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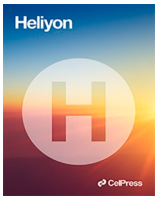

Research article

Novel therapeutic role of Ganoderma Polysaccharides in a septic mouse model - The key role of macrophages

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ABSTRACT

Ganoderma lucidum polysaccharides (G. PS) have been recognized for their immune-modulating properties. In this study, we investigated the impact of G. PS in a sepsis mouse model, exploring its effects on survival, inflammatory cytokines, Treg cell differentiation, bacterial load, organ dysfunction, and related pathways. We also probed the role of macrophages through chlorphosphon-liposome pretreatment. Using the cecal ligation and puncture (CLP) model, we categorized mice into normal, PBS, and G. PS injection groups. G. PS significantly enhanced septic mouse survival, regulated inflammatory cytokines (TNF-α, IL-17A, IL-6, IL-10), and promoted CD4+Foxp3+ Treg cell differentiation in spleens. Additionally, G. PS reduced bacterial load, mitigated organ damage, and suppressed the NF-κB pathway. In vitro, G. PS facilitated $CD4^+$ T cell differentiation into Treg cells via the p-STAT5 pathway. Chlorphosphon-liposome pretreatment heightened septic mortality, bacterial load, biochemical markers, and organ damage, emphasizing macrophages' involvement. G. PS demonstrated significant protective effects in septic mice by modulating inflammatory responses, enhancing Treg cell differentiation, diminishing bacterial load, and inhibiting inflammatory pathways. These findings illuminate the therapeutic potential of G. PS in sepsis treatment.

1. Introduction

Sepsis, described as a systemic organ dysfunction, emerges from a rampant inflammatory reaction in the body, consistently ranking as a leading cause of mortality in critically ill patients [1]. Cutting-edge epidemiological studies reveal that sepsis affects approximately 450 out of every 100,000 individuals [2]. Astonishingly, this incidence surpasses the combined rates of myocardial infarctions and prevalent cancers such as those of the lung, breast, and prostate [3]. Each year, a staggering 150 to 300 million individuals globally are diagnosed with sepsis, with a sobering 20% not surviving this affliction [4]. Even with the meticulous application of sepsis-3 treatment protocols, the mortality statistics associated with this condition have remained disturbingly stable [5].

At the crux of sepsis lies the body's immune mechanisms. Macrophages, often hailed as the immune system's stalwarts, are

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instrumental during the initial stages of most infections. However, their role in sepsis can be ambivalent, at times intensifying the inflammatory cascade rather than curbing it [6,7]. T cells, with a special emphasis on regulatory T cells (Treg), are paramount in striking a harmonious balance between pro-inflammatory and anti-inflammatory actions. A suboptimal T-cell mediated response can propel sepsis into a more severe phase, leading to widespread organ failure and, in many instances, death [8,9]. The detrimental impact of sepsis-induced immune suppression has been spotlighted, prompting a surge in research focusing on anti-inflammatory cytokines, immune cell programmed death, and inhibitory signaling in septic patients, thereby refining immunotherapeutic approaches [10–12]. The essence of the immune dysregulation in sepsis can be distilled down to a precarious imbalance between anti-inflammatory and pro-inflammatory reactions [13,14]. The phenomenon termed "cytokine storm", a vehement release of inflammatory agents, stands out as a primary harbinger of poor outcomes in septic patients [15].

In the face of the plethora of advancements in the medical arena, sepsis remains a formidable challenge to treat. Traditional antimicrobial therapies, while proficient in addressing the root cause of the infection, often fall short in modulating the widespread inflammatory fallout of sepsis. This therapeutic conundrum is further complicated by the looming threat of bacteria that defy multiple drugs [16,17]. This underscores the urgent need to pioneer innovative therapeutic modalities and to pinpoint novel treatment targets.

In the vast compendium of medicinal mushrooms, Ganoderma lucidum, popularly known as 'lingzhi' in China, holds a place of distinction. Revered not only for its therapeutic prowess but also as a culinary delight, it has been a staple in Asian households and traditional medicine practices for over two millennia [18]. As the nexus of modern science and traditional wisdom emerges, contemporary research has focused intently on unraveling the myriad of bioactive compounds nestled within Ganoderma lucidum's fruiting body. This intricate matrix comprises peptides, polysaccharides, proteins, triterpenoids, and sterols, each with its own therapeutic signature [19].

Significantly, G. lucidum is rich in polysaccharides (G. PS), which have emerged as its primary bioactive arsenal. These polysaccharides have demonstrated a spectrum of biological activities, from antioxidant properties [20] to antitumor [21], anti-inflammatory [22], and immune-modulating effects [23], to even influencing lipid metabolism [24]. Delving into the immunoregulatory facets of G. PS, it becomes apparent that they play pivotal roles in diverse cellular processes, including the augmentation of macrophage proliferation, T lymphocyte activation, spleen cell growth enhancement, and the synthesis of antibodies and cytokines [18].

Experimental insights derived from rat models have further accentuated the potential of G. PS. Administered at specific dosages, these polysaccharides have shown a capability to temper the inflammatory cascade, notably by downregulating key inflammatory markers such as IL-6, TNF-α, NF-κB, and iNOS [25]. In a parallel vein, recent discoveries have spotlighted sulfated polysaccharides from Ganoderma lucidum as promising anti-inflammatory agents, with the capacity to mitigate L-selectin-induced inflammation and allay cytokine-driven inflammatory storms.

Yet, despite these promising strides, the intricate interplay of G. PS within septic conditions remains shrouded in mystery. Preliminary observations from our team have hinted at a protective aura that Ganoderma lucidum polysaccharides might bestow in septic mouse models, potentially through modulations in T-cell differentiation dynamics. However, the granular molecular pathways underlying these observations beckon a more thorough exploration.

In this study, we conducted a CLP-induced sepsis model to investigate the specific role of Ganoderma lucidum polysaccharides (GLP) in a mouse model of sepsis, particularly their impact on the differentiation and function of macrophages and T cells. Through this research, we aim to provide important theoretical evidence and molecular targets for novel therapeutic approaches to sepsis. Moreover, this study may also reveal the potential application of GLP as an immunomodulator in other inflammatory diseases.

2. Materials and methods

2.1. Establishment and grouping of sepsis mouse model

C57BL/6 male mice, aged between 8 and 12 weeks old and weighing 20–24 g, were acquired from the Experimental Animal Center at Chongqing Medical University in Chongqing, China (2021020). Each mouse is housed in specific pathogen-free (SPF) conditions, with a controlled relative humidity of 50%–60% at 24 °C and a 12-h light/12-h dark cycle. They are allowed to eat and drink freely. Throughout the experiment, all of the mice remained in a healthy condition without any signs of infection. Every animal is treated according to the Care and Use Guidelines for Chinese Experimental Animals. All experiments involving mice were approved by the Animal Ethics Committee of Chongqing Three Gorges Medical College (No. SYYZ-A-2212-0005).

To simulate sepsis, we employed the cecal ligation and puncture (CLP) method, a technique previously described in the literature [26]. Mice were anesthetized with chloramphenicol (80 mg per kg body weight, Sigma) and thiopental (10 mg per kg body weight, Sigma). The cecum was then exposed, ligated, and perforated once using a 21-gauge needle (BD Biosciences). Subsequently, the cecum was replaced into the abdominal cavity and the incision was sutured using Ethicon thread. To accelerate recovery, mice were administered subcutaneous injections of saline solution (5 mL per 100 g body weight, Sigma).

Depletion of macrophages using sodium clodronate liposomes: A total of 200 μL of sodium clodronate liposomes (Clo-lip, Sigma) was injected into the peritoneal cavity of mice. Four days later, a second dose of 100 μL of Clo-lip was administered. After 48 h, the effect of Clo-lip on macrophage depletion was assessed using immunofluorescence staining (Thermo Fisher Scientific).

Intraperitoneal Administration of G. PS: Following two administrations of Clo-lip, mice underwent cecal ligation and puncture (CLP) surgery 48 h later. Two hours after the CLP procedure, mice were intraperitoneally injected with G. PS (100 mg/kg, Chengdu Desite Biotechnology Co., Ltd). As a control group, mice were injected with PBS (Sigma). Twenty-four hours after CLP modeling, organs and body fluids were collected from the mice for subsequent analysis.

2.2. Serum biochemical testing

Blood was collected from mice within 24 h after CLP using the retro-orbital venous extraction method following anesthesia. The collected blood was then stored in heparin-containing tubes (BD Biosciences). The levels of creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined using a Roche biochemical analyzer.

The expression of inflammatory factors TNF-α, IL-6, IL-17A, and IL-10 in serum, peritoneal fluid (PF), and bronchoalveolar lavage fluid (BALF) was detected using the Cytokine Magnetic Bead Panel assay kit (eBioScience).

Serum levels of immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) were measured using ELISA kits obtained from Kangrung Biotech Company in Shanghai, China, following the instructions provided by the manufacturer.

2.3. Measurement of bacterial colony forming units (CFU)

First, we performed peritoneal lavage using PBS (5 mL, Sigma) to obtain PF. Subsequently, the diluted PF or peripheral blood was plated on blood agar plates (BD Biosciences) and incubated at 37 °C for 24 h. The colony-forming units (CFUs) were then counted and recorded. Each data point was independently measured three times.

2.4. Flow cytometry

Splenic T cells were counted and adjusted to a cell density of 1×10^6 cells per group. Subsequently, they were washed with PBS and further prepared as cell pellets. Staining was performed using monoclonal antibodies against Foxp3 and CD4. Anti-Foxp3-APC (eBioScience, USA), anti-CD4-FITC (eBioScience, USA), and the Fixation/Permeabilization kit (eBioScience, USA) were used according to the manufacturer's instructions. Flow cytometry was performed using a FACScan instrument (Becton Dickinson), and the data were analyzed using FlowJo software version 10.8.

2.5. Purification and culture of $CD4^+$ T cells

Mouse spleens were collected and homogenized in pre-cooled HBSS solution. The homogenate was centrifuged at 600g for 5 min, and the pellet was collected. Subsequently, lymphocytes were separated using a gradient centrifugation method with ficoll-Hypaque solution (GE Healthcare, USA). Then, 5×10^7 cells were isolated by cell counting and resuspended in pre-cooled MACS buffer. Non-$CD4^+$ cells were labeled using the appropriate magnetic beads (MACS, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) following the manufacturer's instructions. Finally, using the instructions provided, we used the $CD4^+$ T Cell Isolation Kit II (STEMCELL Technologies, Canada) to isolate naïve $CD4^+$ T cells ($\sim 1 \times 10^5$). The purity of naïve $CD4^+$ T cells was confirmed by flow cytometry (>90%), and all procedures were performed according to the manufacturer's instructions. The cells were stimulated with anti-CD28 and cultured in RPMI 1640 medium (Gibco, Grand Island, New York, USA) supplemented with 10% fetal bovine serum. The cells were incubated at 37 °C in a 5% CO_2 humidified incubator for 3–4 days before subsequent experiments.

2.6. Generation of treg cell subsets

Due to the limited number of Treg cells obtained directly from the circulatory system, we employed the magnetic-activated cell sorting (MACS) method to isolate and enrich Treg cells in mice using their high expression of CD25 (at least 1×10^5 Treg cells were isolated per mouse). Subsequently, Treg cells were cultured in the presence of TGF-β (2.5 ng/mL), IL-2 (50 U/mL), anti-CD3 (5 μg/mL), anti-CD28 (2 μg/mL), L-glutamine (2 mM), and β-mercaptoethanol (50 mM) to induce Treg cell subtypes, with a total co-culture duration of 3 days. Cell counting was performed, and 1×106 cells were selected per group for flow cytometric analysis of surface marker levels and intracellular staining, ensuring the induction effect of G. PS was achieved by adding it to the culture medium.

2.7. Measurement of NO levels

Mouse peritoneal macrophages were collected and cell counting was performed to ensure equal cell numbers (1×10^5 cells per group). The cells were then lysed using cold PBS and an ultrasonic cell disruptor, followed by centrifugation at 16,000×*g* for 20 min. The supernatant was collected and the NO levels were determined using the assay kit (A012, Nanjing Jiancheng Bioengineering Research Institute). The absorbance at 550 nm was measured using an enzyme-linked immunosorbent assay (ELISA) reader (Labserv K3 Touch, Thermo Fisher), and the results were expressed as fold changes relative to the control group. Each experiment was independently performed three times.

2.8. Histological staining of tissues

After a 24-h modeling period, the kidney, lung, liver, and colon tissues from mice were dissected and fixed in 10% formalin, followed by embedding in paraffin. Subsequently, 4 μm sections were prepared and subjected to hematoxylin and eosin (HE) staining. An independent pathologist, blinded to the grouping conditions, performed the analysis of these slides.

Frozen tissue (lung, liver, kidney) slices should be thawed using PBS. Subsequently, incubate the slices with 0.3% Triton X-100 (50 μL) for 15 min to disrupt the cell membranes. Antigens are extracted using sodium citrate and then incubated with sliced normal goat

serum. The slice should be treated with F4/80 antibody (rabbit anti-mouse) and incubated at 4 °C overnight. Reheat for 1 h at room temperature (RT). Next, add goat anti-rabbit antibody conjugated with APC and incubate for 1 h at room temperature. Next, label the cell nuclei with DAPI. Neutral sealant sealed these parts. Observations and photographs were captured using a fluorescence microscope (FV-1000/ES, Olympus, Japan). The quantitative method measured the fluorescence coverage area under a 40 × objective lens with a fixed field of view. Six fields of view were captured for each group, and the average value was calculated. The fluorescence intensity in the images was quantified using ImageJ software.

2.9. Western blot

To investigate the molecular mechanisms underlying the protective effects of Ganoderma lucidum polysaccharides (G. PS) in septic mice, total protein was extracted from tissues (lung, liver, or kidney) or splenic T lymphocytes using the protein extraction kit (Beyotime, China). The activation levels of the classical pro-inflammatory signaling pathways, namely the NF-κB pathway in macrophages or the STAT5 pathway in T lymphocytes, were then determined by Western blot analysis. Total protein extracts were quantified using the BCA protein assay kit (Pierce, USA) to ensure equal loading. Since the phosphorylation levels of p65 and STAT5 positively correlate with their activation and protein function, the phosphorylation status of these proteins was examined to reflect the activity of the respective pathway. To exclude the possibility that changes in protein content affected the phosphorylation levels, total protein levels of p65 and STAT5 were also detected as controls. Protein samples were separated by 10% SDS-PAGE gel and transferred

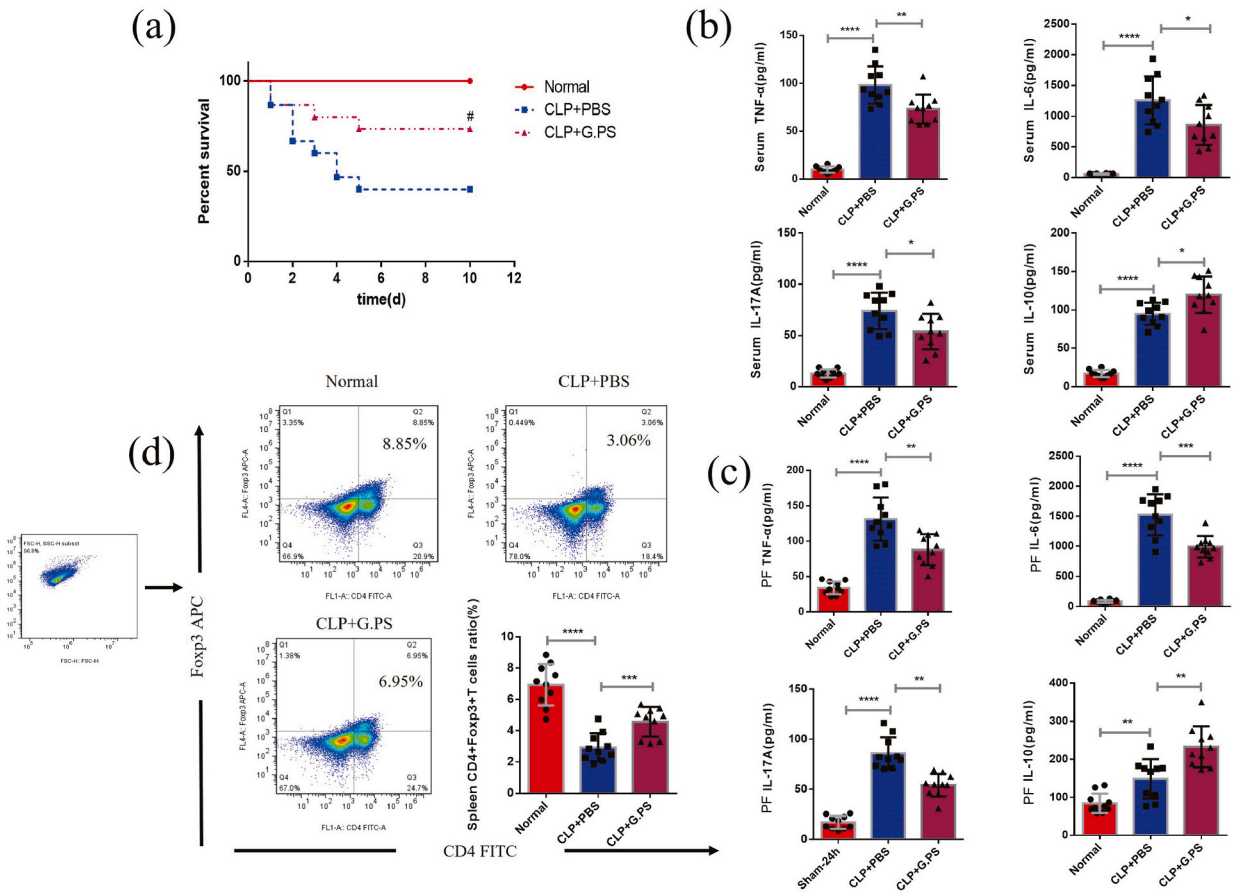

Fig. 1. The Regulatory Effect of G. PS on Inflammatory Response in CLP-induced Septic Mice. Note: CLP + PBS and CLP + G.PS treatment experiments were conducted in C57BL/6 mice. After CLP, each animal was intraperitoneally injected with 100 mg/kg G PS or PBS at 2 h post-CLP. (a) Survival challenge experiment of septic mice treated with G. PS or PBS (n = 15 per group). Comparison between different groups was done using Log-rank test and Kaplan-Meier analysis. Compared to septic mice treated with PBS, #$p < 0.05$. (b, c) G. PS administration reduced the production of pro-inflammatory cytokines in septic mice but enhanced the generation of anti-inflammatory cytokines. Cytokine levels in the PF or serum of G. PS-treated mice were measured 24 h after septic onset using mouse cytokine Magnetic Luminex Assay Kit and compared with PBS-treated control mice. The upregulation of IL-10 and downregulation of IL-6, IL-17A, and TNF-α in serum or PF after G. PS treatment were features of sepsis. (d) Isolation of splenic T cells and subsequent measurement of Treg cell proportion by flow cytometry. Figure d shows a representative dot plot. A significant increase of splenic Tregs was observed at 24 h post-CLP and PBS or G. PS treatment. One-way ANOVA followed by LSD multiple comparison test was used for group comparisons. Compared to CLP-induced septic mice treated with PBS, $p < 0.05$, $p < 0.01$, $p < 0.001$, **$p < 0.0001$.

to nitrocellulose membranes. Subsequently, the membranes were incubated with 5% (w/v) skim milk in tris-buffered saline containing 0.05% Tween-20 at 4 °C. Afterward, the membranes were incubated with GAPDH (#5174), total NF-κB p65 (#8242), phosphorylated NF-κB p65 (#3033), total STAT5 (#25656), and phosphorylated STAT5 (#4322; purchased from CST, USA) antibodies. ChemiDoc XRS Plus luminescent image analyzer (Bio-Rad, https://www.bio-rad.com/zh-cn) was used for imaging. The Western blot bands were quantified for grayscale using Image J analysis software, with GAPDH used as an internal control. Each experiment was independently repeated three times.

2.10. Analysis of phagocytosis by macrophages

Initially, 5 mL of PBS was injected into each mouse to isolate peritoneal macrophages. Subsequently, the samples were subjected to plastic adhesion to separate macrophages from peritoneal fluid. Next, *Escherichia coli* (*E. coli*, purchased from ATCC) was labeled with FITC (0.5 mg/mL, Sigma). The FITC-labeled bacteria, at a multiplicity of infection (MOI) of 100, were then incubated with 1×10^5 peritoneal macrophages at 37 °C for 30 min. After cultivation, the cells were washed, and nuclear staining was performed using DAPI (Invitrogen). The cells were observed using a confocal laser scanning microscope (LSM 510, Zeiss), and 300 cells per well were analyzed for quantification of the proportion of engulfed bacteria. Prior to infecting FITC-labeled *E. coli*, peritoneal macrophages were

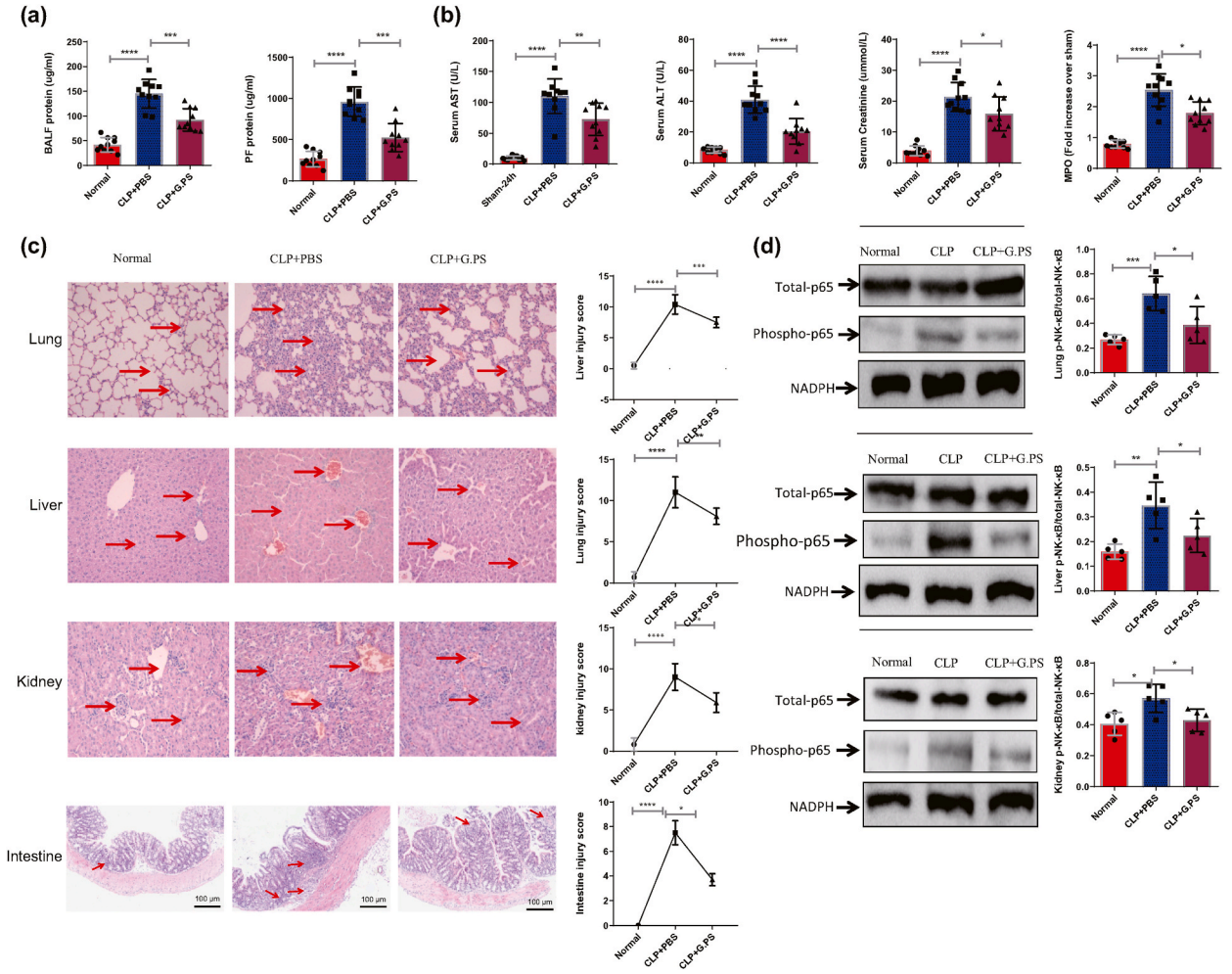

Fig. 2. Protective Effects of G. PS and Regulation of NF-κB Pathway in CLP-induced Septic Mice. Note: (a) Blood vessel permeability was measured by quantifying protein content in PF and BALF obtained from mice 24 h after CLP. These mice received normal control, G. PS, or PBS treatment (n = 10 per group). (b) Organ injury markers such as creatinine, ALT, MPO, and AST were measured in the CLP model after 24 h of normal control, G. PS, or PBS treatment (n = 10 per group). (c) Histological scores of lung, kidney, and liver were analyzed in the septic mouse model treated with G. PS or PBS and the normal control group (n = 10 per group). (d) Levels of NF-κB-related activated protein p-P65 in the liver, lung, and kidney decreased after G. PS treatment compared to the CLP + PBS group. Total-P65, p-P65, and GAPDH levels in tissues at 24 h after CLP-induced sepsis were measured by Western blot analysis. One-way analysis of variance (ANOVA) followed by an LSD multiple comparison test was used for inter-group comparisons. Compared to the CLP + PBS group, $p < 0.05$, $p < 0.01$, $p < 0.001$, $^{**}p < 0.0001$. Full, The non-adjusted WB images can be found in the supplementary materials.

treated with Ganoderma lucidum polysaccharides.

2.11. Macrophages kill bacteria

The *E. coli* strain used in our experiment was obtained from the American Type Culture Collection (ATCC, #BAA-2471). Peritoneal macrophages (1 × 105) were infected with live *Escherichia coli* (MOI, 10) and incubated at 37 °C for 1 h. To eliminate extracellular bacteria, rinse with a buffer solution containing tetracycline at 100 μg/mL. Then, use lysis buffer (Promega) to lyse the cells. Next, viable bacteria were quantified in the lysate to assess bacterial adsorption (t = 0 h) and intracellular killing (t = 2 h). The calculation formula follows, based on the colony ratio at t = 2 h and the bactericidal rate at t = 0 h: 100 - (CFU at t = 2 h/CFU at t = 0 h). In certain experiments, peritoneal macrophages were pre-treated with G. PS before infection with live *E. coli* bacteria.

2.12. Statistical analysis

Data analysis was conducted utilizing SPSS 17.0 (IBM, Armonk, New York, USA). The findings were presented as either mean ± SD or median (interquartile range). The heterogeneity of specific groups can be evaluated using the Mann-Whitney *U* test, while survival curves can be assessed using the log-rank (Mantel-Cox) test. The statistical analysis utilized a one-way analysis of variance (ANOVA), followed by multiple comparisons using the least significant difference (LSD) test to compare multiple groups. $P < 0.05$ indicates a statistically significant difference between the groups.

3. Results

3.1. The impact of G.PS on host defense against sepsis and immune regulation in mice

To investigate the role of G. PS in the host defense of septic mice, we categorized the mice into three groups: the normal group, the PBS group, and the G. PS injection group. Compared to the PBS group, the survival rate of mice receiving G. PS and CLP treatments was significantly improved (Fig. 1A). Further investigation revealed that after 24 h of CLP-induced sepsis, inflammatory factors TNF-α, IL-17A, and IL-6 in the blood (Fig. 1B) and peritoneal fluid (Fig. 1C) of septic mice significantly decreased, while IL-10 did not show a significant decrease, instead maintaining relative stability or a slight increase (Fig. 1B–C). As a critical immune organ, the spleen

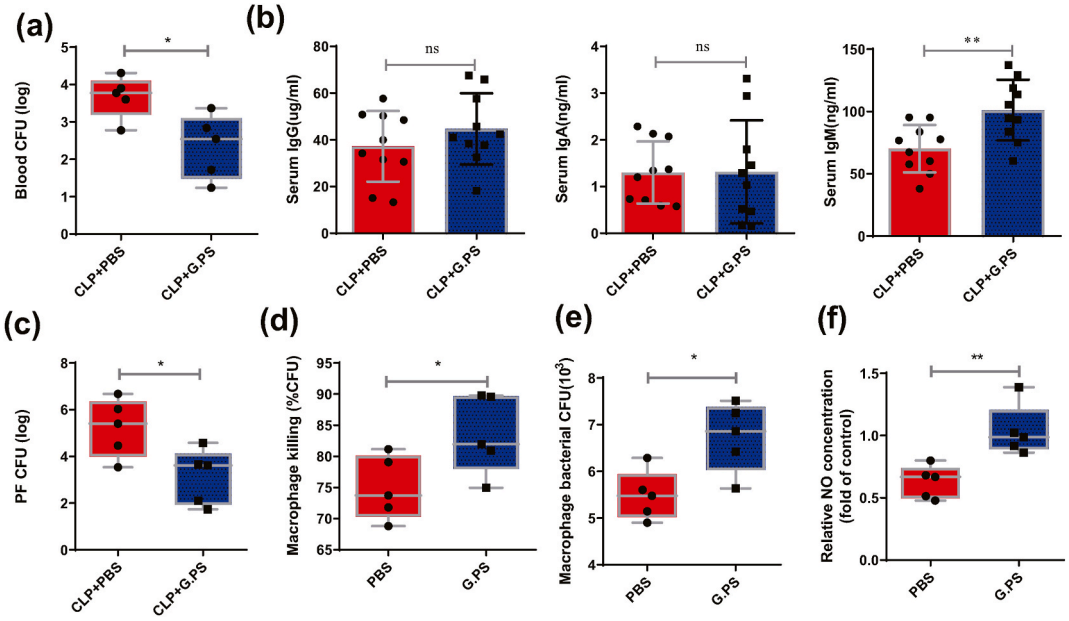

Fig. 3. Antibacterial Effects of G. PS and Its Mechanism of Action in Macrophages of CLP-induced Septic Mice.
Note: (a) Bacterial counts in blood samples of C57BL/6 mice 24 h post-CLP (n = 10 per group) displayed mice receiving G. PS treatment and those without treatment. (b) Concentrations of IgA, IgG, and IgM in serum samples were determined by ELISA. (c) Bacterial counts in peritoneal fluid of C57BL/6 mice 24 h post-CLP (n = 10 per group) displayed mice receiving G. PS treatment and those without treatment. (d) Mice peritoneal macrophages were induced with 100 μg/mL G PS for 12 h, followed by challenge with FITC-labeled *Escherichia coli* at 37 °C for 30 min. Absorbed FITC-labeled *Escherichia coli* counts were calculated based on the description. (e) Mice peritoneal macrophages were pretreated with G. PS (100 μg/mL) for 12 h and then infected with *Escherichia coli* (MOI, 10). Bacterial killing within cells was determined based on the description (t = 2 h). (f) Impact of G. PS on NO production induced by *Escherichia coli* in mice peritoneal macrophages. Compared to the PBS control group, $p < 0.05$, *$p <$ 0.01 (Mann-Whitney *U* test).

potentially plays a vital role in sepsis. After 24 h of treatment with G. PS, a significant increase in the differentiation of $CD4^{+}Foxp3^{+}$(Treg) T lymphocytes in the spleen was observed (Fig. 1D). These results demonstrate the beneficial effects of G. PS in enhancing the survival rate of mice with sepsis, regulating inflammatory cytokine levels, and promoting the differentiation of Treg cells.

3.2. The protective effect of G.PS on mouse organ function

Multiple organ dysfunction is a leading cause of mortality in sepsis. Following the observation of G. PS host defense mechanisms, we investigated its potential impact on mouse organ function to better understand its role in sepsis. After inducing sepsis through cecal ligation and puncture (CLP), the animals were treated with G. PS. The results demonstrated a significant decrease in protein levels in both the PF and the BALF of mice treated with G. PS at 2 h after CLP. Furthermore, creatinine, ALT, MPO, and AST levels also decreased (Fig. 2A–B).

Additionally, the observed damage in the lungs, liver, intestines, and kidneys was consistent with the aforementioned biochemical indicators (Fig. 2C). Furthermore, comprehensive studies have demonstrated that G. PS effectively inhibits the NF-κB inflammatory signaling pathway in multiple mouse tissues, including the liver, lung, and kidney. It is supported by a significant decrease in the levels of phosphorylated NF-κB-P65 (Fig. 2D). The results above demonstrate that treatment with G. PS led to a substantial improvement in multi-organ function and a reduction in tissue damage in a sepsis mouse model and effectively suppressed inflammatory signaling pathways.

3.3. The role of G.PS in reducing bacterial burden in septic mice is investigated

The elevated mortality rate observed in septic mice correlates with an augmented bacterial load in their blood and peritoneum. We observed a significant reduction in the bacterial load in the blood of mice treated with G. PS compared to the PBS group. It is shown in Fig. 3A. IgA, IgM and IgG are the three major types of immunoglobulins that specifically bind to these pathogenic microorganisms, making the pathogens more likely to be engulfed by several phagocytes [27]. Further investigation revealed that treatment with G. PS significantly increased the level of IgM in mouse serum, while levels of IgA and IgG remained stable (Fig. 3B). This analysis implies that G. PS can potentially diminish the bacterial load in the bloodstream through the augmentation of IgM levels.

Moreover, mice treated with G. PS significantly reduced bacterial load in the PF (Fig. 3C). Furthermore, our findings revealed that G. PS can improve intracellular bactericidal and phagocytic functions, which can be attributed to its ability to induce the production of nitric oxide (NO) by macrophages (Fig. 3D–F). The results above indicate that G. PS significantly reduces the bacterial load in septic mice.

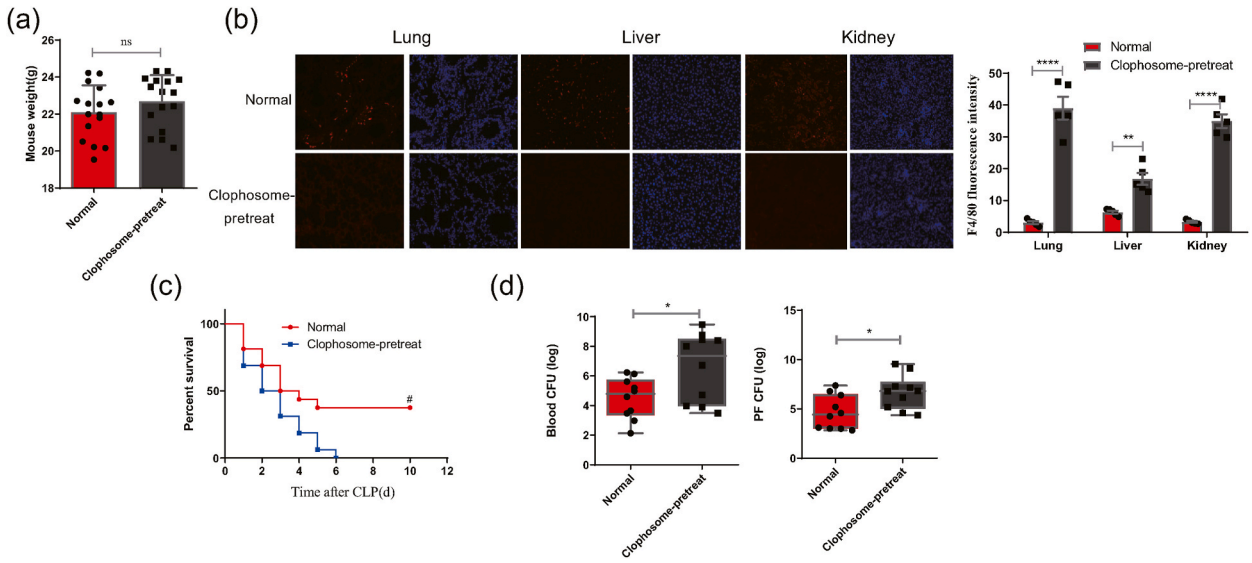

Fig. 4. Effects of Chlorpyrifos-Liposome Pretreatment on CLP-induced Septic Mice and Mechanism Study.
Note: (a) Weight changes in mice of the normal group and chlorpyrifos-liposome pretreatment group. (b) The lungs, liver, and kidney of the two groups of mice were collected for immunofluorescence analysis to quantify the staining intensity of F4/80 macrophages. Compared to the normal group, $^{**}p < 0.01$, $^{*}p < 0.0001$ (Mann-Whitney *U* test). (c) Survival rate observed for 10 days after CLP ($n = 15$ mice per group). Group comparisons were done using Log-rank test and Kaplan-Meier analysis. Compared to the normal group, $\#\#p < 0.01$. (d) Bacterial counts in blood and peritoneal fluid of C57BL/6 mice 24 h post-CLP, with or without chlorpyrifos-liposome pretreatment ($n = 10$ mice per group). Compared to the normal control group, $p < 0.05$, $p < 0.01$, $p < 0.001$, $^{*}p < 0.0001$ (Mann-Whitney *U* test).

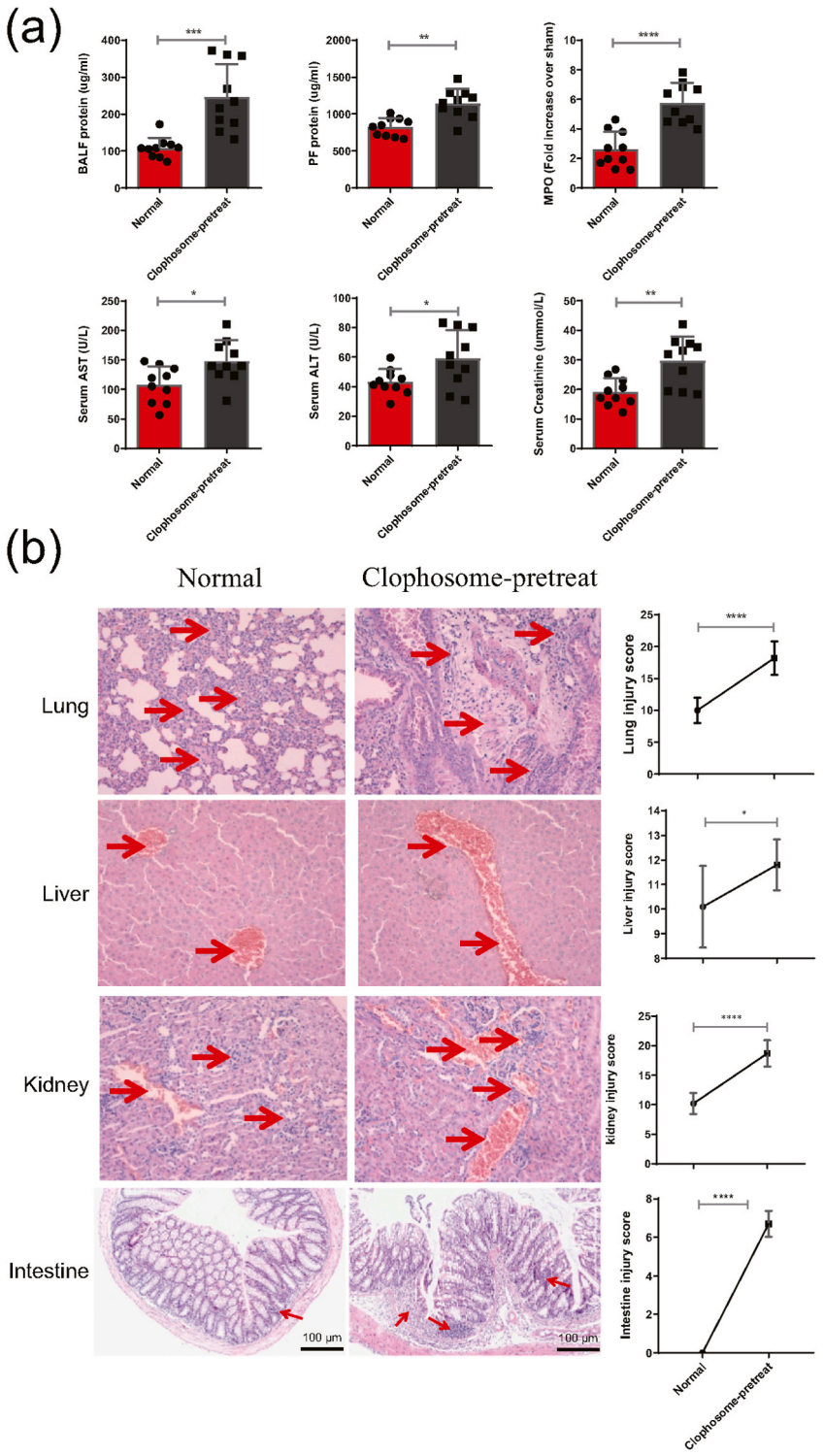

Fig. 5. Correlation Analysis of Macrophage Clearance and the Increase in Organ Dysfunction and Injury Scores in Sepsis (a) Vascular permeability was measured in the PF and BALF protein content obtained from mice 24 h after CLP-induced sepsis, with or without chlorpyrifos-liposome pretreatment (n = 10 mice per group). Organ injury markers, including ALT, AST, MPO, and creatinine at 24 h post-CLP, were measured in two groups: chlorpyrifos-liposome pretreatment or normal healthy control group (n = 10 mice per group). (b) Histological scores of lung, kidney, and liver in the two septic mouse models. Compared to the normal control group, $p < 0.05$, $p < 0.01$, $p < 0.001$, *$p < 0.0001$ (Mann-Whitney *U* test).

3.4. Eliminating macrophages has detrimental effects on sepsis progression

To further investigate the role of macrophages in protecting against sepsis, we pretreated mice with chlorphosphonic acid liposomes to deplete their macrophage population. The results indicated that, following the intervention, there was no significant change in the body weight of the mice. However, the macrophages in various organs were nearly eliminated (Fig. 4A–B). Next, we induced sepsis models in two groups of mice through cecal ligation and puncture (CLP). Compared to the control group, chlorphosphine liposomes significantly increased the mortality rate (Fig. 4C) and the bacterial load (Fig. 4D) in CLP-induced septic mice.

Furthermore, several biochemical indicators, including creatinine, ALT (Alanine Aminotransferase), MPO (Myeloperoxidase), and AST (Aspartate Aminotransferase) exhibited increased levels, suggesting organ damage (Fig. 5A). Consistent with the results above (Fig. 5B), the injury scores of different organs align. The removal of macrophages markedly worsens the severity of sepsis, leading to elevated mortality rates, increased bacterial load, and substantial organ injury.

3.5. G. Prostaglandin ps promotes the differentiation of $CD4^+$ T cells into regulatory T (treg) cells through the p-STAT5 signaling pathway

Through our *in vitro* experiments, we have observed that G. PS can facilitate the regeneration of Treg cells in the spleen. To further investigate the mechanism of action, *in vitro* experiments were conducted to determine if G. PS directly affects the differentiation of naive $CD4^+$ T cells. Naive $CD4^+$ T cells were extracted from the spleens of mice and subsequently cultured. Notably, after three days of culture, more undeveloped $CD4^+$ T cells matured into Treg cells when subjected to G. PS treatment. This finding offers valuable insights into the possible regulatory role of G. PS in immune responses (Fig. 6A).

To further investigate the signaling mechanism, we analyzed the phosphorylated STAT5 (p-STAT5) levels in T cells, a critical signaling molecule involved in regulating the differentiation of T cells. Furthermore, the p-STAT5 levels in T cells significantly increased as the concentration of G. PS gradually increased. This exciting finding confirms that G. PS promotes the conversion of immature $CD4^+$ T cells into Treg cells through the p-STAT5 signaling pathway, as illustrated in Fig. 6B. The above results indicate that G. PS promotes the recovery of Treg cells *in vivo* and directly promotes the differentiation of immature $CD4^+$ T cells *in vitro*. Its mechanism may be related to the activation of the p-STAT5 signaling pathway.

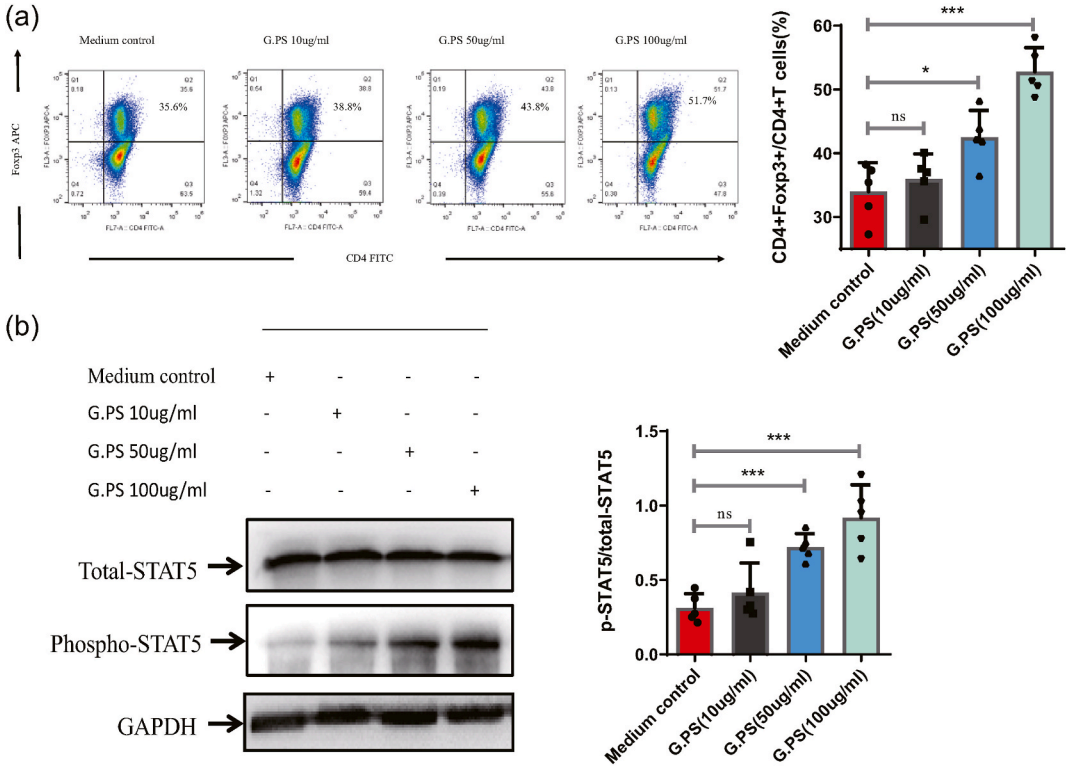

Fig. 6. Promotion of Differentiation of Primary $CD4^+$ T Lymphocytes into Treg Cells by G. PS.
Note: (a) Addition of Ganoderma polysaccharide (G.PS) increased the proportion of Tregs relative to the untreated group. (b) Western blot analyzed the expression of total-STAT5, p-STAT5, and GAPDH in $CD4^+$ T lymphocytes. One-way analysis of variance (ANOVA) followed by an LSD multiple comparison test was used for comparison between the two groups. Represented as ns for no significance, $p < 0.05$, $^{**}p < 0.001$. The non-adjusted WB images can be found in the supplementary materials.

4. Discussion

Sepsis, a profound systemic inflammatory syndrome, arises primarily due to infections. The crux of its pathogenesis lies in an unchecked inflammatory reaction, where the body's innate immune system overreacts to bacterial pathogens, leading to an overproduction of pro-inflammatory factors in the early stages [28–30]. This dysregulated immune response can escalate to multi-organ dysfunction, introducing severe, potentially fatal complications [31].

Historically, Reishi, a traditional medicine prevalent in diverse regions like North America, Europe, and East Asia, has been revered for its potential to bolster the body's defenses [32]. Dive deeper into its components, and *Ganoderma lucidum* emerges as a promising agent. Its polysaccharides (G. PS) have been shown to quell inflammation, offering protection against organ damage [33,34]. Notably, G. PS curtails the expression of the NLRP3 protein in liver tissues, dampening inflammation in mice with liver injuries and providing substantial protection against tissue damage [35]. This is further supported by the observed reduction in inflammatory markers, such as IL-8 and TNF-α, in rat brain tissues, indicative of its protective role against cerebral ischemia-reperfusion-induced inflammation [36].

Ganoderma lucidum polysaccharides have been found to exhibit remarkable anti-cancer activity. They can inhibit the proliferation and migration of cancer cells through various mechanisms, such as blocking the DNA damage repair pathway within the cancer cells [37]. Furthermore, the aqueous extract of G. lucidum polysaccharides has been discovered to activate the expression levels of the NKG2D/NCR receptors within NK cells, thereby enhancing the cytotoxic effect of NK cells against tumor cells [38]. In addition to its regulatory effects on NK cells, Ganoderma lucidum polysaccharides can also activate signaling pathway cascades within macrophages, including the PI3K/Akt and MAPK signaling pathways, or directly bind to receptors on their surface, leading to the activation of macrophages [39].

Recent advances in sepsis research have unveiled the intricate involvement of immune factors in the disease's progression [40]. Central to this immune response are T lymphocytes, which play pivotal roles across cell-mediated immunity, humoral immunity, and overall immune regulation [8,41]. A characteristic feature of sepsis initiation is the marked decline in certain T lymphocyte subpopulations, especially CD4 cells, signaling a shift towards an immunosuppressed state [42]. Within the T lymphocyte spectrum, Regulatory T cells (Tregs) are particularly intriguing. These cells, under physiological conditions, are instrumental in maintaining immune balance [43,44]. The therapeutic potential of Tregs was highlighted by Stieglitz and colleagues, where Treg transplantation in CLP mouse models led to improved survival rates, suggesting their critical immunoregulatory function during the onset of sepsis [45].

The balance between Tregs and Th17 cells is intricate and holds significant implications in sepsis [46]. In our quest to understand this balance, we investigated how G. PS influences the differentiation of splenic lymphocytes in septic mice. With triterpenes and polysaccharides identified as *Ganoderma lucidum*'s bioactive components, the role of polysaccharides in immune modulation becomes evident [47,48]. G. PS not only amplifies the proliferation of both B and T lymphocytes but also showcases intricate immune regulatory roles [49,50]. Xiang et al. provided further insights, demonstrating G. PS's capacity to boost T lymphocyte proliferation and enhance the secretion of key cytokines like IFN-γ and IL-2. These effects can be attributed to its interaction with multiple pathways, notably the MAPK and Ca2+/calcineurin (CaN) pathways [51].

Our in-depth *in vitro* analyses revealed G. PS's potential in fostering the differentiation of splenic Treg cells, primarily via the phosphorylated STAT5 signaling cascade—a pivotal pathway in sepsis. This underscores G. PS's ability to attenuate inflammation in septic models. Intriguingly, Ganoderma polysaccharides showcased remarkable therapeutic benefits without discernible side effects, elevating its potential clinical utility, especially in managing multifaceted conditions like sepsis.

Polysaccharides have garnered significant attention for their inhibitory properties against a wide array of microorganisms [52]. A primary mechanism hypothesized for this activity is the affinity between polysaccharides and specific sugar residues and proteins that populate the microbial cell wall. This interaction is believed to induce notable aggregation on bacterial surfaces. Such aggregation, beyond mere physical clustering, can substantially alter the metabolic dynamics of both bacterial and fungal entities. This metabolic interference can hinder growth and reproductive processes, often culminating in microbial mortality [53–55].

Moreover, another intriguing interaction posits polysaccharides binding directly with bacterial DNA or ribosomes. Such engagements can critically impede bacterial genomic function, especially pivotal cellular processes like replication and transcription. The end result of such interactions invariably undermines bacterial cellular integrity [56,57].

In our recent animal-centric investigations, we observed that the intraperitoneal administration of G. PS led to a marked reduction in bacterial concentration within septic mice. This revelation prompted us to delve deeper into the potential multifaceted impacts of G. PS, specifically its influence on phagocytosis and bactericidal activities. Our findings indicate a dual role for G. PS; it not only mitigates bacterial presence in the circulatory system but also augments immune defensive mechanisms, exemplified by the enhanced secretion of immunoglobulin IgM.

Our subsequent experiments focused on the role of G. PS in modulating the phagocytic prowess of intraperitoneal macrophages. Given the documented correlation between intracellular cytotoxicity, bacterial phagocytosis, and nitric oxide (NO) synthesis, we were keen to ascertain G. PS's role in influencing NO secretion in macrophages. Our data suggests that G. PS acts as a potent enhancer of NO production, thereby amplifying macrophage-mediated phagocytosis and cytotoxicity against bacterial invaders (Fig. 3).

To further underscore the indispensable role of macrophages in sepsis progression, we designed an experiment wherein macrophages were depleted in mice prior to sepsis induction. The outcomes were stark; macrophage-deprived mice exhibited heightened mortality rates, amplified bacterial loads, and aggravated organ dysfunction, underscoring the protective role of G. PS in sepsis scenarios.

Although meaningful results have been obtained in this study, there are still some limitations that need to be acknowledged. For instance, the use of a mouse model may not fully reflect the effects in humans. Furthermore, the accuracy of the results could be

influenced by experimental conditions and sample size. For example, we used a G. PS concentration below the safe dose of 100 μg/mL. However, it remains to be explored whether an increase in G. PS dosage could lead to more pronounced protective and therapeutic effects, as well as potential side effects. This exploration could be a valuable direction for future research. Building upon the findings of this study, further investigation is warranted to elucidate the specific mechanisms of action of Ganoderma lucidum polysaccharides and their effects in other disease models. Additionally, considering the combination of Ganoderma lucidum polysaccharides with other treatment modalities may enhance therapeutic efficacy.

5. Conclusion

In summary, Ganoderma lucidum polysaccharides play a key role in sepsis prevention by modulating the dynamic balance between anti-inflammatory and pro-inflammatory cytokines. These molecules enhance Treg differentiation in the spleen, contributing to inflammation control in sepsis models. Furthermore, G. PS has been demonstrated to reduce bacterial burden and procalcitonin levels in the blood of septic mice, while downregulating inflammatory signaling pathways in tissues, thereby protecting organs from sepsis impact (Fig. 7). These findings provide important theoretical basis and molecular targets for novel therapeutic approaches in sepsis. However, further research is needed to validate their clinical application. Despite certain limitations in this study, it provides valuable

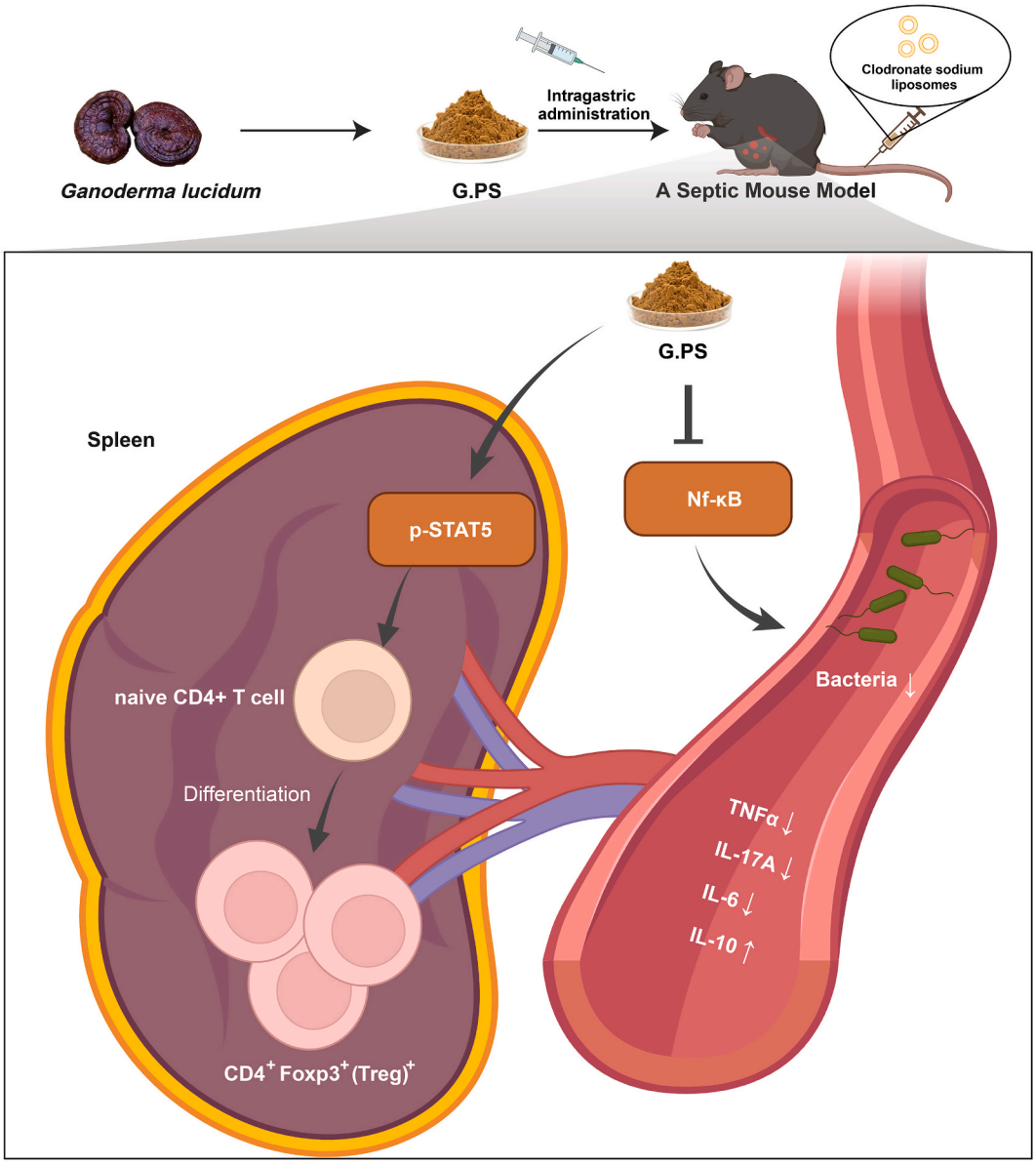

Fig. 7. Molecular mechanism diagram illustrating the protective effect of G. PS in CLP-induced septic mice.

directions for future investigations and enhances our confidence in the use of Ganoderma lucidum polysaccharides for the treatment of inflammatory diseases. In conclusion, Ganoderma lucidum polysaccharides are an effective compound for sepsis treatment.

Data availability statement

Data will be made available on request.

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Ethical statement

All experiments involving mice were approved by the Animal Ethics Committee of Chongqing Three Gorges Medical College (No. SYYZ-A-2212-0005).

CRediT authorship contribution statement

Wei Xiong: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jing Xia:** Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Xiaoyuan Peng:** Writing – original draft, Supervision, Methodology, Formal analysis, Data curation. **Ying Tan:** Writing – review & editing, Visualization, Validation, Formal analysis, Data curation. **Wansong Chen:** Writing – review & editing, Visualization, Validation, Supervision, Software. **Minghua Zhou:** Writing – review & editing, Visualization, Validation, Formal analysis, Data curation. **Ce Yang:** Writing – review & editing, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Wenxiang Wang:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26732.

References

[1] M. Singer, C.S. Deutschman, C.W. Seymour, et al., The third international consensus definitions for sepsis and septic shock (Sepsis-3), JAMA 315 (8) (2016) 801–810, https://doi.org/10.1001/jama.2016.0287.
[2] C. Fleischmann, A. Scherag, N.K. Adhikari, et al., Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations, Am. J. Respir. Crit. Care Med. 193 (3) (2016) 259–272, https://doi.org/10.1164/rccm.201504-0781OC.
[3] R.L. Siegel, K.D. Miller, N.S. Wagle, A. Jemal, Cancer statistics, 2023, Ca - Cancer J. Clin. 73 (1) (2023) 17–48, https://doi.org/10.3322/caac.21763.
[4] N.K. Adhikari, R.A. Fowler, S. Bhagwanjee, G.D. Rubenfeld, Critical care and the global burden of critical illness in adults, Lancet 376 (9749) (2010) 1339–1346, https://doi.org/10.1016/S0140-6736(10)60446-1.
[5] M.E. Nunnally, A. Patel, Sepsis - what's new in 2019? Curr. Opin. Anaesthesiol. 32 (2) (2019) 163–168, https://doi.org/10.1097/ACO.0000000000000707.
[6] K. Yang, M. Fan, X. Wang, et al., Lactate promotes macrophage HMGB1 lactylation, acetylation, and exosomal release in polymicrobial sepsis, Cell Death Differ. 29 (1) (2022) 133–146, https://doi.org/10.1038/s41418-021-00841-9.
[7] C. Song, J. Xu, C. Gao, W. Zhang, X. Fang, Y. Shang, Nanomaterials targeting macrophages in sepsis: a promising approach for sepsis management, Front. Immunol. 13 (2022) 1026173, https://doi.org/10.3389/fimmu.2022.1026173.
[8] S. Huang, D. Liu, J. Sun, et al., Tim-3 regulates sepsis-induced immunosuppression by inhibiting the NF-κB signaling pathway in CD4 T cells, Mol. Ther. 30 (3) (2022) 1227–1238, https://doi.org/10.1016/j.ymthe.2021.12.013.
[9] Y.L. Gao, Y. Yao, X. Zhang, et al., Regulatory T cells: angels or demons in the pathophysiology of sepsis? Front. Immunol. 13 (2022) 829210 https://doi.org/10.3389/fimmu.2022.829210.
[10] X. Chen, Q. Wei, Y. Hu, C. Wang, Role of Fractalkine in promoting inflammation in sepsis-induced multiple organ dysfunction, Infect. Genet. Evol. 85 (2020) 104569, https://doi.org/10.1016/j.meegid.2020.104569.

[11] K.E. Rudd, S.C. Johnson, K.M. Agesa, et al., Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study, Lancet 395 (10219) (2020) 200–211, https://doi.org/10.1016/S0140-6736(19)32989-7.

[12] C. Wang, K. Xie, K. Li, S. Lin, F. Xu, Potential therapeutic effects of interleukin-35 on the differentiation of naïve T cells into Helios^{+}Foxp3^{+} Tregs in clinical and experimental acute respiratory distress syndrome, Mol. Immunol. 132 (2021) 236–249, https://doi.org/10.1016/j.molimm.2021.01.009.

[13] B.G. Chousterman, F.K. Swirski, G.F. Weber, Cytokine storm and sepsis disease pathogenesis, Semin. Immunopathol. 39 (5) (2017) 517–528, https://doi.org/10.1007/s00281-017-0639-8.

[14] J. Brady, S. Horie, J.G. Laffey, Role of the adaptive immune response in sepsis, Intensive Care Med Exp 8 (Suppl 1) (2020) 20, https://doi.org/10.1186/s40635-020-00309-z.

[15] J.B. Dartiguelongue, Systemic inflammation and sepsis. Part I: storm formation. Inflamación sistémica y sepsis. Parte I: generación de la tormenta, Arch. Argent. Pediatr. 118 (6) (2020) e527–e535, https://doi.org/10.5546/aap.2020.eng.e527.

[16] A. Perner, A.C. Gordon, D. De Backer, et al., Sepsis: frontiers in diagnosis, resuscitation and antibiotic therapy, Intensive Care Med. 42 (12) (2016) 1958–1969, https://doi.org/10.1007/s00134-016-4577-z.

[17] R.Y. Kim, A.M. Ng, A.K. Persaud, et al., Antibiotic timing and outcomes in sepsis, Am. J. Med. Sci. 355 (6) (2018) 524–529, https://doi.org/10.1016/j.amjms.2018.02.007.

[18] J. Lu, R. He, P. Sun, F. Zhang, R.J. Linhardt, A. Zhang, Molecular mechanisms of bioactive polysaccharides from Ganoderma lucidum (Lingzhi), a review, Int. J. Biol. Macromol. 150 (2020) 765–774, https://doi.org/10.1016/j.ijbiomac.2020.02.035.

[19] J. Zhang, Y. Liu, Q. Tang, S. Zhou, J. Feng, H. Chen, Polysaccharide of ganoderma and its bioactivities, Adv. Exp. Med. Biol. 1181 (2019) 107–134, https://doi.org/10.1007/978-981-13-9867-4_4.

[20] X. Zeng, P. Li, X. Chen, et al., Effects of deproteinization methods on primary structure and antioxidant activity of Ganoderma lucidum polysaccharides, Int. J. Biol. Macromol. 126 (2019) 867–876, https://doi.org/10.1016/j.ijbiomac.2018.12.222.

[21] S. da Silva Milhorini, D. de Lima Bellan, M. Zavadinack, et al., Antimelanoma effect of a fucoxylomannan isolated from Ganoderma lucidum fruiting bodies, Carbohydr. Polym. 294 (2022) 119823, https://doi.org/10.1016/j.carbpol.2022.119823.

[22] M.N. Bin-Jumah, M.S. Nadeem, S.J. Gilani, S.S. Imam, S. Alshehri, I. Kazmi, Novelkaraya gum micro-particles loaded Ganoderma lucidum polysaccharide regulate sex hormones, oxidative stress and inflammatory cytokine levels in cadmium induced testicular toxicity in experimental animals, Int. J. Biol. Macromol. 194 (2022) 338–346, https://doi.org/10.1016/j.ijbiomac.2021.11.072.

[23] G. Liu, J. Zhang, Q. Kan, et al., Extraction, structural characterization, and immunomodulatory activity of a high molecular weight polysaccharide from ganoderma lucidum, Front. Nutr. 9 (2022) 846080, https://doi.org/10.3389/fnut.2022.846080.

[24] L. Li, J.X. Xu, Y.J. Cao, et al., Preparation of Ganoderma lucidum polysaccharide-chromium (III) complex and its hypoglycemic and hypolipidemic activities in high-fat and high-fructose diet-induced pre-diabetic mice, Int. J. Biol. Macromol. 140 (2019) 782–793, https://doi.org/10.1016/j.ijbiomac.2019.08.072.

[25] K. Wu, K. Na, D. Chen, Y. Wang, H. Pan, X. Wang, Effects of non-steroidal anti-inflammatory drug-activated gene-1 on Ganoderma lucidum polysaccharides-induced apoptosis of human prostate cancer PC-3 cells, Int. J. Oncol. 53 (6) (2018) 2356–2368, https://doi.org/10.3892/ijo.2018.4578.

[26] D. Rittirsch, M.S. Huber-Lang, M.A. Flierl, P.A. Ward, Immunodesign of experimental sepsis by cecal ligation and puncture, Nat. Protoc. 4 (1) (2009) 31–36, https://doi.org/10.1038/nprot.2008.214.

[27] H. Eibel, H. Kraus, H. Sic, A.K. Kienzler, M. Rizzi, B cell biology: an overview, Curr. Allergy Asthma Rep. 14 (5) (2014) 434, https://doi.org/10.1007/s11882-014-0434-8.

[28] M. Cecconi, L. Evans, M. Levy, A. Rhodes, Sepsis and septic shock, Lancet 392 (10141) (2018) 75–87, https://doi.org/10.1016/S0140-6736(18)30696-2.

[29] M. Huang, S. Cai, J. Su, The pathogenesis of sepsis and potential therapeutic targets, Int. J. Mol. Sci. 20 (21) (2019) 5376, https://doi.org/10.3390/ijms20215376.

[30] S.M. Opal, X. Wittebole, Biomarkers of infection and sepsis [published correction appears in crit care clin. 2020 jul;36(3):xi], Crit. Care Clin. 36 (1) (2020) 11–22, https://doi.org/10.1016/j.ccc.2019.08.002.

[31] R. Pool, H. Gomez, J.A. Kellum, Mechanisms of organ dysfunction in sepsis, Crit. Care Clin. 34 (1) (2018) 63–80, https://doi.org/10.1016/j.ccc.2017.08.003.

[32] A.L. Loyd, C.W. Barnes, B.W. Held, et al., Elucidating "lucidum": distinguishing the diverse laccate Ganoderma species of the United States, PLoS One 13 (7) (2018) e0199738, https://doi.org/10.1371/journal.pone.0199738.

[33] M. Song, Z.H. Li, H.S. Gu, et al., Ganoderma lucidum spore polysaccharide inhibits the growth of hepatocellular carcinoma cells by altering macrophage polarity and induction of apoptosis, J Immunol Res 2021 (2021) 6696606, https://doi.org/10.1155/2021/6696606. Published 2021 Mar 5.

[34] C. Guo, D. Guo, L. Fang, et al., Ganoderma lucidum polysaccharide modulates gut microbiota and immune cell function to inhibit inflammation and tumorigenesis in colon, Carbohydr. Polym. 267 (2021) 118231, https://doi.org/10.1016/j.carbpol.2021.118231.

[35] Y.S. Chen, Q.Z. Chen, Z.J. Wang, C. Hua, Anti-inflammatory and hepatoprotective effects of ganoderma lucidum polysaccharides against carbon tetrachloride-induced liver injury in kunming mice, Pharmacology 103 (3–4) (2019) 143–150, https://doi.org/10.1159/000493896.

[36] W. Zhang, Q. Zhang, W. Deng, et al., Neuroprotective effect of pretreatment with ganoderma lucidum in cerebral ischemia/reperfusion injury in rat hippocampus, Neural Regen Res 9 (15) (2014) 1446–1452, https://doi.org/10.4103/1673-5374.139461.

[37] M. Wang, F. Yu, Research progress on the anticancer activities and mechanisms of polysaccharides from ganoderma, Front. Pharmacol. 13 (2022) 891171, https://doi.org/10.3389/fphar.2022.891171.

[38] C.C. Lu, Y.J. Hsu, C.J. Chang, et al., Immunomodulatory properties of medicinal mushrooms: differential effects of water and ethanol extracts on NK cell-mediated cytotoxicity, Innate Immun. 22 (7) (2016) 522–533, https://doi.org/10.1177/1753425916661402.

[39] I.A. Schepetkin, M.T. Quinn, Botanical polysaccharides: macrophage immunomodulation and therapeutic potential, Int. Immunopharm. 6 (3) (2006) 317–333, https://doi.org/10.1016/j.intimp.2005.10.005.

[40] L.K. Torres, P. Pickkers, T. van der Poll, Sepsis-induced immunosuppression, Annu. Rev. Physiol. 84 (2022) 157–181, https://doi.org/10.1146/annurev-physiol-061121-040214.

[41] W. He, K. Xiao, J. Xu, et al., Recurrent sepsis exacerbates CD4^{+} T cell exhaustion and decreases antiviral immune responses, Front. Immunol. 12 (2021) 627435, https://doi.org/10.3389/fimmu.2021.627435.

[42] C.J. Wang, M. Zhang, H. Wu, S.H. Lin, F. Xu, IL-35 interferes with splenic T cells in a clinical and experimental model of acute respiratory distress syndrome, Int. Immunopharm. 67 (2019) 386–395, https://doi.org/10.1016/j.intimp.2018.12.024.

[43] U. Khan, H. Ghazanfar, T lymphocytes and autoimmunity, Int Rev Cell Mol Biol 341 (2018) 125–168, https://doi.org/10.1016/bs.ircmb.2018.05.008.

[44] S. Sakaguchi, N. Mikami, J.B. Wing, A. Tanaka, K. Ichiyama, N. Ohkura, Regulatory T cells and human disease, Annu. Rev. Immunol. 38 (2020) 541–566, https://doi.org/10.1146/annurev-immunol-042718-041717.

[45] D. Stieglitz, T. Schmid, N.F. Chhabra, B. Echtenacher, D.N. Männel, S. Mostböck, TNF and regulatory T cells are critical for sepsis-induced suppression of T cells, Immun Inflamm Dis 3 (4) (2015) 374–385, https://doi.org/10.1002/iid3.75.

[46] Q. Zou, M. Yang, M. Yu, C. Liu, Influences of regulation of miR-126 on inflammation,Th17/treg subpopulation differentiation, and lymphocyte apoptosis through caspase signaling pathway in sepsis, Inflammation 43 (6) (2020) 2287–2300, https://doi.org/10.1007/s10753-020-01298-7.

[47] S.P. Wasser, Medicinal mushroom science: current perspectives, advances, evidences, and challenges, Biomed. J. 37 (6) (2014) 345–356, https://doi.org/10.4103/2319-4170.138318.

[48] Z. Xiao, W. Zhou, Y. Zhang, Fungal polysaccharides, Adv. Pharmacol. 87 (2020) 277–299, https://doi.org/10.1016/bs.apha.2019.08.003.

[49] X.F. Bao, X.S. Wang, Q. Dong, J.N. Fang, X.Y. Li, Structural features of immunologically active polysaccharides from Ganoderma lucidum, Phytochemistry 59 (2) (2002) 175–181, https://doi.org/10.1016/s0031-9422(01)00450-2.

[50] L. Ren, J. Zhang, T. Zhang, Immunomodulatory activities of polysaccharides from Ganoderma on immune effector cells, Food Chem. 340 (2021) 127933, https://doi.org/10.1016/j.foodchem.2020.127933.

[51] Q.D. Xiang, Q. Yu, H. Wang, et al., Immunomodulatory activity of ganoderma atrum polysaccharide on purified T lymphocytes through Ca^{2+}/CaN and mitogen-activated protein kinase pathway based on RNA sequencing, J. Agric. Food Chem. 65 (26) (2017) 5306–5315, https://doi.org/10.1021/acs.jafc.7b01763.

[52] Y. Yu, M. Shen, Q. Song, J. Xie, Biological activities and pharmaceutical applications of polysaccharide from natural resources: a review, Carbohydr. Polym. 183 (2018) 91–101, https://doi.org/10.1016/j.carbpol.2017.12.009.
[53] F. Ji, Y. Shen, L. Tang, Z. Cai, Determination of intracellular metabolites concentrations in Escherichia coli under nutrition stress using liquid chromatography-tandem mass spectrometry, Talanta 189 (2018) 1–7, https://doi.org/10.1016/j.talanta.2018.06.050.
[54] X. Chen, L. Tao, Y. Ru, et al., Antibacterial mechanism of Tetrastigma hemsleyanum Diels et Gilg's polysaccharides by metabolomics based on HPLC/MS, Int. J. Biol. Macromol. 140 (2019) 206–215, https://doi.org/10.1016/j.ijbiomac.2019.08.097.
[55] M. Chen, D. Xiao, W. Liu, et al., Intake of Ganoderma lucidum polysaccharides reverses the disturbed gut microbiota and metabolism in type 2 diabetic rats, Int. J. Biol. Macromol. 155 (2020) 890–902, https://doi.org/10.1016/j.ijbiomac.2019.11.047.
[56] Q. Bingjun, J. Jung, Y. Zhao, Impact of acidity and metal ion on the antibacterial activity and mechanisms of β- and α-chitosan, Appl. Biochem. Biotechnol. 175 (6) (2015) 2972–2985, https://doi.org/10.1007/s12010-014-1413-1.
[57] M. Krupkin, I. Wekselman, D. Matzov, et al., Avilamycin and evernimicin induce structural changes in rProteins uL16 and CTC that enhance the inhibition of A-site tRNA binding, Proc. Natl. Acad. Sci. U. S. A. 113 (44) (2016) E6796–E6805, https://doi.org/10.1073/pnas.1614297113.