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Astragalus regulates the intestinal immune response during sepsis by mediating ILC3 proliferation through RORγt

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ABSTRACT

Background: Sepsis is a common complication of many diseases and is associated with high morbidity and mortality rates. Astragalus can improve humoral and innate immunity, inhibit inflammatory responses, and protect immune cells and organs from damage. However, to the best of our knowledge there are no reports on whether astragalus can regulate intestinal innate immune function during sepsis.

Methods: In this study, a rat cecal ligation and puncture model of sepsis was used to investigate the effects of astragalus treatment, following which the apoptosis rate of lymphocytes from Peyer's patches (PP) was determined. Type 3 innate lymphoid cells (ILC3) were cultured in vitro to further evaluate the effects and mechanisms of astragalus.

Results: The apoptosis level of lymphocytes from PP in rats with sepsis was significantly increased, and the number of ILC3 was significantly reduced, compared with the sham operation group, which aggravated intestinal injury and ultimately led to the death of rats. Astragalus treatment significantly inhibited the apoptosis of lymphocytes from PP, increased the number of ILC3, and improved the intestinal inflammatory environment compared to the sepsis group. RT-PCR revealed that astragalus and the retinoic acid-related orphan receptor γt (RORγt) agonist LYC-55716 both promote the expression of interleukin (IL)-17A, IL-17F, IL-22, interferon-γ, and granulocyte-macrophage colony-stimulating factor mRNA. Mechanistically, astragalus promotes the proliferation of ILC3 through RORγt, thereby reducing intestinal inflammatory damage.

Conclusion: Astragalus, via RORγt, promotes the generation of ILC3, improves the inflammatory environment in rats with sepsis.

1. Introduction

Sepsis refers to the systemic inflammatory response syndrome (SIRS) caused by infection with various pathogenic microorganisms [1]. It is a common complication of many clinical diseases and is often encountered in the intensive care unit. The high incidence, mortality, and cost of treatment make sepsis a serious public health issue. Previous studies have revealed that in patients being treated for multi-organ dysfunctional syndrome, the incidence of sepsis was 39.7% [2–4]. However, the pathophysiology and pathogenesis of sepsis have not been fully elucidated.

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The current preferred hypothesis is that the high mortality rate is mainly caused by excessive apoptosis of immune cells leading to the suppression of immune function, which worsens the infection [5,6]. Clinical studies have shown that a large decrease in lymphocytes in the peripheral circulation of patients with sepsis is associated with lymphocyte apoptosis [7–9]. A paper by Bone confirmed that anti-inflammatory cytokines produced during sepsis result in immunosuppression [10]. Recent studies have found that extensive apoptosis of lymphocytes and intestinal epithelial cells occurs in patients who die from sepsis, but not in patients without sepsis, which indicates that the apoptosis of intestinal tract lymphocytes may be an indicator of sepsis. Although new treatment strategies, such as broad-spectrum antibiotics to fight infection and restore tissue oxygen supply, have been adopted, the mortality rate remains as high as 30% [11]. As it has been confirmed that as well as inflammatory damage caused by pathogens and their toxins, sepsis causes immunosuppression [12,13] in patients, immunotherapy may be a potential therapeutic strategy for the treatment of sepsis. Current immunomodulatory therapy is primarily targeted at specific monocytes, with inconclusive results.

Astragalus sp. is a perennial herb [14,15]. Astragalus has been used in traditional Chinese medicine for more than 2000 years [14,15]. It may enhance the immune system, protect the liver, lower blood pressure, and have anti-aging, anti-stress, and antibacterial effects [14,15]. Many clinical trials have confirmed that astragalus is safe, causes few adverse reactions, and has wide clinical applicability [14,15]. Studies have found that astragalus injection can correct immune responses in patients with burns [16,17]. Moreover, it has been confirmed in animal models of sepsis that astragalus can significantly improve both acquired and innate immunity, suppress inflammatory responses, and protect immune cells and organs [18,19]. Studies have further confirmed that astragalus injection has immunoregulatory effects in patients with sepsis and can improve the health and prognosis of patients. Therefore, astragalus induces long-term effects with few adverse reactions [14], and may play an important role in regulating and augmenting the immune system. However, whether astragalus has an effect on the intestinal immune system during sepsis remains poorly understood.

In the present study, we used a rat model to determine the effect of astragalus on the intestinal immune system in sepsis and explore the possible mechanisms of these effects, in order to provide a more solid theoretical basis for clinical application.

2. Materials and methods

2.1. Animal model and study design

Healthy male Sprague Dawley rats weighing 250 ± 20 g were purchased from the Laboratory Animal Science Department of the School of Medicine, Nanchang University, and were used in the experiment after acclimating for one week in a constant temperature environment. Randomly divide 80 rats into three groups: sham operation, sepsis, and drug (astragalus or LYC-55716) treatment. After anesthetizing the rats with 10% chloral hydrate solution, all rats in the ligation and puncture (CLP)-induced sepsis and drug treatment groups underwent the following surgical procedure: the cecum was ligated in equal proportions and pierced with a needle. A portion of the intestinal contents was squeezed out, return to the intestinal tube, then the abdominal wall was sutured one layer at a time. All rats in the drug treatment group were intraperitoneally injected with astragalus (10 g/kg/d, Dali Pharmaceutical Co., Ltd., Dali, China) or LYC-55716 (150 mg/kg/d, MedChemExpress, Monmouth Junction, NJ, USA) 30 min before surgery. Rats in the sham operation group were anesthetized using the same method. After successful anesthesia, the abdominal wall was opened and closed. Rats in the sepsis and sham operation groups were intraperitoneally injected with the same volume of saline. All protocols were approved by the Animal Care Ethics Committee of Nanchang University and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animal experiments were performed under specific sterile barrier conditions in accordance with institutional guidelines, and the experimental protocols were approved by the Ethics Committee of Animal Experimentation of Nanchang University.

2.2. Sampling

Eight rats were randomly selected from each group at 12, 24, and 72 h after surgery. After the rats were killed by cervical dislocation, the required tissues (Peyer's patches [PP] and intestine) were collected and immediately fixed in a 10% formaldehyde solution. The fixed tissues were washed, dehydrated, paraffin-embedded, and sectioned for immunohistochemistry. In addition, for the cell assays, approximately 10 cm of the upper small intestine of the rat ileocecal area was collected, and the specimen was carefully washed twice with 20 mL ice-cold isotonic saline. The samples were centrifuged at 2700 r/min at 4 °C for 30 min, and the supernatant were stored in liquid nitrogen or a −80 °C freezer until further use.

2.3. Terminal deoxynucleotidyl transferase dUTP nick-end labeling assay

Freshly prepared 3% H_2O_2 was added to the tissue sections for 15 min at 15–25 °C, followed by rinsing with 0.01 M PBS 3 times, for 2 min each time. The terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay was then performed according to the kit manufacturer's instructions (K403-50, Biovision), following which the sections were dehydrated, cleared, and mounted. Finally, the cells were examined under a microscope.

2.4. Immunohistochemistry

Intestinal tissues were fixed in 4% paraformaldehyde at 4 °C for 24 h and embedded in paraffin. The samples were cut into sections 5–6 μm thick and rehydrated with xylene and declining grades of ethanol for 5–6 min. The sections were washed three times with PBS

for 5 min. Immunohistochemical staining for caspase-3 (catalog no. ab184787; Abcam, Cambridge, UK) was then performed. Followed by Goat Anti-Rabbit IgG H&L (catalog no. ab97051; Abcam, Cambridge, UK) secondary antibody at 1/500 dilution. Counter stained with Hematoxylin. Finally, take photos under a microscope and count the percentage of apoptotic cells in all cells.

2.5. Cell counting kit-8 analysis

The Cell Counting Kit-8 (CCK-8) produced by Beyotime Institute of Biotechnology (catalog no. C0038; Shanghai, China) was used to detect cell viability. Briefly, 20 μL CCK-8 solution was added to a 96-well plate and incubated for 2 h in a cell culture incubator to test the survival rate of type 3 innate lymphoid cells (ILC3). Thereafter, the absorbance was measured at a wavelength of 450 nm.

2.6. Intestinal innate lymphoid cell extraction

Intestinal innate lymphoid cells (ILCs) were extracted as previously described [20]. The specific steps were as follows: the rat small intestine was collected and placed in ice-cold PBS, and PP and adipose tissue were removed. The intestinal tissue was separated and digested, and the cells were extracted and added to the Percoll solution. A Pasteur pipette was used to remove the upper layer of fluid containing fat and cell debris and then collect the opaque middle layer, which contained the ILCs.

2.7. Establishment of an in vitro sepsis model using type 3 innate lymphoid cells

For in vitro culture of type 3 innate lymphoid cells (ILC3), interleukin (IL)-2 (1000 U/mL; catalog no. 400-02; Proteintech, U.S.), IL-15 (10 ng/mL; catalog no. 400-24; Proteintech, U.S.), IL-7 (50 ng/mL; catalog no. 400-07; Proteintech, U.S.), and IL-23 (50 ng/L; catalog no. HY-P73195; MedChemExpress, Monmouth Junction, NJ, USA) [21]. Lipopolysaccharide (LPS; 200 ng/mL; catalog no. 437650; Sigma-Aldrich, U.S.) was added to cultures for 24 h to establish an in vitro sepsis model. Next, we collected cells and supernatants after 24 h of treatment with 50 μM astragalus to evaluate the effect of astragalus.

2.8. Enzyme-linked immunosorbent assay

After the blood was placed in the test tube, centrifuge at 4000r/min for 10min at 4 °C. IL-1β (catalog no. RK00009; ABclonal, Wuhan, China), IL-6 (catalog no. RK00020; ABclonal, Wuhan, China) and C-Reactive Protein (CRP) (catalog no. RK00195; ABclonal, Wuhan, China) was purchased from ABclonal Technology Co.,Ltd. Detect its concentration according to the method provided by the kit.

2.9. Flow cytometric analysis

Single-cell suspensions of the lamina propria of the small intestine were obtained, as described above. The cell surface was labeled with LinLD (catalog no. 423102; Biolegend, U.S.), CD45 (catalog no. A15395; ThermoFisher, U.S.), TCRβ,γδ (catalog no. MA5-28524; ThermoFisher, U.S.), NKp46 (catalog no. 250802; Biolegend, U.S.), CD127 (catalog no. PA5-79511; ThermoFisher, U.S.), RORγt (catalog no. ab104950; Abcam, Cambridge, UK), and GATA3 (catalog no. 386902; Abcam, Cambridge, UK). Cells were analyzed using LSRFortessa X-20 multi-dimensional high-definition flow cytometry (BD, Franklin Lakes, NY, USA), and the data were analyzed using FIowJo software version 10 (FlowJo, LLC, Ashland, OR, USA).

2.10. Total RNA extraction and real-time quantitative PCR

Total RNA was extracted from tissues and cells using TRIzol reagent (catalo no. 15596026; Invitrogen, Waltham, MA, USA), and 2 μg of this RNA was reverse-transcribed into cDNA using the Prime-Script RT kit (catalog no. RR036A; Takara Bio, Inc., Kusatsu, Japan). PCR amplification was performed using a CFX96 real-time PCR system (Bio-Rad Laboratories, Hercules, CA, USA). The total reaction volume was 10 μL, which included 5 μl SYBR Green, 1 μL cDNA, 0.5 μL forward primer, 0.5 μL reverse primer, and 3 μL ddH_2O. The following two-step PCR amplification protocol was used: 39 cycles of 95 °C for 30 s, 95 °C for 5 s, and 60 °C for 30 s. Relative gene expression was normalized to that of β-actin or 18s RNA using the standard $2^{-\Delta\Delta Ct}$ quantification method. Primer sequences are listed in Table S1.

2.11. Western blotting

Peyer's patches tissue was harvested and placed in radioimmunoprecipitation assay lysis buffer containing 1 mM phenylmethanesulfonyl fluoride. Protein samples were separated using 10% and 15% SDS-PAGE and transferred to polyvinylidene difluoride membranes (Jiangsu Well Biotech Co., Ltd., Changzhou, China). The membranes were blocked with 5% bovine serum albumin in TBST for 2 h and incubated overnight at 4 °C with the following primary antibodies: anti-BAX (catalog no. ab3191; Abcam, Cambridge, UK), anti-cleaved caspase-3 (catalog no. ab214430; Abcam, Cambridge, UK), and anti-β-actin (catalog no. 4970S; Cell Signaling Technology, Danvers, MA, USA). The membranes were then incubated at 24 °C for 1.5 h with horseradish peroxidase-conjugated secondary antibody. Proteins were detected using Immobilon Western Chemiluminescent HRP Substrate (MilliporeSigma, Burlington, MA, USA) and gel images were captured using an ImageQuant LAS 4000 Mini Biomolecular Imager (GE HealthCare, Chicago, IL, USA). The

detailed methods have been described previously [22–24].

2.12. Quantification and statistical analysis

Data are expressed as mean ± standard error of the mean. Statistical analyses were conducted using GraphPad Prism software version 8.0 (GraphPad Software, San Diego, CA, USA). The normality of the data distribution was tested using the Kolmogorov–Smirnov test. The Mann–Whitney *U* test was used when the group data were not normally distributed or when the group variances were unequal. The homogeneity of variance was analyzed using Levene's test. Continuous data with normal distribution were assessed using a Student's t-test, one-way analysis of variance with a post hoc test, or two-way analysis of variance followed by a post hoc test (Tukey–Kramer test). The detailed methods have been described previously [22–24].

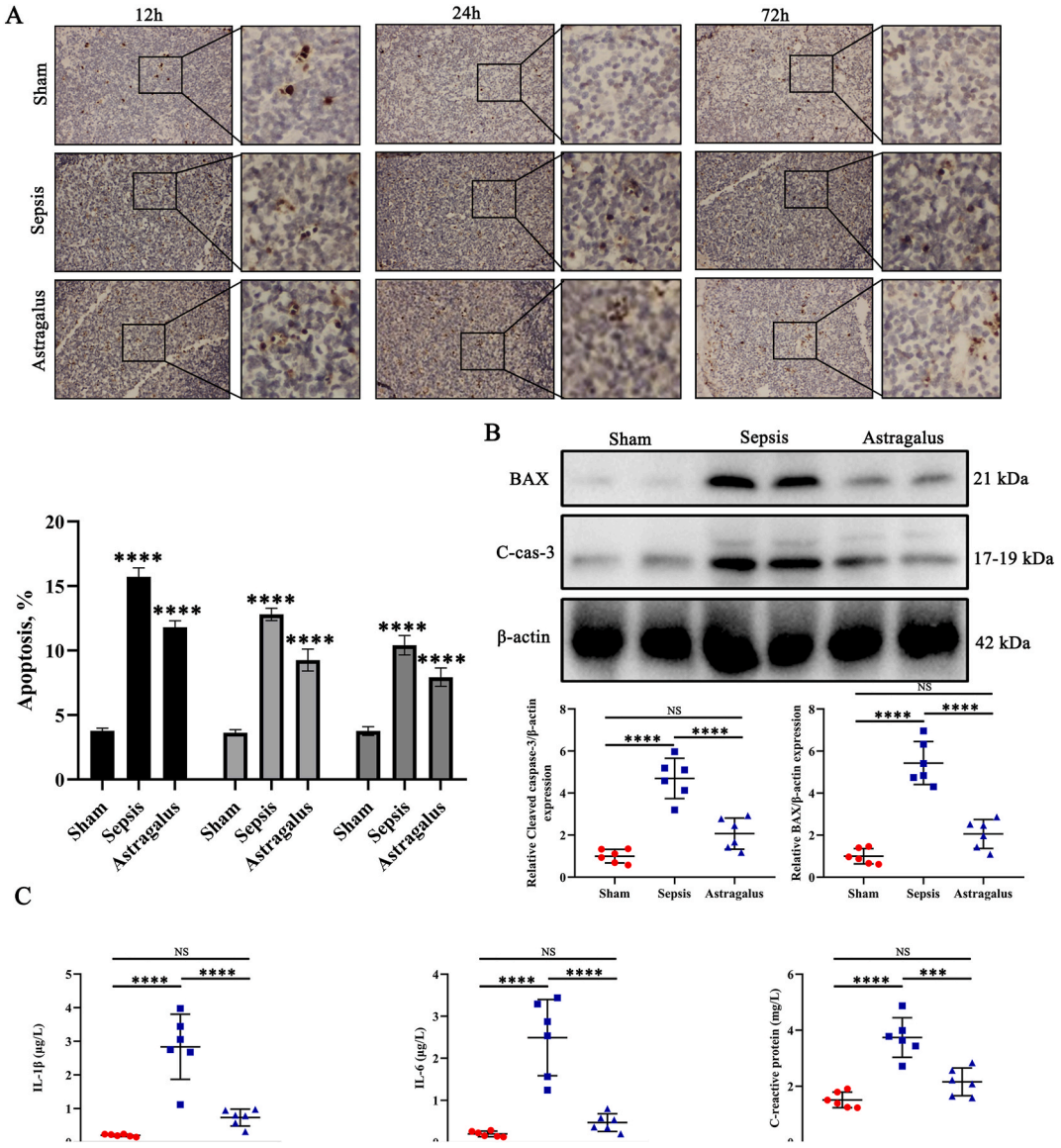

Fig. 1. Astragalus inhibits the apoptosis rate of PP node lymphocytes. (A) Apoptosis level of peyer's patches (PP) node lymphocytes in septic rats after astragalus treatment (n = 8). (B) Western blot analysis of the apoptosis protein expression levels of Cleaved caspase-3 and BAX levels in septic rats after astragalus treatment (n = 6). (C) Detection of pro-inflammatory mediators IL-1β, IL-6 and C-reactive protein (CRP) by ELISA (n = 6). Data are depicted as the mean ± SEM. Statistical significance was determined by one-way ANOVA with a post-hoc Holm-Sidak test, ns, not significant; *P < 0.05; **P < 0.05; ***P < 0.001; ****P < 0.0001.

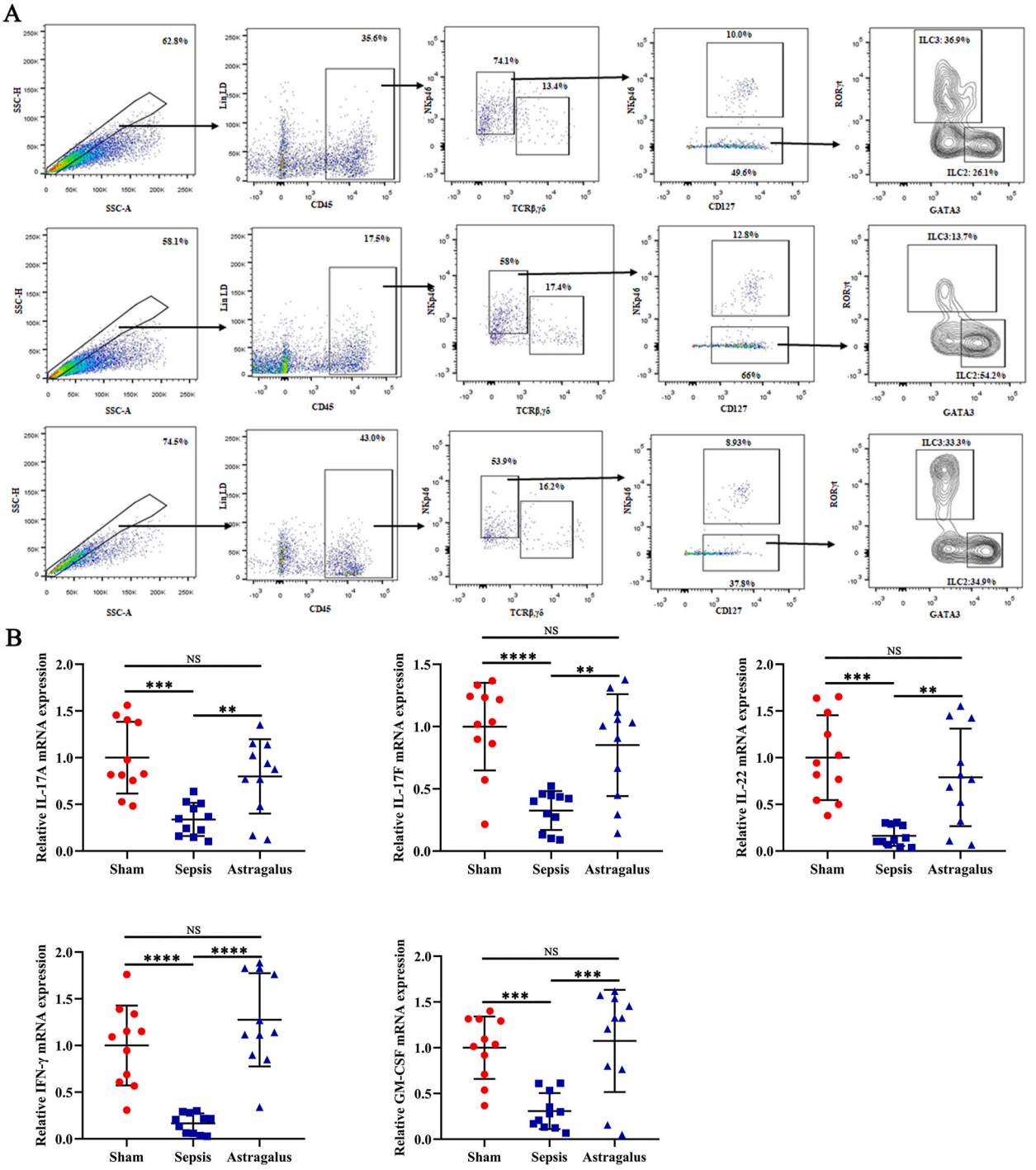

Fig. 2. Astragalus promotes proliferation of PP node ILC3 in rats with sepsis. (A) Flow cytometry was used to detect the number of group 3 innate lymphoid cells (ILC3) in septic rats after treatment with astragalus. (C) RT-qPCR analysis of the mRNA expression levels of IL-17A, IL-17F, IL-22, IFN-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF) in septic rats after treatment with astragalus (n = 10). Data are depicted as the mean ± SEM. Statistical significance was determined by one-way ANOVA with a post-hoc Holm-Sidak test, ns, not significant; *P < 0.05; **P < 0.05; ***P < 0.001; ****P < 0.0001.

3. Results

3.1. Astragalus inhibits the apoptosis rate of lymphocytes from PP

Following the TUNEL assay, cells with brownish yellow-stained nuclei were counted as apoptotic. The number of apoptotic and non-apoptotic lymphocytes were counted in five random high-power fields (magnification, × 400) of each PP. The apoptosis rate of lymphocytes in the PP of the sham operation group was 3.78 ± 0.20% at 12 h, 3.63 ± 0.22% at 24 h, and 3.75 ± 0.35% at 72 h, with no statistically significant differences within the group. The apoptosis rate of lymphocytes from PP in the sepsis group at 12 h, 24 h and 72 h were 15.7 ± 0.71%, 12.8 ± 0.47%, and 10.4 ± 0.75%, respectively, and the apoptosis rate at each time point was significantly higher than that of sham operation group ($p < 0.05$). The apoptotic rate of lymphocytes from PP in the astragalus treatment group was 11.8 ± 0.51% at 12 h, 9.25 ± 0.85% at 24 h, and 7.93 ± 0.71% at 72 h, significantly higher than in the sham operation group and significantly lower than in the sepsis group at each time point ($p < 0.05$), as shown in Fig. 1A. Therefore, astragalus treatment for 12 h significantly inhibited lymphocyte apoptosis in PP.

To examine the effect of different doses of astragalus on sepsis mortality, we monitored rat survival for 7 days after CLP under different doses of Astragalus treatment (1, 10 or 50 g/kg/d) (Supplementary Figure 1A). Based on Kaplan-Meier survival curves, rat with CLP-induced sepsis presented a 7-day survival rate of 10%. Compared with untreated septic rat, the 7-day survival rate of 1 g/kg/d astragalus treatment was 30%, and the 7-day survival rate of 50 g/kg/d astragalus treatment was 50%, while 10 g/kg/d astragalus treatment increased the 7-day survival rate to 70% (Supplementary Figure 1B). As a result of this experiment, we selected 10 g/kg/d astragalus and 12h after CLP as the end-point in subsequent experiments. Next, we examined the levels of the apoptotic proteins cleaved caspase-3 and BAX in PP (Fig. 1B), which were reduced by astragalus treatment compared with the sepsis group. In addition, we detected the serum levels of IL-1β, IL-6 and CRP in rats. We found that the levels of IL-6, IL-8 and CRP were significantly increased in CLP-induced sepsis rats, and astragalus treatment could reduce the levels of IL-1β, IL-6 and CRP, thereby attenuate sepsis injury (Fig. 1C).

3.2. Astragalus promotes proliferation of ILC3 from PP in rats with sepsis

ILCs are a newly discovered group of lymphocytes, of which there are three distinct subgroups: ILC1, ILC2, and ILC3. Using flow

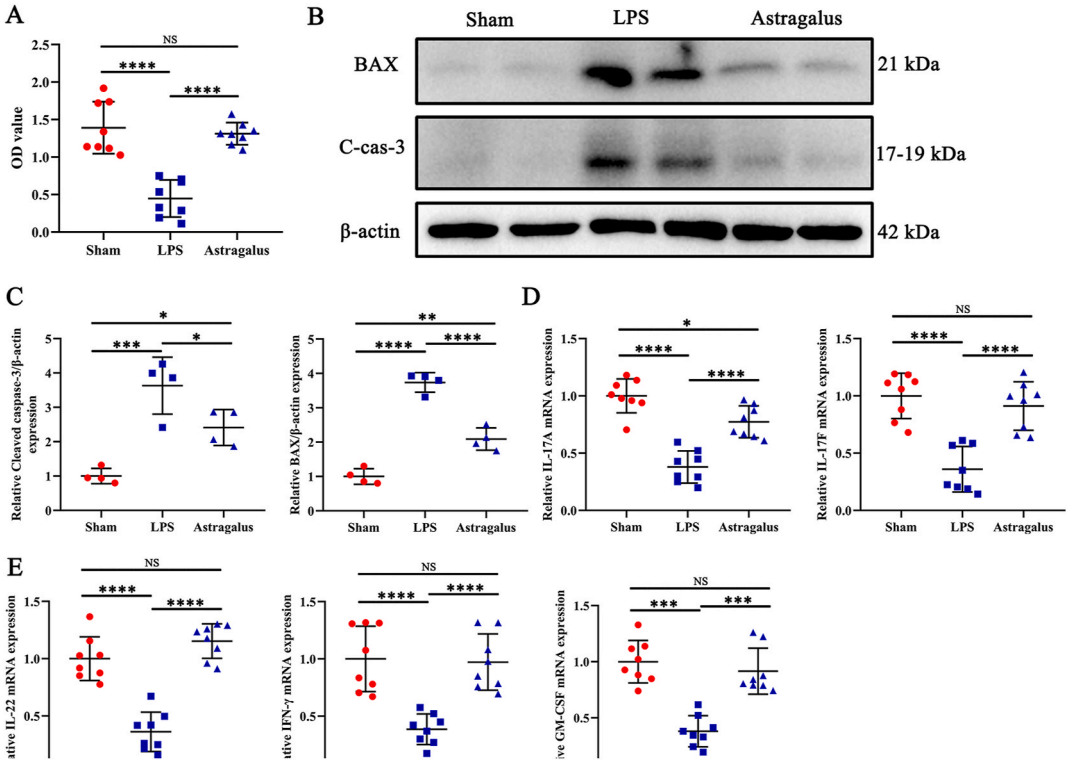

Fig. 3. Astragalus promotes the proliferation of ILC3 cells. (A) CCK-8 detected ILC3 cell survival after 24 h of LPS treatment (n = 8). (B and C) Western blot analysis of the apoptosis protein expression levels of Cleaved caspase-3 and BAX levels in ILC3 after astragalus treatment (n = 4). (D and E) RT-qPCR analysis of the mRNA expression levels of IL-17A, IL-17F, IL-22, IFN-γ, and GM-CSF in ILC3 after treatment with astragalus (n = 8). Data are depicted as the mean ± SEM. Statistical significance was determined by one-way ANOVA with a post-hoc Holm-Sidak test, ns, not significant; *P < 0.05; **P < 0.05; ***P < 0.001; ****P < 0.0001.

cytometry we found that the proportion of ILC3 in lymph nodes from sepsis group rats was significantly reduced (Fig. 2A); when treated with astragalus, this proportion increased significantly (Fig. 2A). ILC3 are similar to T helper 17 cells and produce cytokines such as IL-17A, IL-17F, IL-22, interferon (IFN)-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF), which play an important role in defending against gastrointestinal infection and maintaining intestinal tract homeostasis [25,26]. Therefore, we examined the expression of IFN-γ, IL-17A, IL-17F, IL-22 and GM-CSF mRNA in ILC3 in PP. The results showed that astragalus significantly upregulated the expression of IFN-γ, IL-17A, IL-17F, IL-22, and GM-CSF in PP compared with the sepsis group (Fig. 2B).

Next, we isolated ILC3 from healthy rat intestine and constructed a sepsis model in vitro. CCK-8 results showed that after LPS treatment, the survival of ILC3 was significantly reduced, whereas astragalus inhibited this effect (Fig. 3A). The level of apoptotic proteins further revealed that astragalus reduced the level of LPS-induced ILC3 apoptosis (Fig. 3B and C). In addition, the levels of IFN-γ, IL-17A, IL-17F, IL-22, and GM-CSF were significantly increased after astragalus treatment (Fig. 3D and E) compared with the LPS-only group. Taken together, these results demonstrate that astragalus treatment can significantly increase ILC3 levels and improve the inflammatory microenvironment in septic rats.

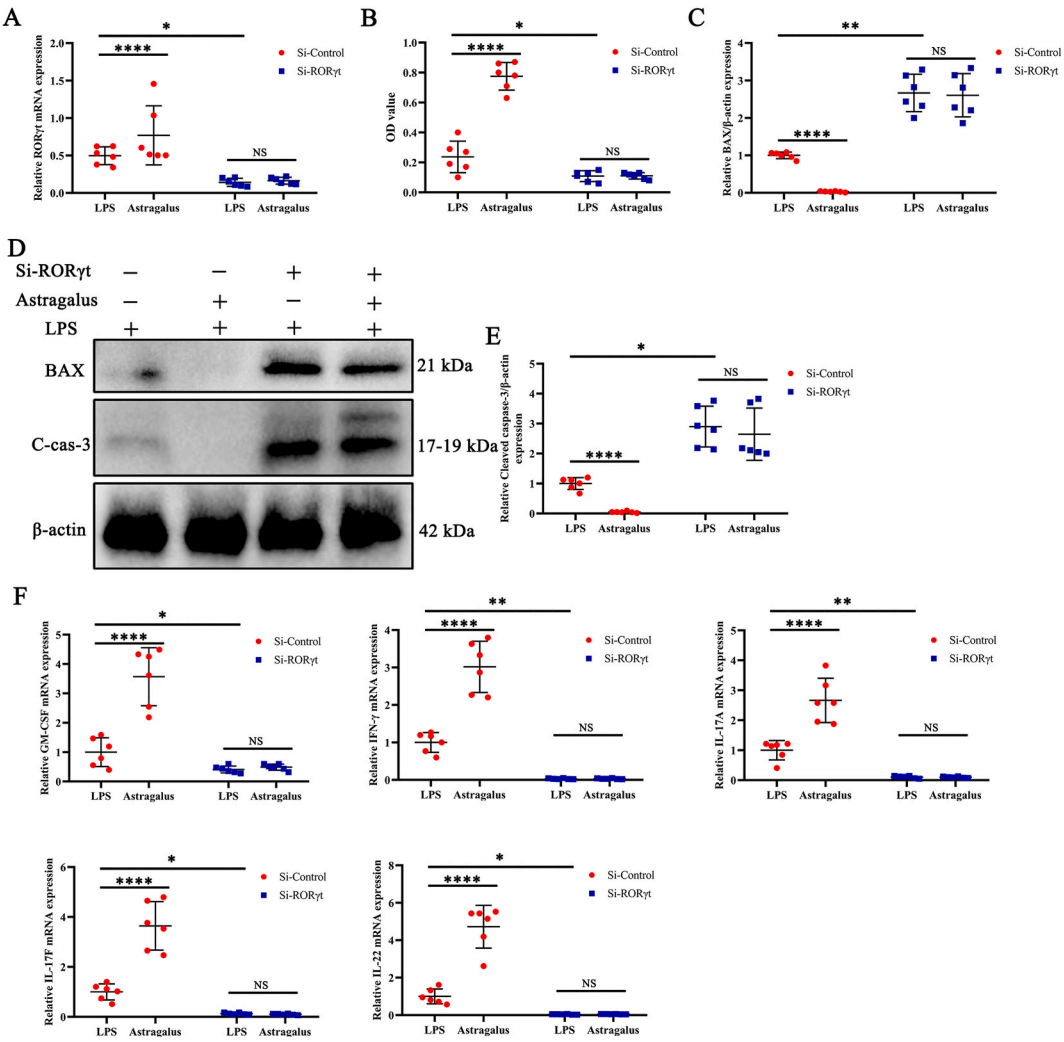

Fig. 4. Astragalus promotes ILC3 cytokines secretion and inhibits apoptosis through RORγt. (A) RT-qPCR analysis of the mRNA expression levels of retinoic acid-related orphan receptor γt (RORγt) after treatment with astragalus and Si-RORγt (n = 6). (B) CCK-8 detected ILC3 cell survival after 24 h of RORγt knockout and astragalus treatment (n = 8). (C–E) Western blot analysis of the apoptosis protein expression levels of Cleaved caspase-3 (D and E, n = 6) and BAX (C and D, n = 6) levels in ILC3 after RORγt knockout and astragalus treatment. (F) RT-qPCR analysis of the mRNA expression levels of GM-CSF, IFN-γ, IL-17A, IL-17F and IL-22 in ILC3 after RORγt knockout and astragalus treatment (n = 6). Data are depicted as the mean ± SEM. Statistical significance was determined by two-way ANOVA with a post-hoc Holm-Sidak test, ns, not significant; *P < 0.05; **P < 0.05; ***P < 0.001; ****P < 0.0001.

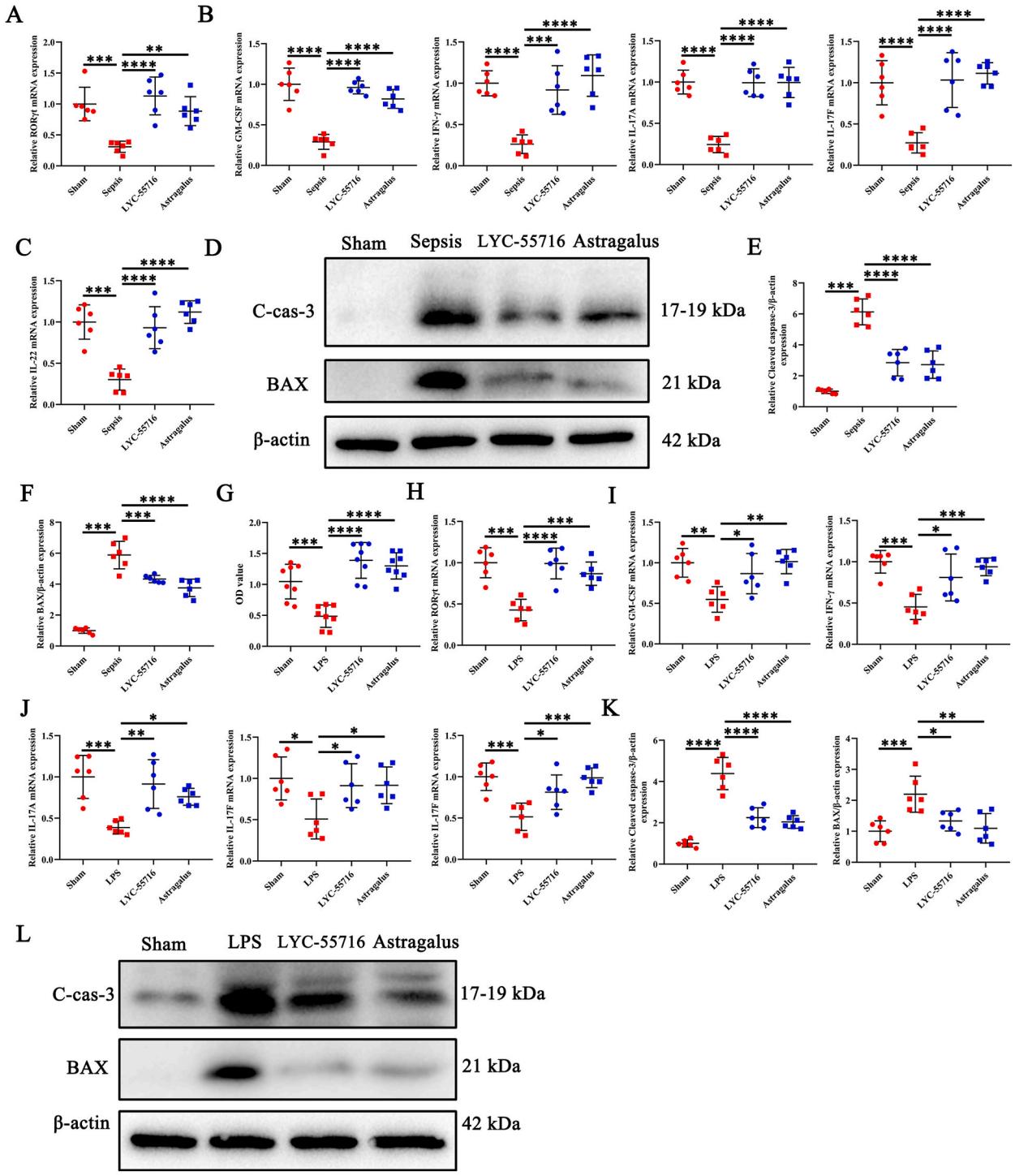

Fig. 5. Astragalus and LYC-55716 promote ILC3 proliferation by activating RORγt and regulate the inflammatory response in sepsis. (A) RT-qPCR analysis of the mRNA expression levels of RORγt after treatment with astragalus and RORγt agonist (LYC-55716, n = 6). (B and C) RT-qPCR analysis of the mRNA expression levels of GM-CSF, IFN-γ, IL-17A, IL-17F and IL-22 in ILC3 after treatment with astragalus and LYC-55716 (n = 6). (D–F) Western blot analysis of the apoptosis protein expression levels of Cleaved caspase-3 (D and E, n = 6) and BAX (D and F, n = 6) levels in septic rats after treatment with astragalus and LYC-55716. (G) CCK-8 detected ILC3 cell survival after treatment with astragalus treatment and LYC-55716 (n = 6). (H–J) RT-qPCR analysis of the mRNA expression levels of RORγt, GM-CSF, IFN-γ, IL-17A, IL-17F and IL-22 in ILC3 after treatment with astragalus treatment and LYC-55716 (n = 6). (K and L) Western blot analysis of the apoptosis protein expression levels of Cleaved caspase-3 and BAX levels in ILC3 after treatment with astragalus and LYC-55716 (n = 6). Data are depicted as the mean ± SEM. Statistical significance was determined by one-way ANOVA with a post-hoc Holm-Sidak test, ns, not significant; *P < 0.05; **P < 0.05; ***P < 0.001; ****P < 0.0001.

3.3. Astragalus promotes ILC3 cytokine secretion and inhibits apoptosis through retinoic acid-related orphan receptor γt

Retinoic acid-related orphan receptor γt (RORγt) is a key transcription factor for the generation of ILC3, and the loss of RORγt can severely block the differentiation and development of ILC3 [27]. To explore whether astragalus promoted the expression of RORγt, we measured the level of RORγt-mRNA in ILC3. The results showed that astragalus significantly promoted RORγt-mRNA expression (Fig. 4A). The CCK-8 assay showed that astragalus could promote the survival of ILC3, while after RORγt knockout, astragalus had no effect on the survival of ILC3 (Fig. 4B). Western blotting also revealed that the effect of astragalus on the apoptosis of ILC3 was affected by RORγt blockade (Fig. 4C–E). In addition, after RORγt knockout, astragalus failed to increase the levels of IL-17A, IL-17F, IL-22, IFN-γ, and GM-CSF mRNA expression in ILC3 (Fig. 4F).

3.4. Astragalus and LYC-55716 promote ILC3 proliferation by activating RORγt and regulating the inflammatory response in sepsis

To further clarify the role of RORγt in the effect of astragalus on the inflammatory response in sepsis, we used a RORγt agonist (LYC-55716) to compare its effects with those of astragalus. This experiment showed that both astragalus and LYC-55716 treatment promoted the expression of RORγt mRNA, with no significant difference between the two (Fig. 5A). RT-PCR further showed that astragalus and LYC-55716 both significantly promoted the expression of IL-17A, IL-17F, IL-22, IFN-γ, and GM-CSF mRNA (Fig. 5B and C). Western blotting showed that astragalus and LYC-55716 both inhibited the apoptosis of lymphocytes in PP (Fig. 5D–F).

In vitro experiments also demonstrated that the proliferation of ILC3 significantly increased after incubation with astragalus and LYC-55716 (Fig. 5G). The results of RT-PCR were consistent with the results of the in vivo experiments. Astragalus and LYC-55716 significantly promoted the expression of RORγt (Fig. 5H), IL-17A, IL-17F, IL-22, IFN-γ, and GM-CSF mRNA in ILC3 (Fig. 5I and J). Western blotting showed that astragalus and LYC-55716 inhibited apoptosis in ILC3 (Fig. 5K and L). These results indicate that astragalus and LYC-55716 both promote the proliferation of ILC3 and regulate the inflammatory response in sepsis by activating RORγt.

4. Discussion

The intestine is the largest and most important immune organ in the human body, and the mucosal barrier is the main component of the intestinal immune system [28]. Its functional structure includes intestinal mucosa, submucosal intestinal lymph nodes, and independent lymph nodes [28–30]. Studies have confirmed that the pathophysiology of sepsis is mainly attributed to uncontrolled SIRS, and intestinal barrier dysfunction also plays a key role in this process. The early stages of sepsis involve an excessive inflammatory response, while patients in the latter stages of sepsis are usually immunosuppressed, making them susceptible to secondary infections [31,32]. Our study confirmed for the first time that Astragalus can mediate the proliferation of ILC3, reduce the apoptosis of lymphocytes in PP of septic rats, and improve the intestinal homeostasis of septic rats. This is supported by the CLP-induced sepsis model and the comparative treatment of the two drugs. In conclusion, our findings suggest that the intestinal immune barrier plays an important role in sepsis, and Astragalus can correct intestinal immune barrier dysfunction and improve prognosis.

PP are part of the mucosal tissue of the small intestine [33]. They recognize and presents antigens and can eliminate a variety of pathogenic microorganisms [33,34]. Lymphocytes within these structures maintain the integrity of the intestinal epithelium and play an important role in intestinal immune responses. In sepsis, the intestinal mucosa is in an ischemic and hypoxic state, which promotes apoptosis of lymphocytes from PP, aggravating the condition [35,36]. Our results showed that during sepsis, a large number of lymphocytes in PP undergo apoptosis, which leads to the suppression of intestinal immune function and worsens sepsis. The apoptosis rate of lymphocytes in the PP of the astragalus treatment group was lower than that in the sepsis group at all time points. This shows that astragalus can inhibit the apoptosis of lymphocytes in the PP of septic rats, thereby enhancing the intestinal immune function in these animals.

The innate immune response mediated by ILCs is the first line of defense against various pathogenic microorganisms, and plays an important role in antimicrobial infection [37]. Based on the expression of transcription factors and cytokines secreted by ILCs, they can be divided into three different groups: ILC1, ILC2, and ILC3 [37,38]. Among them, ILC3 are mainly present in the intestinal tract and can play an important role in the formation of lymphoid organs, the integrity of the epithelial barrier, the regulation of intestinal inflammation, and the antibacterial response by secreting cytokines such as IL-17A, IL-17F, IL-22, IFN-γ, and GM-CSF [39]. In this study, we found that the numbers of ILC3 in the PP of septic rats was decreased, thereby inhibiting the expression of IL-17A, IL-17F, IL-22, IFN-γ, and GM-CSF mRNA. However, after astragalus treatment, the numbers of ILC3 increased, as did the expression of IL-17A, IL-17F, IL-22, IFN-γ, and GM-CSF mRNA, which should lead to improved intestinal homeostasis.

RORγt is a key transcription factor for the conversion of progenitor cells to ILC3, and an important target in infection and inflammation [27]. Interactions between ILC3 and RORγt^{+} regulatory T cells are compromised in inflammatory bowel disease [27,40]. These results define a paradigm whereby ILC3 select for antigen-specific RORγt^{+} regulatory T cells and against T helper 17 cells, thereby establishing immune tolerance to the microbiota and gut health [41]. Our results further confirm the role of RORγt in sepsis [42]. We found that during sepsis the expression of RORγt is inhibited, thereby suppressing the proliferation of ILC3. In addition, we discovered a new mechanism by which astragalus mediates regulation of intestinal inflammation by ILC3. Astragalus has a similar effect on the intestinal tract to the RORγt activator LYC-55716, which can promote the production of ILC3 by activating the expression of RORγt, thereby improving the intestinal inflammatory environment. After knocking down RORγt expression, the effect of astragalus on ILC3 disappeared, further confirming the mechanism by which astragalus regulates the intestinal inflammatory response.

In this study, astragalus effectively inhibited ILC3 in septic rats. This indicates that astragalus has an immunoregulatory effect on

the intestinal immune function of rats with sepsis, and suggests that astragalus can be used as an immunomodulator of sepsis. However, this study was primarily conducted on rats and no further human trials were conducted. The role of astragalus in patients with sepsis will be further investigated in future studies.

Author contribution statement

Jin Li, Xi Chen and Jun Fan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Lidong Wu and Liang He: Performed the experiments.

Juan Tu: Contributed reagents, materials, analysis tools or data.

Shufang Chen: Analyzed and interpreted the data.

Data availability statement

Data will be made available on request.

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.

Abbreviations

PP	Peyer's patches
ILC3	Type 3 innate lymphoid cells
RORγt	Retinoic acid-related orphan receptor γt
CLP	Ligation and puncture

Appendix B. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e17766.

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