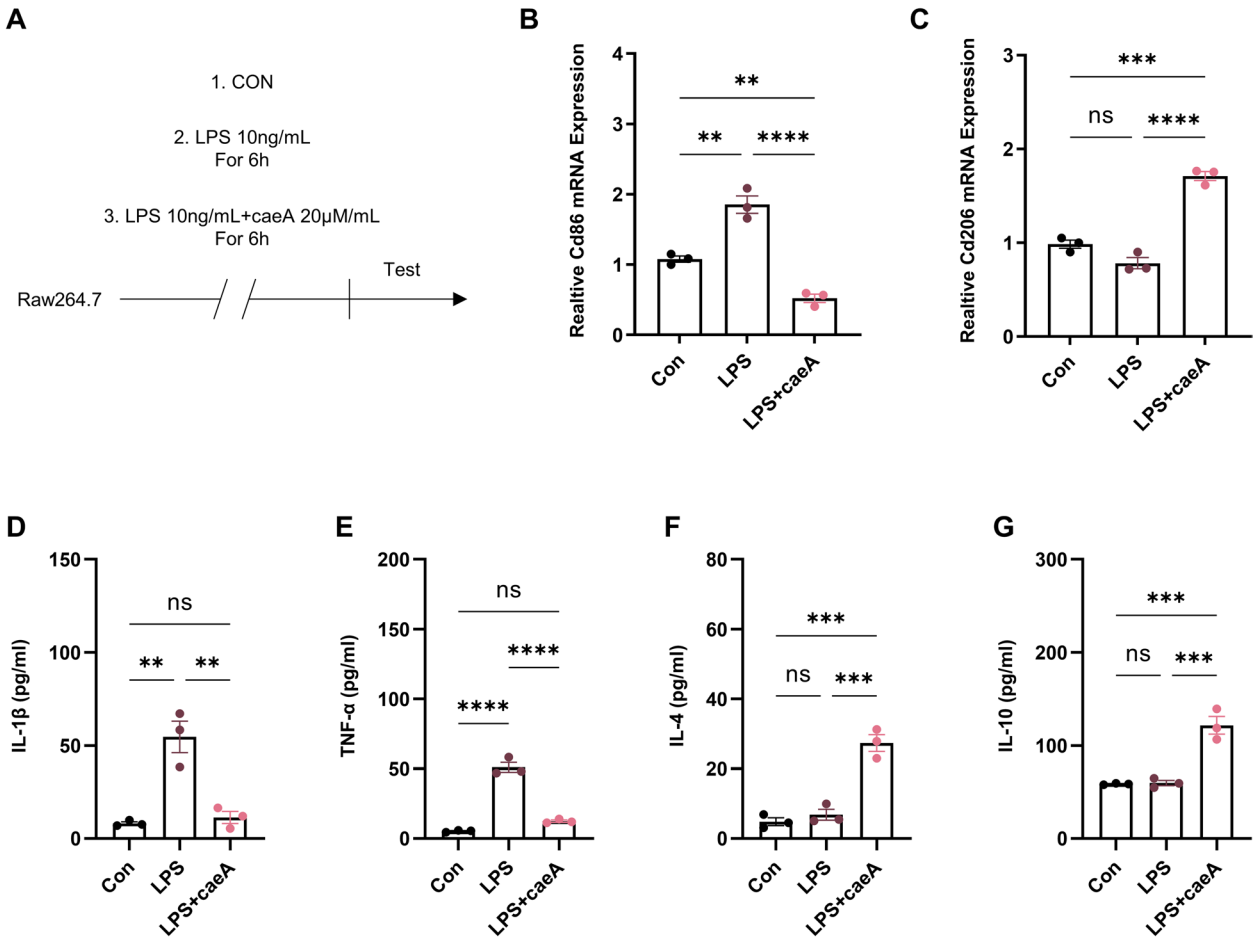

Fig. 5 caeA inhibits pro-inflammatory cytokine expression in sepsis. **A** Experimental flowchart of cells in control, LPS-stimulated, and LPS + caeA-treated groups. **B** Expression levels of CD86 mRNA in the three treatment groups.**C** Expression levels of CD206 mRNA in the three treatment groups. **D** The levels of IL-1β in the three treatment groups. **E** The level of TNF-α in the three treatment groups. **F** The level of IL-4 in the three treatment groups. **G** The level of IL-10 in the three treatment groups. Con refers to untreated RAW264.7 cells to be used as a control group, LPS group refers to RAW264.7 cells treated in 10 ng/mL of LPS for 6 h, and LPS + caeA group refers to RAW264.7 cells treated in 10 ng/mL of LPS and 20 μM of caeA for 6 h. All data of three independent trials were expressed as mean ± standard deviation

of mice with sepsis, demonstrating its potential to modulate immune responses and suppress inflammation. These findings reveal the potential of caeA as a novel drug for sepsis treatment and provide a basis for further clinical studies.

It has been found that in the context of sepsis, M1-type macrophages are usually associated with pro-inflammatory processes, whereas M2-type macrophages are associated with deinflammation and tissue repair [22, 23]. M1 macrophages, also known as classically activated macrophages, fulfill critical functions in the initial host response to pathogens and primarily implicated in pro-inflammatory responses and are potent effector cells for killing microbes and tumor cells [24, 25]. In sepsis, M1 macrophages are rapidly activated by LPS and other bacterial components through pattern recognition receptors such as TLR4 and produce high levels of pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6, which are critical in the early stages of sepsis but may lead to a cytokine storm and the subsequent tissue damage in severe cases [26, 27]. M2 macrophages or activated macrophages typically play a role in resolving inflammation and promoting tissue repair and healing. In sepsis, their role is more complex. By secreting anti-inflammatory cytokines such as IL-10 and TGF-β, they can help suppress the excessive inflammatory response triggered by M1 macrophages. However, their increased activation may also lead to the immunosuppression observed in the later stages of sepsis, which may increase the likelihood of secondary infections [28–31]. The balance between M1 and M2 macrophages is critical in sepsis. Excessive M1 responses may lead to uncontrolled inflammation and organ damage, while overactive M2 responses may lead to failure of bacterial clearance

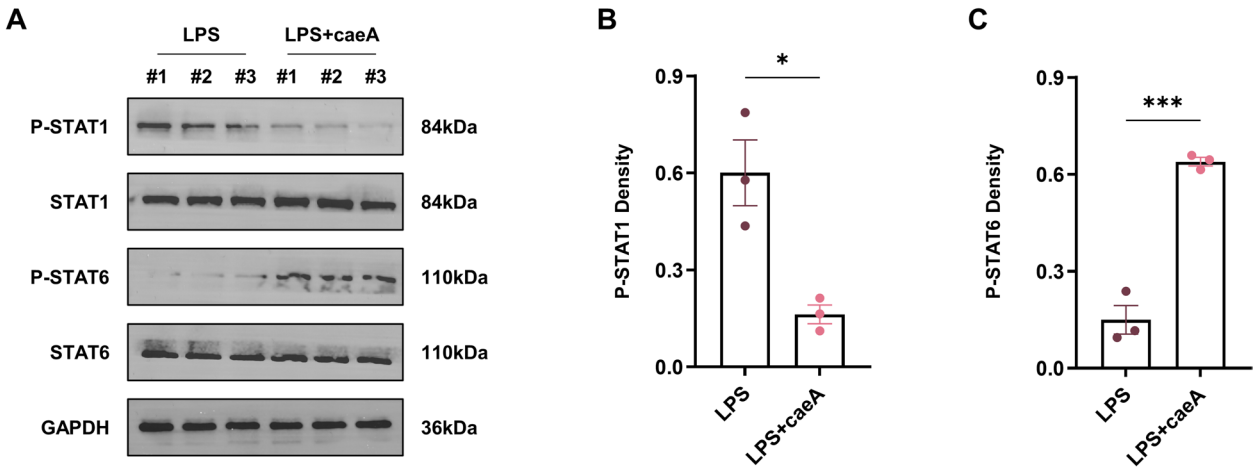

Fig. 6 Caerulomycin A inhibits STAT1 phosphorylation and thus macrophage M1 polarization. **A** Levels of protein phosphorylation in the JAK-STAT signaling pathway in control (LPS-treated) and caeA-treated (LPS + caeA) groups. **B** Detection of STAT1 phosphorylation level in control (LPS-treated) and caeA-treated groups (LPS + caeA). **C** Detection of STAT6 phosphorylation level in control (LPS-treated) and caeA-treated groups (LPS + caeA). LPS group refers to RAW264.7 cells treated in 10 ng/mL of LPS for 6 h, and LPS + caeA group refers to RAW264.7 cells treated in 10 ng/mL of LPS and 20 μM of caeA for 6 h. All data of three independent trials were expressed as mean ± standard deviation

and increased susceptibility to new infections [25, 32]. Understanding and regulating such a balance may contributes to the design of new therapies for sepsis, such as drugs that can modulate macrophage polarization.

caeA is a naturally occurring antibiotic compound that has attracted interest due to its immunosuppressive and potentially anti-inflammatory properties. One of the most studied aspects of caeA is its immunosuppressive effects. caeA could inhibit Th17 cell differentiation, a helper T cell that plays a key role in autoimmune inflammation. By inhibiting Th17 differentiation, caeA can be used to treat autoimmune diseases, for instance, rheumatoid arthritis and multiple sclerosis [15, 16]. Recent studies, including those in the context of sepsis, have explored the potential of caeA to modulate immune responses more broadly. CaeA was found to affect macrophage polarization from pro-inflammatory M1 to anti-inflammatory M2 types. This role is particularly important, because macrophage polarization plays a crucial role in the inflammatory response and can influence the outcome of diseases such as sepsis in which uncontrolled inflammatory responses could damage tissues and result in organ failure [14]. CaeA acts by mechanisms that involve the modulation of signaling pathways that are critical to immune cell function. Specifically, it has been shown to inhibit the phosphorylation of STAT1, a transcription factor that is critical for the activation of a variety of immune responses, including those leading to inflammation and autoimmunity [15, 16]. Given its ability to modulate immune responses and its antibiotic properties, caeA is a potential therapeutic agent for treating a range of diseases that require careful control or modulation of immune responses. This includes not only autoimmune and inflammatory diseases, but also diseases such as sepsis, which require a balanced immune response to prevent infection and excessive inflammation. These are consistent with the trend of the studies in this study that caeA can inhibit macrophage M1 polarization through the STAT signaling pathway. Our study provides evidence that caeA can be effective as a mitigator of sepsis progression. These findings reveal the potential of using caeA as a new drug to treat sepsis, providing a basis for further clinical studies.

(See figure on next page.)

Fig. 7 Mouse test further confirming that caeA inhibits macrophage M1 polarization. **A** Survival curve of sepsis mice after caeA treatment. **B** CD86 mRNA expression level in sepsis mice after caeA treatment.**C** Expression level of NOS2 mRNA in sepsis mice after caeA treatment. **D** Expression level of CD206 mRNA in sepsis mice after caeA treatment. **E** Expression level of ARG1 mRNA in sepsis mice after caeA treatment. **F** Expression level of IL-1β protein in sepsis mice after caeA treatment. **G** Expression level of IL-6 protein in sepsis mice after caeA treatment. **H** Expression level of TNF-α protein in sepsis mice after caeA treatment. **I** Expression level of IL-4 protein in sepsis mice after caeA treatment. **J** Expression level of IL-10 protein in sepsis mice after caeA treatment. Sepsis group refers to mice induced into a sepsis model by intraperitoneal injection of 10 mg/kg LPS, and Sepsis + caeA group refers to LPS induction followed by intraperitoneal injection of Caerulomycin A at 20 mg/kg. All data of three independent trials were expressed as mean ± standard deviation

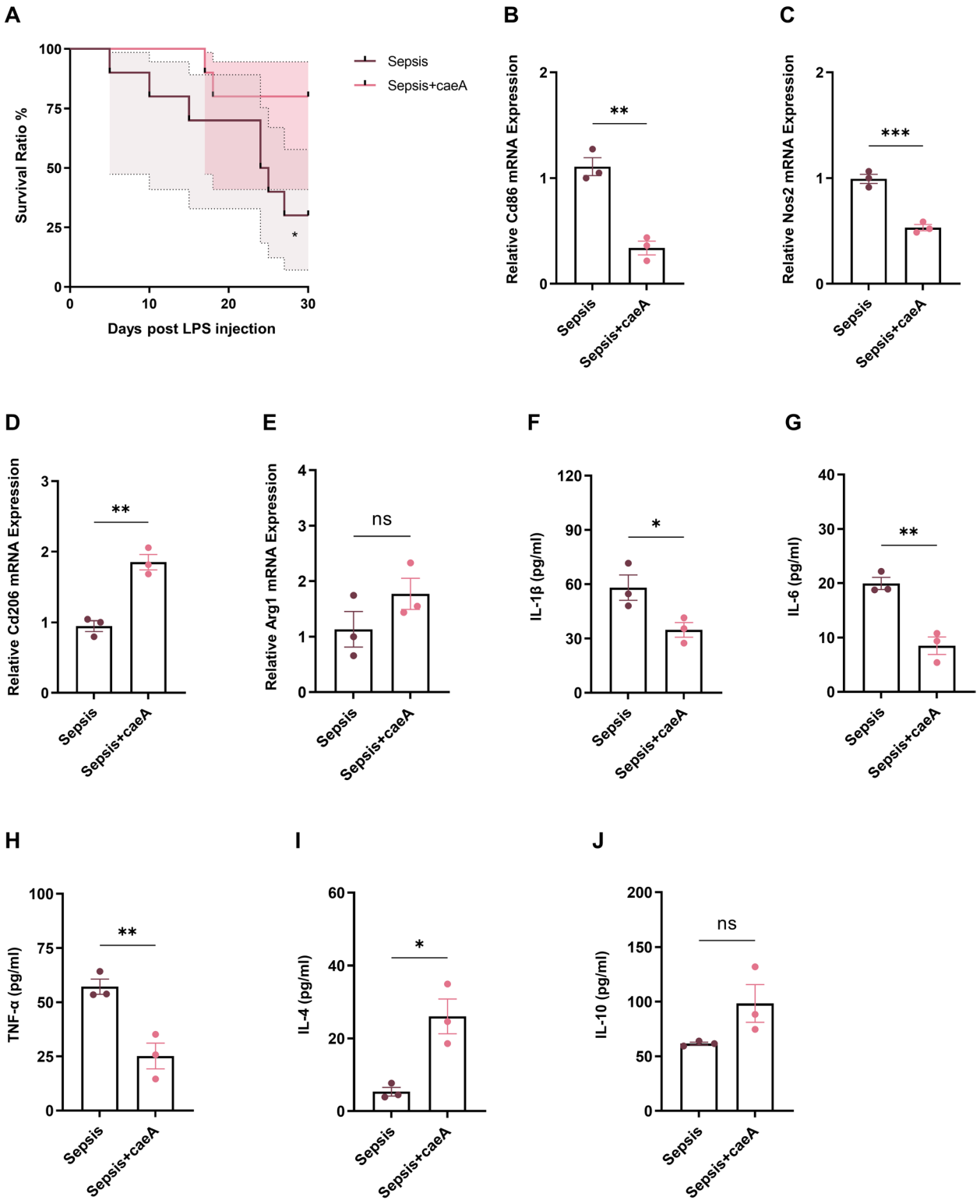

Fig. 7 (See legend on previous page.)

However, there are some limitations to our study. For example, the number of mice used in each group of animal experiments was small, and future studies speak to expanding the experimental sample size to improve the statistical robustness of the data and the reliability of the results. In addition, this study focused on the inflammatory response and macrophage polarization at the molecular and cellular levels and did not monitor behavioral parameters such as body temperature and body weight in mice. Therefore, further studies will introduce behavioral parameters (e.g., temperature changes, body weight monitoring, activity levels, etc.) to more comprehensively assess disease progression and therapeutic effects of caeA. This study focused on the effect of caeA on macrophage polarization and did not explore its potential regulatory role on other immune cells such as T cells, B cells and neutrophils. Future studies will systematically analyze the effects of caeA on different immune cell populations by techniques such as single-cell RNA sequencing and multicolor flow cytometry to reveal the comprehensive mechanisms of its immunoregulation.

Conclusion

This study reveals for the first time that caeA plays an anti-inflammatory role in the LPS-induced sepsis model by regulating macrophage polarization. caeA inhibits STAT1 phosphorylation and activates STAT6 by regulating the JAK–STAT signaling pathway, significantly suppressing LPS-induced macrophage polarization and pro-inflammatory factors in M1-type macrophages, and promotes up-regulation of M2-type macrophages and anti-inflammatory factors, thereby improving the inflammatory response and increasing the survival rate of mice. This study not only expands the potential application of caeA as an immunomodulator, but also provides new molecular targets and drug options for anti-inflammatory treatment of sepsis.

Abbreviations

caeA	Caerulomycin A
LPS	Lipopolysaccharide
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal bovine serum
PS	Penicillin–streptomycin
DMSO	Dimethyl sulfoxide
ELISA	Enzyme-linked immunosorbent assay
qRT-PCR	Real-time quantitative polymerase chain reaction
cDNA	Complementary DNA
qPCR	Quantitative real-time PCR

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None

Author contributions

All authors contributed to this present work: [JZ] & [SYT] designed the study, [JZ] & [SYT] acquired the data, [JZ] & [SYT] made substantial contributions to analysis and interpretation of data. [JZ] & [SYT] improved the figure quality. [JZ] & [SYT] drafted the manuscript, [JZ] & [SYT] revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The experimental data is available upon reasonable request from the corresponding author Jun Zhang.

Declarations

Ethics approval and consent to participate

Animal-involved experiments were approved by the Animal Experimental Ethical Committee of Jinhua Central Hospital (Approval number: (Research) 2022-Ethics Review-44), adhering strictly to the guidelines for humane care outlined by the National Institutes of Health.

Competing interests

The authors declare no competing interests.

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