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Original Article

Elabela mitigates the early stage of inflammation in sepsis by inhibiting pyroptosis

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Abstract: Objectives: This study aims to investigate the potential therapeutic role of Elabela (ELA) in mitigating the sepsis-induced inflammatory storm, a phenomenon commonly associated with multiple organ dysfunction syndrome (MODS) and increased mortality. Our findings show the pathogenesis of sepsis, identifying ELA as a promising therapeutic target. Methods: We conducted a comprehensive analysis of electronic medical records and blood samples from septic patients to assess the incidence of severe organ complication and characterize the inflammatory response. Subsequently, we measured the expression levels of ELA and various inflammatory factors in serum, and performed correlation analysis to explore the relationship between them, aiming to identify the cells and inflammatory pathways targeted by ELA. Furthermore, animal and cellular experiments were conducted to investigate the molecular mechanism underlying the therapeutic effect of ELA. Results: Our findings revealed a higher prevalence of severe organ complications among septic patients, contributing to adverse prognoses and increased mortality. Notably, these patients exhibited significantly elevated levels of inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-1β (IL-1β) in their sera, indicating a robust inflammatory response. Correlation analysis revealed a negative correlation between ELA and IL-1β in septic patients. Through animal and cellular experiments, we demonstrated that ELA inhibits the cleavage of caspase-1 and gasdermin D (GSDMD), thereby attenuating pyroptosis and the inflammatory response. Conclusions: ELA is a promising therapeutic agent for mitigating the deleterious effects of sepsis. Its ability to inhibit macrophage pyroptosis and suppress the inflammatory response offers a novel approach.

Keywords: Elabela, sepsis, inflammatory response, pyroptosis

Introduction

Sepsis is a common clinical critical illness caused by severe infection, which induces a systemic inflammatory response and multiple organ dysfunction syndrome (MODS). It carries a high incidence and mortality rate in intensive care units (ICUs), standing as a leading cause of death among hospitalized patients [1]. Rudd KE et al. showed a detailed analysis on the incidence and mortality of sepsis in the Lancet in 2020 and the number of deaths due to sepsis in recent years has doubled compared to previous estimates, with 48.9 million new cases of sepsis worldwide in 2017, resulting in 11 million sepsis-related deaths, equivalent to 19.7% of the total global mortality [2]. This underscores sepsis as an acute syndrome still posing a significant threat to human health. The pathogenesis of sepsis is intricate. The latest international consensus on the definitions of Sepsis and Septic Shock (referred to as Sepsis-3) has redefined sepsis as life-threatening organ failure caused by the uncontrolled response to infection [3]. Studies have shown that sepsis is mainly caused by infection or other attacks, leading to the excessive activation of the innate immune system, instigating an inflammatory storm in the early stage of sepsis, which stands as the primary cause of organ dysfunction and mortality [4]. Despite numerous studies on anti-inflammatory strategies for sepsis, they most often focus on a singular inflammatory factor. Trials employing single strategies such as antagonizing tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), among others, have consis-

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tently ended in failure [5-7]. Therefore, there is a critical need for breakthroughs in the diagnosis and treatment of the early inflammatory response in sepsis.

Pyroptosis, also known as inflammatory cell death, is primarily instigated by cell membrane perforation and subsequent release of large quantities of inflammatory factors within the cell, inciting an inflammatory cascade, resulting in damage to various organ functions [8, 9]. The team of Shao et al elucidated the mechanisms of pathogenic bacterial infection and natural immune defense, confirming that caspase-1, and caspase-4/-5/-11 act as intracellular receptors for bacterial endotoxins during the course of sepsis. Upon activation, they can cleave gasdermin D (GSDMD) protein to activate its N-terminal, leading to the formation of activated GSDMD protein fragments capable of puncturing holes in the cell membrane, thereby disrupting it from within the eukaryotic cell. Membrane rupture triggers the extravasation of numerous inflammatory factors, such as interleukin-1β (IL-1β) and interleukin-18 (IL-18), from the cytoplasm, resulting in a robust inflammatory response [10, 11]. This discovery suggests that pyroptosis may occur at the early stage of sepsis, forming a "cellular and cytokine network", leading to an early inflammatory storm, progressing to tissue and organ damage [12].

Elabela (ELA) represents a novel endogenous ligand of angiotensin II type I receptor. Comprising 54 amino acids, it undergoes cleavage by the Golgi apparatus to yield a mature active polypeptide of 32 amino acids with a relative molecular mass of approximately 3872. ELA is primarily expressed in adult kidneys and pluripotent stem cells [13, 14]. In our study, we observed a significant decrease in the mortality rate of septic mice treated with Ela32. Previous studies have demonstrated that ELA can significantly inhibit DNA damage and inflammatory responses in renal tubular cells after ischemia/reperfusion injury, activate G protein-coupled receptors to trigger various G protein-related signaling pathways, and affect the oxidative stress effects of various cell death progression-related diseases and inflammation [15-18]. In this study, we found a significant decrease in the expression level of ELA in septic patients, with the strongest correlation observed with the inflammatory factor IL-1β. We synthesized the ELA molecule and administered it through intraperitoneal injection to investigate its efficacy in modulating inflammatory response and enhancing bacterial clearance in septic mice.

Materials and methods

Ethics approval statement

This research was approved by the Ethics Committee of the Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University (Jiangsu, China) and performed in accordance with the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (Approval number: YX-Z-2022-006-01, 2022-02-22).

Participants recruitment

Following the third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), patients exhibiting systemic signs of infection and a Sequential Organ Failure Assessment (SOFA) (originally the Sepsis-related Organ Failure Assessment) score of 2 or higher were classified as having sepsis [19]. Blood samples were collected upon enrollment into the study, and clinical data were recorded through the Hospital Information System (HIS). The SOFA score is composed of scores from six organ systems (respiratory, renal, hepatic, cardiovascular, coagulation and neurologic) graded from 0 to 4. If the score exceeds two points, it signifies organ injury. Exclusion criteria encompassed individuals under the age of 18, pregnant individuals, those with end-stage organ failure, and individuals with terminal illness. During hospitalization, if patients experienced worsening organ dysfunction indicated by a change in baseline of the total SOFA score of 2 points or more, or the occurrence of new complications such as acute respiratory distress syndrome (ARDS), septic shock, disseminated intravascular coagulation (DIC), multiple organ dysfunction syndrome (MODS) or death, we categorized them as having deterioration of sepsis. Finally, 43 patients with sepsis were recruited from our ICU, along with 20 healthy donors from the Health Examination Center of the affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University during December 2022 to October 2023. The study was approved

by the Ethics Committee of the Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University, and informed consent was obtained from each participant, in adherence to the principles outlined in the Helsinki Declaration.

Reagents

FAM-FLICA® Caspase-1 Assay Kit was procured from ImmunoChemistry Technologies (FAM-YVAD-FMK 655). FLICA probes contain a specific 3 or 4 amino acid sequence that can be specifically recognized by cleaved-caspase 1. PE Annexin V Apoptosis Detection Kit I and 7AAD was obtained from BD PharmingenTM, while Lipopolysaccharides from Escherichia coli0111: B4 were sourced from Sigma. The anti-cleaved N-terminal GSDMD antibody (EPR20829-408) was purchased from Abcam, and Caspase-1 p20 was acquired from Proteintech Group, Inc., based in Rosemont, IL, USA.

Animals

C57BL/6 mice, aged 6-8 weeks and weighing between 18-25 g, were purchased from the Jiangsu Aniphe Biolaboratory Inc. These mice were subsequently transported to the Animal Center of the affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University one week prior to commencement of the experiment. Upon arrival, they were caged in a temperature-controlled environment (22±2°C) with a 12/12 hour light/dark cycle with ad libitum access to food and water. For euthanasia, mice were anesthetized and administered KCL intravenously at a dosage of 1-2 mg/kg. Following specimen collection, all experimental mouse cadavers were centrally gathered within the laboratory premises for environmentally friendly disposal, ensuring compliance with ethical guidelines and regulations.

Sepsis model

The sepsis model was made by cecal ligation and puncture (CLP) as described by Rittirsch in Nature Protocols [20]. Mice were administered intraperitoneal pentobarbital at a dosage of 50 mg/kg for anesthesia. After successful anesthesia, the mice were placed on a temperature-controlled heating pad to maintain body temperature. A midline incision was made just below the xiphoid process using a surgical blade, cutting through the abdominal muscles to enter the abdominal cavity. The cecum was carefully exteriorized using non-toothed forceps, and the intestinal mesentery was dissected to maintain the integrity of the small and large intestines within the abdominal cavity. Subsequently, the cecum was ligated approximately 1 cm from the distal end with a single transfixion using an 18-gauge needle. A small amount of cecal content was gently extruded using forceps or fingers. The cecum was then repositioned into the abdominal cavity, and the incision was closed in two layers, involving the abdominal muscles and skin. A subcutaneous injection of warm physiological saline was administered in the neck and back area. Following closure of the incision, the mice were placed on the heating pad and allowed to recover from anesthesia, demonstrating the ability to flip themselves, before being returned to their cages. For the sham group, identical procedures were followed except for the ligation and puncture of the cecum, serving as a control for the experimental group.

Haematoxylin-eosin (H&E) staining

The organs were fixed in a 4% formaldehyde solution for a minimum of 24 hours to ensure proper preservation. Subsequently, tissue was embedded in paraffin and sectioned at 4 μm. These slices were then stained using hematoxylin and eosin and assessed microscopically.

Flow cytometry

The peritoneal macrophages and peripheral blood immune cells were homogenized to achieve a single-cell suspension. The cells were washed twice and 1×10^6 cells were resuspended in 50 μL of PBS supplemented with 1% FBS, and then stained with FLICA (FAM-VAD-FMK655) to detect Cleaved-caspase 1 for pyroptosis and PE Annexin V Apoptosis Detection Kit I and 7AAD for apoptosis. Following staining, the cells were subjected to analysis using a FACSCalibur flow cytometer. The acquired data were subsequently analyzed utilizing FlowJo software to characterize and quantify the stained cells, facilitating the assessment of pyroptosis and apoptosis.

Synthesis and application of Ela32

Through literature retrieval and the NCBI database, a mature active polypeptide consisting of

32 amino acids of ELA was identified. Suzhou Qiangyao Biotechnology Co., Ltd. was then engaged for the processing, synthesis and purification of the polypeptide. Ela32 was dissolved in 1×PBS at a dose of 1.5 uM/kg.

The sequence of Ela32 is as follows: Gln-Arg-Pro-Val-Asn-Leu-Thr-Met-Arg-Arg-Lys-Leu-Arg-Lys-His-Asn-Cys-Leu-Gln-Arg-Arg-Cys-Met-Pro-Leu-His-Ser-Arg-Val-Pro-Phe-Pro.

Isolation and culture of peritoneal macrophages

After administering 1 mL of sterile 3% thioglycolic acid broth into the abdominal cavity of C57BL/6 mice for three consecutive days, the mice were deeply anesthetized, and then injected with 5 mL of 10% serum RPMI 1640 culture medium into the abdominal cavity, followed by gentle abdominal massage for 5 minutes. The peritoneal cavity lavage fluid was then repeatedly washed twice, and the aspirated abdominal cavity lavage fluid was centrifuged at 1000 rpm for 10 minutes at room temperature. The resulting cell pellet was resuspended in RPMI 1640 culture medium and inoculated at 37°C for 2-3 hours. Following incubation, non-adherent cells were washed away, leaving behind adherent macrophages for further experimentation.

Enzyme linked immunosorbent assay (ELISA)

Serum samples were collected for analysis. The levels of IL-1β and IL-6 were measured using ELISA kits obtained from NeoBioscience, while ELA levels were determined using the [pGlu1]-ELA-32 Kit from Phoenix Pharmaceuticals, INC. All assays were performed in accordance with the manufacturer's instructions to ensure accuracy and reliability of the results.

Western blot

Peritoneal macrophages were lysed in an ice-cold RIPA lysis buffer containing 1% PMSF. The protein concentration was determined using the BCA kit. The protein samples were dissociated by SDS polyacrylamide gel and transferred onto the PVDF membrane, and then sealed with 10% skimmed milk at room temperature for 1 hour, followed by overnight incubation with the appropriate primary antibody at 4°C. After washing the membrane three times with TBST, the secondary antibody was applied and incubated at room temperature for 2 hours. The membrane was washed with TBST three times and then incubated with ECL color solution for 1 minute. Chemiluminescence images were captured using darkroom development technology.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism 10.0 software (GraphPad Software Inc., San Diego, CA, USA). Continuous variables are presented as either mean ± standard deviation (Mean ± SD) or median with interquartile range. The comparisons between groups were performed using one-way analysis of variance (ANOVA) with a post-hoc Turkey's test for multiple comparisons for normally distributed data and Kruskal Wallis test for other data. Survival data were analyzed using the log-rank (Mantel-Cox) test. A P value of 0.05 or less was considered significant.

Results

Inflammatory response and organ injury in patients with sepsis

In our intensive care unit (ICU), we conducted a study involving electronic medical records and blood samples from a cohort of 20 healthy control individuals and 43 patients with sepsis. Among the septic patients, 27 cases showed clinical improvement while 16 cases exhibited deterioration. Notably, septic patients had a higher prevalence of severe organ complications, including circulatory failure, pulmonary, hepatic, renal, coagulation system, and neurologic impairment, which contributed to poor prognosis and a high mortality rate (38%) (**Figure 1A** and **Table 1**). Conversely, patients with fewer complications experienced better prognoses and lower mortality rates (**Figure 1A**). Using ELISA kits, we measured the expression levels of inflammatory factors IL-6, IL-1β, IL-10, and ELA in serum. Septic patients demonstrated significantly elevated levels of IL-6 and IL-1β in serum, with higher levels of inflammatory response associated with worse prognosis and statistically significant differences (**Figure 1B**, **1C**). However, IL-10 showed no significant change (**Figure 1D**). Additionally, patients with sepsis exhibited reduced ELA expression levels compared to the healthy con-

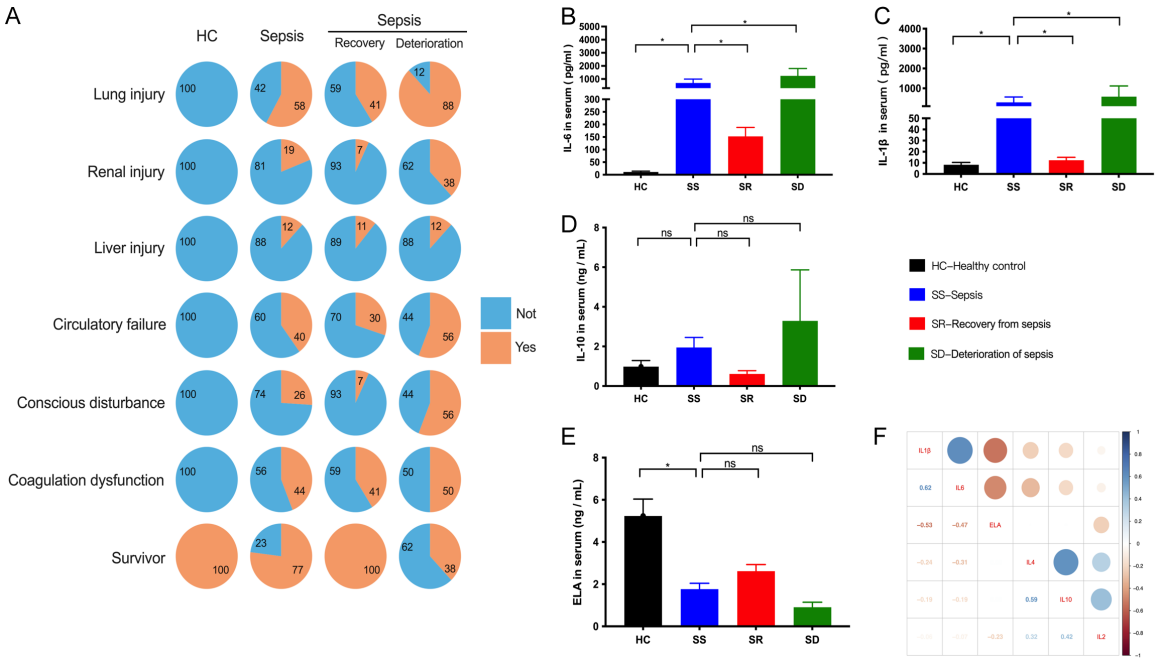

Figure 1. The expression of Elabela (ELA) in septic patients is correlated with inflammatory response and organ injury. (A) Pie charts depict the prevalence of multiple organ injury and mortality in the healthy control (HC) group (n = 20) and sepsis groups (sepsis, n = 43; recovery from sepsis, n = 27; deterioration of sepsis, n = 16). (B-D) Bar graphs illustrate the levels of IL-6 (B), IL-1β (C), and IL-10 (D) in serum of septic patients with different outcomes. (E) The expression levels of ELA in the serum of individuals from different groups. (F) Correlation analysis between ELA and various inflammatory factors in septic patients. Red indicates a negative correlation, while blue indicates a positive correlation. The size and color intensity of the circles reflect the magnitude of the correlation coefficients. Data are presented as the mean ± standard error of the mean. *P<0.05. ns, P>0.05.

Table 1. Characteristics of patients with different outcomes of sepsis

Index	Healthy Donors	Septic patients		*P* value
		Recovery	Deterioration	
At the time of admission:				
No. of individuals	20	27	16	NS
Age (yr)	63 (39-79)	71 (49-82)	76 (30-82)	NS
Male/female	13/7	16/11	11/5	NS
BMI (kg/m^2)	24.7±2.51	22.66±3.13	23.86±4.19	NS
APACHE II score	3.00±1.59	9.96±4.71	17.25±6.89	<0.05
SOFA score	0.15±0.37	6.48±3.27	7.81±3.27	<0.05
Length of ICU stay (d)	NA	5.0 (2-50)	3.5 (1-43)	<0.05
Leukocytes, *10^9/L	6.09±1.54	15.61±10.52	10.20±10.04	<0.05
Platelet (*10^9/L)	200.40±58.19	164.56±88.38	153.81±103.57	NS
PCT (ng/mL)	0.06±0.12	31.15±30.82	39.38±36.04	<0.05
Sepsis due to	NA			
Peritonitis		13	10	
Pneumonia		1	3	
Urinary tract infection		2	1	
Others		11	2	

Abbreviations: BMI, body-mass index mean; APACHE II, acute physiology and chronic health evaluation II; ICU, intensive care unit; PCT, procalcitonin; NA, not applicable; NS, not significant.

trol group (**Figure 1E**). Consequently, Pearson correlation analysis between ELA and various inflammatory factors (IL-6, IL-1β, IL-10, IL2, IL4) in septic patients revealed a negative correlation between ELA concentration and IL-1β content (r = -0.609, *P* = 0.036), while no significant correlation was observed with other inflammatory factors, including IL-6 (*P* = 0.056) (**Figure 1F**).

Pyroptosis of peripheral blood monocytes in patients with sepsis

Following erythrocyte lysis, peripheral blood cells underwent flow cytometry analysis. Initial observations of changes in peripheral blood immune cell count changes were based on forward scatter (FSC) and side scatter (SSC). Statistical analysis revealed a significant decrease in the number of monocytes in the peripheral blood of septic patients (**Figure 2A**). Apoptosis was assessed using Annexin V and 7AAD staining, while pyroptosis was evaluated through Cleaved-caspase 1 and PI staining. The results showed a significantly higher rate of pyroptosis in monocytes of septic patients compared to the healthy control group, with significant differences, while apoptosis showed no significant difference (**Figure 2B**, **2C**).

Intraperitoneal injection of ELA improves the survival rate and attenuates inflammation and organ injury in septic mice

We established a sepsis model in C57BL/6 mice through cecal ligation surgery, immediately followed by intraperitoneal injection of Ela32 (n = 10). The number of surviving mice was recorded over a ten-day period, and survival analysis was performed. Additionally, the expression changes of ELA and the pyroptosis-related inflammatory factor IL-1β in mouse serum were observed at different time points (day 0, 1, 3, 5, 7) (**Figure 3A**). We observed that compared to the CLP group, mice treated with Ela32 exhibited a higher survival rate in sepsis (70%) with a significant difference (**Figure 3B**). Peripheral serological tests revealed a significant decrease in ELA levels in serum from the CLP group on the first day after surgery. However, intraperitoneal injection of Ela32 in the ELA treatment group elevated the serum ELA levels, with a significant difference (**Figure 3C**). When measuring IL-1β levels at different time points, we found that the secretion of IL-1β peaked on the first day after CLP modeling, and the inflammatory response was significantly lower in the group treated with Ela32 (**Figure 3D**). Nutrient agar plate assays were utilized to detect differences in the bacterial counts in the peritoneal lavage fluid of mice on the first day. The results indicated that the number of bacterial colonies in the peritoneal lavage fluid of mice in the CLP group was significantly higher than that in the sham group and the ELA treatment group (**Figure 3E**), suggesting that ELA may enhance the bacterial clearance ability in septic mice. Organ tissues from mice in each group were selected on the first day for H&E staining and injury scoring. Mice in the CLP group exhibited pathologic damage to the lungs and kidneys on the first day of sepsis, while mice in the ELA treatment group showed relatively lighter inflammatory cell infiltration in the lungs, as well as glomerular atrophy and diffuse expansion of renal tubules in the kidney. Pathological injuries in the heart, liver, spleen, and intestines were less apparent on the first day (**Figure 3F-I**).

ELA treatment alleviated the pyroptosis-related infammatory response

Primary peritoneal macrophages were extracted and cultured from C57BL/6 mice. Following pretreatment with 10 μg/mL of Ela32, they were stimulated with 100 ng/ml of LPS for 24 hours. Subsequently, Cleaved-caspase 1 and 7AAD staining, along with flow cytometry, were used to detect the pyroptosis (Cleaved-caspase 1+ 7AAD+) and inflammatory response of peritoneal macrophages. It was observed that after LPS stimulation, the pyroptosis of peritoneal macrophages increased, whereas pretreatment with Ela32 significantly reduced the pyroptosis of peritoneal macrophages (**Figure 4A**, **4B**). Supernatants were extracted to detect the levels of inflammatory cytokines IL-6, IL-1β, and ELA, revealing that Ela32 significantly attenuated the inflammatory response, with a significant differences noted (**Figure 4C-E**). Western blot analysis was utilized to detect the pyroptosis signaling pathway, indicating that ELA could inhibit the cleavage of caspase-1 and GSDMD, thereby reducing the release of pyroptosis and the inflammatory response (**Figure 4F**).

Discussion

Sepsis, a life-threatening condition triggered by an exaggerated immune response to infection,

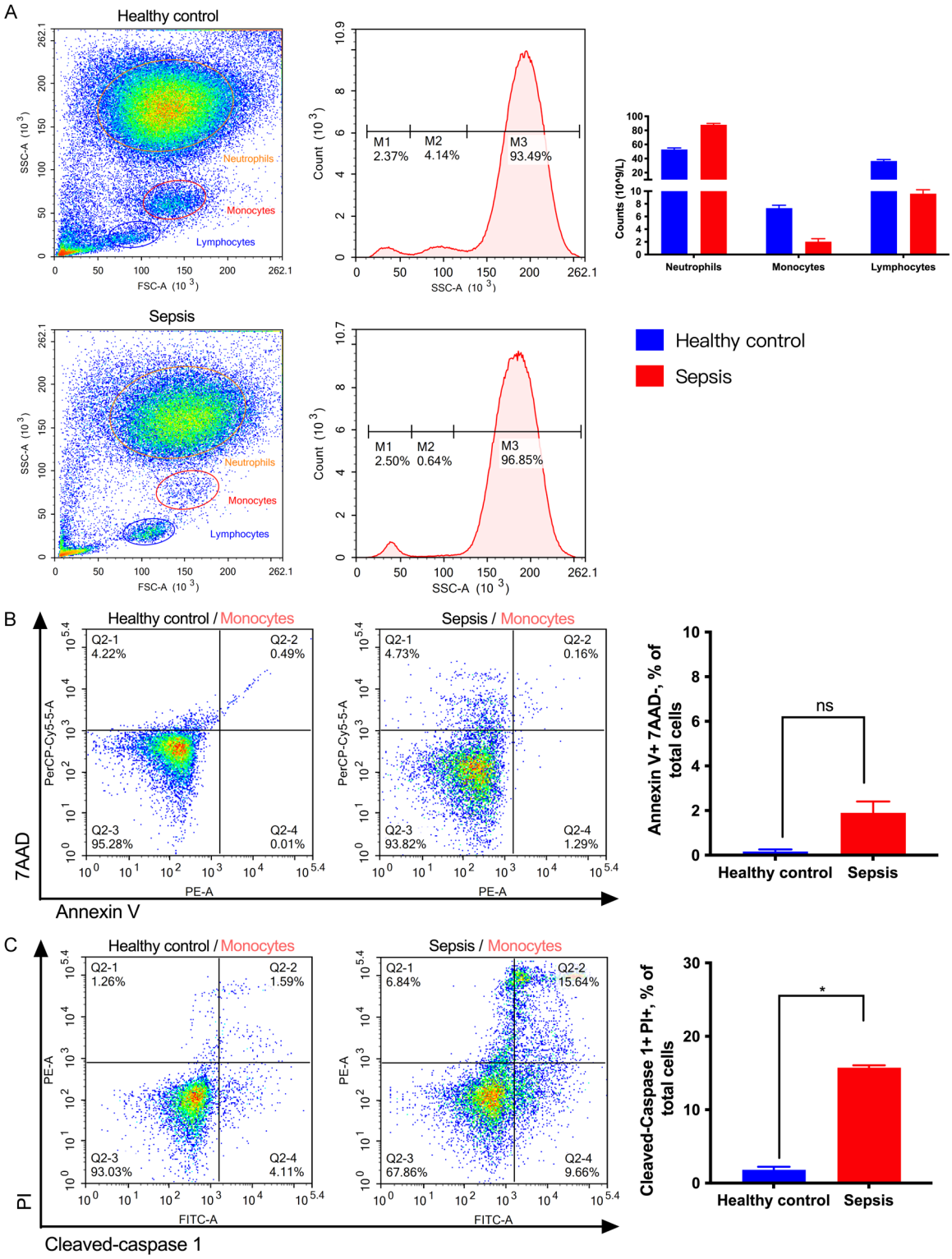

Figure 2. Monocyte pyroptosis significantly increases in peripheral blood of septic patients. (A) Comparison of the number of immune cells in the peripheral blood between septic patients and healthy controls by flow cytometry. (B, C) Quantitative analysis of Annexin V+7AAD- apoptotic cells (B) and of Cleaved-caspase 1+PI+ pyroptotic cells (C) among septic patients and healthy controls. Results are mean ± standard error of the mean. *P<0.05. ns, P>0.05. FSC, Forward Scatter; SSC, Side Scatter.

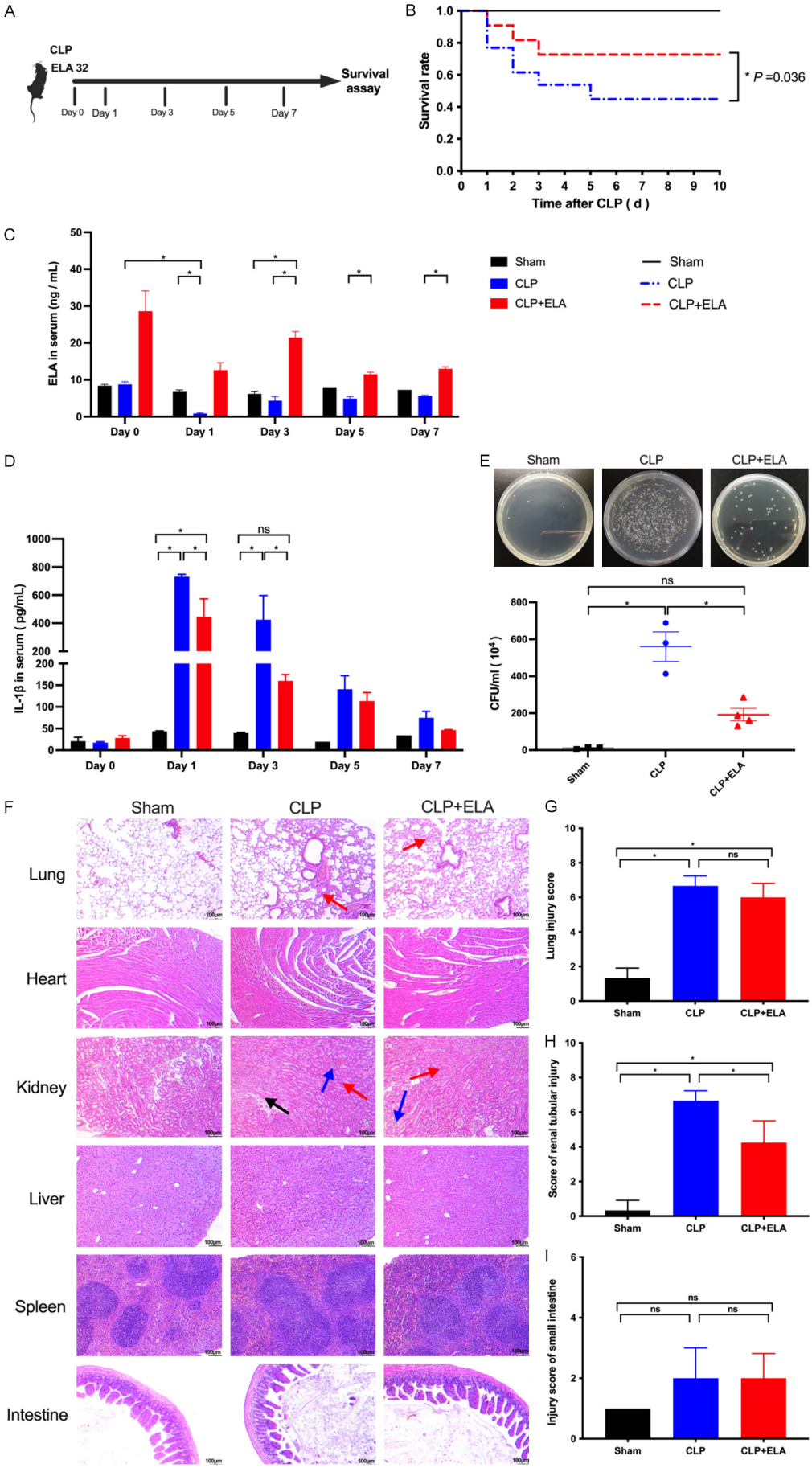
A
CLP
ELA 32
Survival assay
Day 0
Day 1
Day 3
Day 5
Day 7
B
Survival rate
Time after CLP (d)
* P =0.036
C
ELA in serum (ng / mL)
Sham
CLP
CLP+ELA
D
IL-1β in serum (pg/mL)
ns
E
Sham
CLP
CLP+ELA
CFU/ml (10⁴)
F
Lung
Heart
Kidney
Liver
Spleen
Intestine
G
Lung injury score
H
Score of renal tubular injury
I
Injury score of small intestine

Figure 3. ELA plays an important protective role in the early inflammatory response and survival rate of mice with sepsis. (A) Septic mice with or without ELA treatment were subjected to cecal ligation and puncture (CLP) as described in "Methods". Sham-operated mice underwent surgery without CLP. Mice were sacrificed to collect serum and organ specimens at multiple time points (day 0, 1, 3, 5, 7). (B) Survival curves of mice in different groups. (C, D) Bar graphs illustrate the levels of ELA (C) and IL-1β (D) in serum of mice at different times. (E) Smear examination and bacterial culture results of peritoneal lavage fluid of the three groups at 24 h after CLP. (F) Pathologic changes were seen in the lung, heart, kidney, liver, spleen, and intestine of the three groups at 24 h after CLP. Tissue sections were stained with hematoxylin and eosin (H&E) (scale bar = 100 μm); red arrow indicates inflammatory cell infiltration; blue arrow in the kidney images indicates glomerular atrophy; black arrow indicates diffuse expansion of kidney tubules. (G-I) Histologic scoring of the main organ injury as lung (G), kidney (H), and intestine (I) assessed from H&E-stain. All values are mean ± standard error of the mean. *, P<0.05; ns, P>0.05.

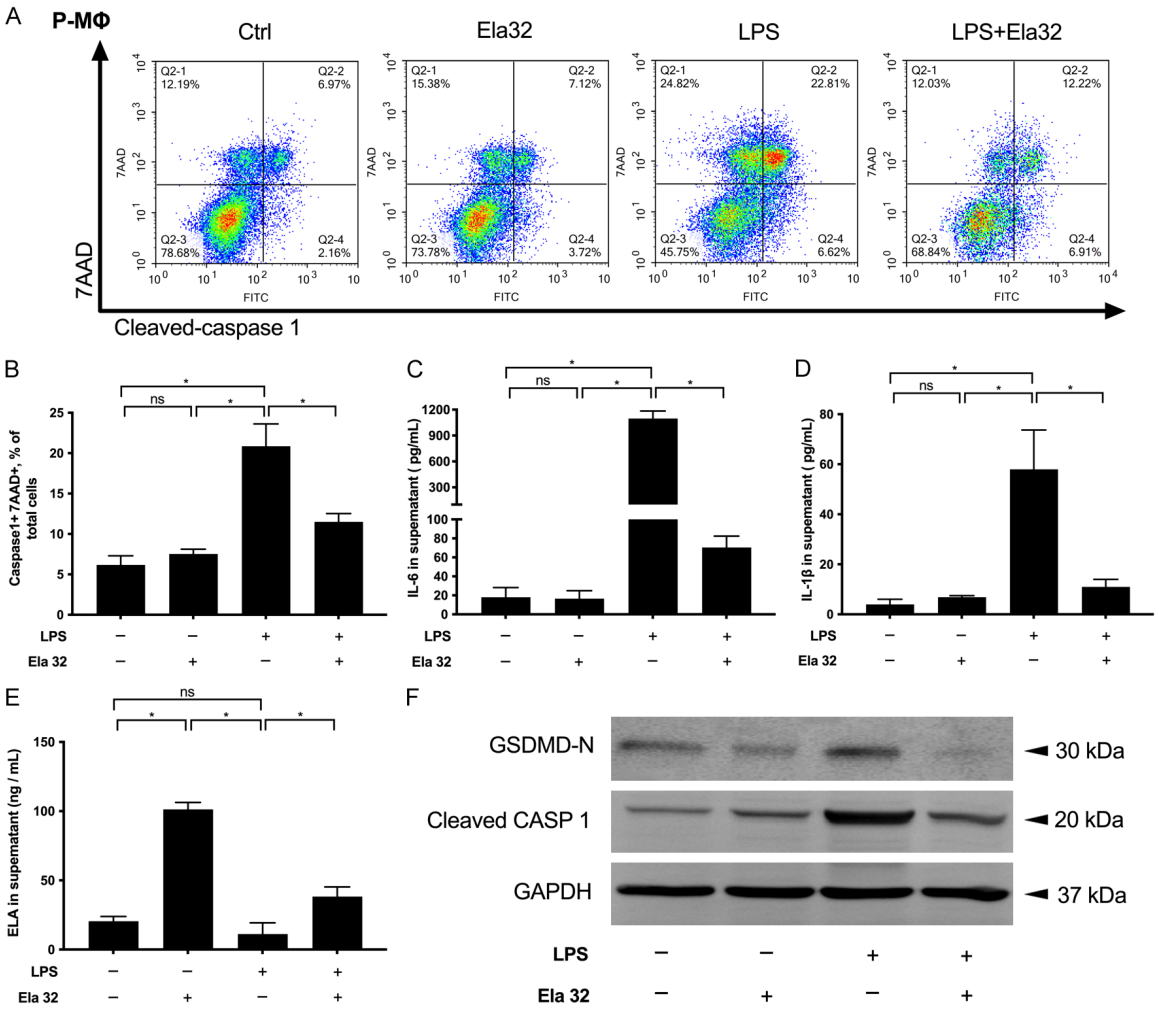

Figure 4. ELA alleviates pyroptosis and the release of inflammatory factors by inhibiting gasdermin D (GSDMD) cleavage. (A) LPS-stimulated peritoneal macrophages were treated with Ela32 (10 μg/mL) and stained with Cleaved-caspase 1 and 7AAD, then measured by flow cytometry. (B) Quantitative analysis of Cleaved-caspase 1+ 7AAD+ pyroptotic cells in the four groups. (C-E) ELISA results for the concentration of IL-6 (C), IL-1β (D), and ELA (E) in supernatants of peritoneal macrophages stimulated with LPS (100 ng/ml) for 24 h. (F) Western blot of Cleaved-caspase 1 and GSDMD-N in each group. Data are mean ± standard error of the mean. *, P<0.05; ns, P>0.05.

often leads to a severe inflammatory response known as the inflammatory storm. This phenomenon is characterized by the excessive release of inflammatory cytokines and mediators, which can induce widespread tissue damage and organ dysfunction. A pivotal cellular event within this inflammatory cascade is pyroptosis, a programmed cell death mecha-

nism resulting in the release of harmful intracellular contents [21, 22]. The pathogenesis of sepsis is intricate, involving a network of immune cells and mediators. Among these, macrophages emerge as central players in the inflammatory response [23-25]. Notably, macrophage pyroptosis stands out as a significant contributor to the early inflammatory surge observed in sepsis.

Recently, ELA, a peptide hormone belonging to the adrenomedullin family, has emerged as a promising therapeutic candidate to mitigate the detrimental effects of sepsis due to its demonstrated anti-inflammatory and vasoprotective properties [26, 27]. Our study reveals that septic patients exhibit a higher incidence of severe organ complications, leading to poor prognosis and elevated mortality. Notably, these patients also manifest significantly elevated levels of inflammatory factors such as IL-6 and IL-1β in serum, indicating a robust inflammatory response. However, ELA expression levels are diminished in septic patients compared to the healthy controls. The mechanism underlying the protective effect of ELA in sepsis is intriguing. It has been demonstrated that ELA effectively inhibits macrophage pyroptosis, thereby suppressing the release of inflammatory cytokines that drive the inflammatory cascade. This inhibition not only curtails tissue damage but also mitigates the development of multi-organ dysfunction, a common complication of sepsis. Hence, it is apparent that ELA plays a pivotal role in modulating the inflammatory response in septic patients. Our analysis further uncovers a negative correlation between ELA concentration and IL-1β content in septic patients, suggesting a regulatory effect of ELA on the inflammatory response in sepsis. Given the central role of macrophages in orchestrating the inflammatory response, we hypothesize that ELA may mitigate the early inflammatory response in sepsis by suppressing macrophage pyroptosis.

Pyroptosis is a form of programmed cell death characterized by the release of inflammatory cytokines and the activation of the immune system [28]. In the context of sepsis, macrophage pyroptosis can exacerbate the exaggerated inflammatory response observed in these patients. Through the inhibition of macrophage pyroptosis, ELA holds the potential to impede the uncontrolled release of inflammatory cytokines and subsequent tissue damage, thus attenuating the inflammatory response in sepsis. Our research has found that ELA may inhibit macrophage pyroptosis by suppressing the cleavage of caspase-1 and GSDMD, thereby reducing the release of inflammatory cytokines and averting the onset of a cytokine storm. The equilibrium between pro- and anti-inflammatory mediators facilitates the resolution of inflammation and fosters the tissue repair processes.

The therapeutic potential of ELA in sepsis is further substantiated by preclinical studies. Animal models of sepsis and peritoneal macrophages treated with synthetic Ela32 have demonstrated reduced macrophage pyroptosis, decreased levels of inflammatory cytokine, and improved survival rates compared to untreated controls. These compelling findings suggest that ELA-based therapies could be effective in mitigating the detrimental effects of sepsis in humans. However, translating these preclinical findings into clinical practice remains challenging. Additional studies are imperative to comprehensively elucidate the molecular mechanisms underlying the anti-inflammatory effects of ELA in sepsis and to investigate its safety and efficacy in human patients. Furthermore, determining the optimal dosing, timing, and route of administration of ELA or its analogs necessitates rigorous investigation through clinical trials.

In conclusion, our findings indicate that ELA may serve as a therapeutic target for mitigating the early inflammatory response in septic patients. Future studies are warranted to delve deeper into the molecular mechanisms underlying the inhibitory effect of ELA on macrophage pyroptosis and to elucidate its therapeutic implication in sepsis. Continued research in this area may facilitate development of novel therapeutic strategies to improve patient outcomes.

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Disclosure of conflict of interest

None.

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