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ORIGINAL RESEARCH

Combination of Glutamine and Ulinastatin Treatments Greatly Improves Sepsis Outcomes

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Background: Sepsis is one of the most dangerous syndromes, has extremely high mortality, and is caused by the body's extreme responses to an infection. The pathogenesis of sepsis is very complex and remains largely unknown and thus the treatments for sepsis are limited. Here, we evaluated the treatment results of two potential drugs, glutamine and ulinastatin, on sepsis.

Methods: CLP rat model was used to study sepsis. Gastrostomy was performed to deliver the drugs. Flow cytometry was employed to measure CD4 and CD8 levels. May–Grünwald–Giemsa staining was used to count the numbers of monocytes and neutrophils in the blood. ELISA assay was performed to assess the levels of PCT, IL-6, TNFα, and IL-1β.

Results: Sepsis was successfully induced with the standard CLP rat model. Both glutamine and ulinastatin treatments greatly improved the outcomes of sepsis, but the combination of both treatments had the maximum therapeutic effect. Mechanistically, PCT, IL-6, TNFα, and IL-1β levels were significantly diminished following glutamine and ulinastatin treatments, suggesting an inhibition of inflammatory responses. Further, CD4 and CD4/CD8 ratio, and the numbers of monocytes and neutrophils were greatly up-regulated by glutamine and ulinastatin, indicating an enhanced immunity.

Conclusion: Glutamine and ulinastatin treatments largely mitigate sepsis shock by suppressing the inflammatory responses of the body and strengthening the immune system. Combination of these two drugs could serve as a potential treatment for sepsis.

Keywords: sepsis, glutamine, ulinastatin, inflammation, immune function

Introduction

Sepsis is a life-threatening syndrome that arises when systemic inflammatory responses to infections are out of balance and trigger damages to the body's own tissues and organs.[1–4] It has been recognized as a deadly menace with extremely high mortality and is one of the major causes of death among hospitalized patients. The pathogenesis of sepsis is very complex and currently is under intense scrutiny. Mechanisms including systemic inflammation, coagulation, disordered fibrinolysis, and overproduction of reactive oxygen and nitrogen species have been proposed.[3,5] Clinically sepsis is divided into several progressive phases based on the symptoms.[2,6] Following infection, a hyperinflammation phase is characterized by systemic inflammatory response syndromes, such as high body temperature, elevated pulse rate, and abnormal white blood cell number. Without appropriate treatments, the disease can develop into the severe phase during which the immune system gets suppressed instead and widespread organ dysfunction is present. Despite numerous studies and great advances in research, the mortality rate associated with sepsis still remains high and thus there is a clear need for better therapeutic treatments.

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Glutamine is the most abundant free amino acid in the blood circulation that is used for biosynthesis of proteins.[7] It has many important functions.[7,8] For example, it can regulate the metabolism of liver and small intestine, and help gut function.[8,9] In addition, it is the essential amino acid for lymphocyte proliferation and secretion.[10–13] Previous studies also indicate that glutamine has antioxidant effects and can improve intestinal immunity by stimulating the release of lymphocytes within the intestinal wall.[14,15] Moreover, glutamine can promote the synthesis and secretion of immunoglobulin, and increase the production of gastrointestinal-related hormones.[16,17] Therefore, glutamine has been used as an anti-inflammatory treatment.[18,19]

Ulinastatin is a glycoprotein purified from the fresh urine of an adult healthy male and is an intrinsic trypsin inhibitor.[20] As an acidic protein, ulinastatin can inhibit the activity of various enzymes and the production of many inflammatory factors and mediators.[21–23] In addition, it can directly bind with toxins and protect the body. Consequently, ulinastatin has been used to treat acute pancreatitis, acute circulatory failure, acute lung injury and many other severe infection-related diseases.[20,24,25]

In the present study, we sought to investigate the effects of glutamine and ulinastatin treatments on sepsis and examine the underlying mechanisms. We found that both glutamine and ulinastatin greatly alleviated sepsis syndromes and that combination of both treatments had the biggest effect. Mechanistically, we showed that glutamine and ulinastatin inhibited the inflammatory responses by decreasing levels of procalcitonin, IL-6, TNFα, and IL-1β. Further, they can strengthen the immune system by up-regulating CD4, CD4/CD8 ratio, and the numbers of monocytes and neutrophils. Our study demonstrates that the combination of glutamine and ulinastatin tremendously improves the outcome of sepsis and thus they can be used as a potential therapeutic avenue for sepsis in the future.

Materials and Methods

Cecal Ligation and Puncture (CLP) Rat Model and Gastrostomy

Sprague Dawley wild-type rats were purchased from Animal Center of Inner Mongolia Medical University. All animal protocols were approved by Inner Mongolia Medical University Committee of Animal Care and Use, and animal experiments were done according to national and institutional animal care and ethical guidelines. Sepsis was induced by the standardized CLP as described previously.[26,27] Briefly, adult male rats (8–12 weeks, 240–260g) were fasted overnight and then anesthetized by 10% chloral hydrate (4mL/kg). The abdominal region was cut open and the cecum was located and separated from the surrounding tissues. The cecum was ligated with a 3.0 silk ligature at the middle part. A 22-G needle was used to make one perforating puncture between the ligation and the tip of the cecum. The cecum was then put back.

The stomach was located afterwards and was cut open between the anterior gastric wall and the bottom of gastric body. A spherical headed silicon tube (2-mm in diameter) was inserted into the stomach and attached with the stomach. The other end of the tube was put behind the neck through the lateral abdominal wall. Then, the abdomen was closed.

Following the surgery, the saline group (15 animals) was injected with normal saline (30mL/kg) via the tail vein; Ulinastatin (Tianpu Shenghua Medical Co., Ltd. Guangdong, China) group (15 animals) was injected with ulinastatin (100,000 U/kg) via the tail vein; Glutamine (Linco Pharmaceutical Group, Hainan, China) group (15 animals) was fed with glutamine (0.6g/kg) through the silicon tube; Glutamine plus ulinastatin group (15 animals) was injected with ulinastatin (100,000U/kg) via the tail vein and fed with glutamine (0.6g/kg) through the silicon tube. The rats in the control group were anesthetized by 10% chloral hydrate (4mL/kg) without any additional surgeries. No animals died during the surgery and the animals were sacrificed at 24 h after surgery for further experiments.

Sample Collection

Blood was collected through the fundus veins at 6h, 12h and 24h after surgery. A fraction of blood was collected and gently mixed in the EDTA-anticoagulant tube; Another fraction was put in the centrifuge tube at room temperature for 10–20 mins to allow for coagulation. Then, the blood was centrifuged at 3,000 rpm for 10 mins and the supernatant was collected and stored at −80 °C for further experiments.

White Blood Cell Counts

Blood was used to determine the total white blood cell (WBC) counts in different groups. Five microliters of blood were stained with May–Grünwald–Giemsa (Solarbio Life Sciences, China) according to the manufacturer's protocol and then smeared onto a microscope glass.

The percentage of monocytes and neutrophils was counted using a microcell counter.

Flow Cytometry

CD4 and CD8 levels were measured through flow cytometry. Briefly, the blood was incubated with specific fluorochrome-conjugated antibodies (CD4 [Zehao Biotech CO., Ltd. China), CD8 [Zehao Biotech CO., Ltd. China] and control mouse IgG1 [Zehao Biotech CO., Ltd. China]) for 15 mins at room temperature first, and then incubated with fluorescence-activated cell scanner (FACS) lysis solution for another 10 mins, followed by analysis on the FACS Calibur flow cytometer (SpringDe Electronic Technology CO., Ltd. China).

Enzyme-Linked Immunosorbent Assay (ELISA)

Plasma was collected and plasma procalcitonin (PCT), IL-6, TNFα, and IL-1β levels were determined with commercial specific ELISA kits (Shanghai Qiyi Biotech CO., Ltd, China) according to the manufacturer's protocols. Briefly, 100 μL standard samples and collected samples were added into the 96-well plate and incubated at 37 °C for 1h. One hundred microliters of specific antibodies (Shanghai Qiyi Biotech CO., Ltd, China) were added afterwards and incubated for another 1h, followed by 30 min of incubation with 100 μL horseradish peroxidase-linked secondary antibody. Finally, the plate was washed with TBST and analyzed with a spectrophotometer.

Statistical Analysis

All statistical analyses were analyzed with software SPSS22.0 and plotted in GraphPad Prism 7. Data distribution was tested for normality and all the data showed normal distribution. Comparison was made using two-way ANOVA with Bonferroni's multiple comparisons test. The Data were presented as Mean ± SEM. All experimented were performed at least three times.

Results

Ulinastatin and Glutamine Improved Sepsis Symptoms

To investigate the effects of glutamine and ulinastatin on sepsis, we induced sepsis by using the standard CLP rat model. Immediately following the surgery, we treated the rats with different drugs and examined their responses. We observed that all rats showed tremor, retardation, piloerection, tachypnea and abdominal swelling starting 3 hrs after the surgery, indicating the success of sepsis induction. However, the group treated with normal saline exhibited the severest symptoms, including shortness of breath, very rare activity, and obvious distension of the abdomen (Table 1). The ulinastatin group and glutamine group showed general symptoms with fast breathing, reduced activity, and some abdominal distension (Table 1). The combined group that was treated with ulinastatin and glutamine had the most minor symptoms. They showed regular breath, general activity and very light abdominal distension (Table 1). We sacrificed the rats after 24 h and examined the cecum. In the saline group, the cecum turned black with severe edema (Table 1). In the ulinastatin and glutamine groups, both the color of the cecum and the degree of edema were lighter compared to the saline group (Table 1). In the combined group, the cecum showed the lightest darkness and exhibited the least edema (Table 1). Together, these results show that treatment with ulinastatin and glutamine can improve the outcomes of sepsis and that combination of both treatments is the most effective way.

Ulinastatin and Glutamine Suppressed Inflammatory Responses in Sepsis

Next, we sought to explore the mechanisms underlying the protective effects of ulinastatin and glutamine on sepsis. We examined the inflammatory responses as they are greatly involved in sepsis.[6] Procalcitonin (PCT), IL-6, TNFα, and IL-1β are important inflammatory markers and PCT has been used as diagnosis of sepsis.[28,29] Their expressions are suppressed in the absence of infection but get highly enhanced following infection. As expected, in the control

Table 1 Symptoms of Rats Following CLP Surgery and Drug Treatments

Group	Breath	Activity	Color of Cecum	Abdominal Distension	Edema
Saline	Tachypnea	Rare	Dark	Obvious	Severe
Ulinastatin	Fast	Reduced	Brown	Some	Some
Glutamate	Fast	Reduced	Brown	Some	Some
Combined	Regular	General	Light	Little	Little

group in which no sepsis was induced, the levels of PCT, IL-6, TNFα, and IL-1β were very low, and they were tremendously elevated in rats that underwent CLP surgery (Figure 1A–D), indicating that CLP surgery induces robust inflammatory responses. However, we found that both ulinastatin treatment and glutamine treatment significantly reduced the levels of PCT, IL-6, TNFα, and IL-1β at all time points (6, 12, 24 h) compared to saline-treated animals (Figure 1A–D). Moreover, the combination of both treatments further decreased the levels of PCT, IL-6, TNFα, and IL-1β in the plasma (Figure 1A–D). Therefore, we conclude that ulinastatin and glutamine treatments inhibit the inflammatory responses during sepsis.

Ulinastatin and Glutamine Enhanced Immune Responses Following Sepsis

To further understand the underlying mechanisms, we examined how ulinastatin and glutamine affected the immune system during sepsis. CD4 and CD8 positive T helper cells play an important role in the development of immunity against infection.[30] The number of CD4 positive cells and CD4:CD8 ratio are highly associated with immune function. Increased CD4 positive cells and CD4/CD8 ratio indicate strong immune system.[31,32] We found that treatment with ulinastatin and glutamine significantly increased the percentage of CD4 positive cells compared with the saline-treated rats (Figure 2A and B), and that combination of both treatments further up-regulated the number (Figure 2A and B). More importantly, CD4/CD8 ratio was greatly increased following ulinastatin and glutamine treatments and rats treated with both ulinastatin and glutamine had the highest ratio (Figure 2A and C). We also counted the numbers of monocytes and neutrophils in the blood as they are good indicators of immune system as well. We found that CLP surgery greatly decreased the numbers of monocytes and neutrophils compared to control rats (Figure 2D and E). Nevertheless, ulinastatin and glutamine treatments recovered the numbers (Figure 2D and E), and the combination of both treatments increased the numbers the most (Figure 2D and E). Taken

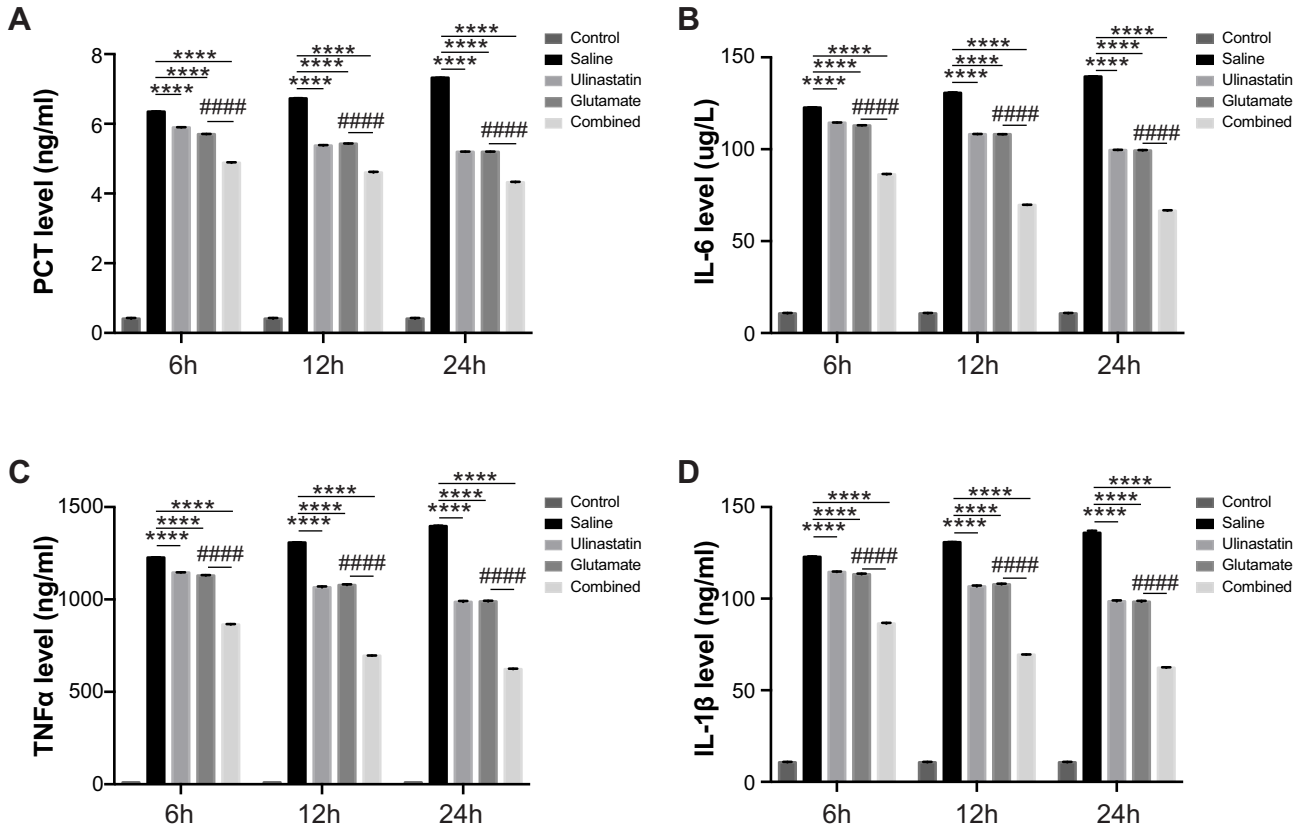

Figure 1 PCT, IL-6, TNFα, and IL-1β levels in rats following CLP surgery and drug treatments.

Notes: (**A**) PCT levels in different groups of rats at different time points following CLP surgery (n = 15 in each group). (**B**) IL-6 levels in different groups of rats at different time points following CLP surgery (n = 15 in each group). (**C**) TNFα levels in different groups of rats at different time points following CLP surgery (n = 15 in each group). (**D**) IL-1β levels in different groups of rats at different time points following CLP surgery (n = 15 in each group). Data are presented as mean ± SEM. ****$P < 0.0001$; ####$P < 0.0001$.

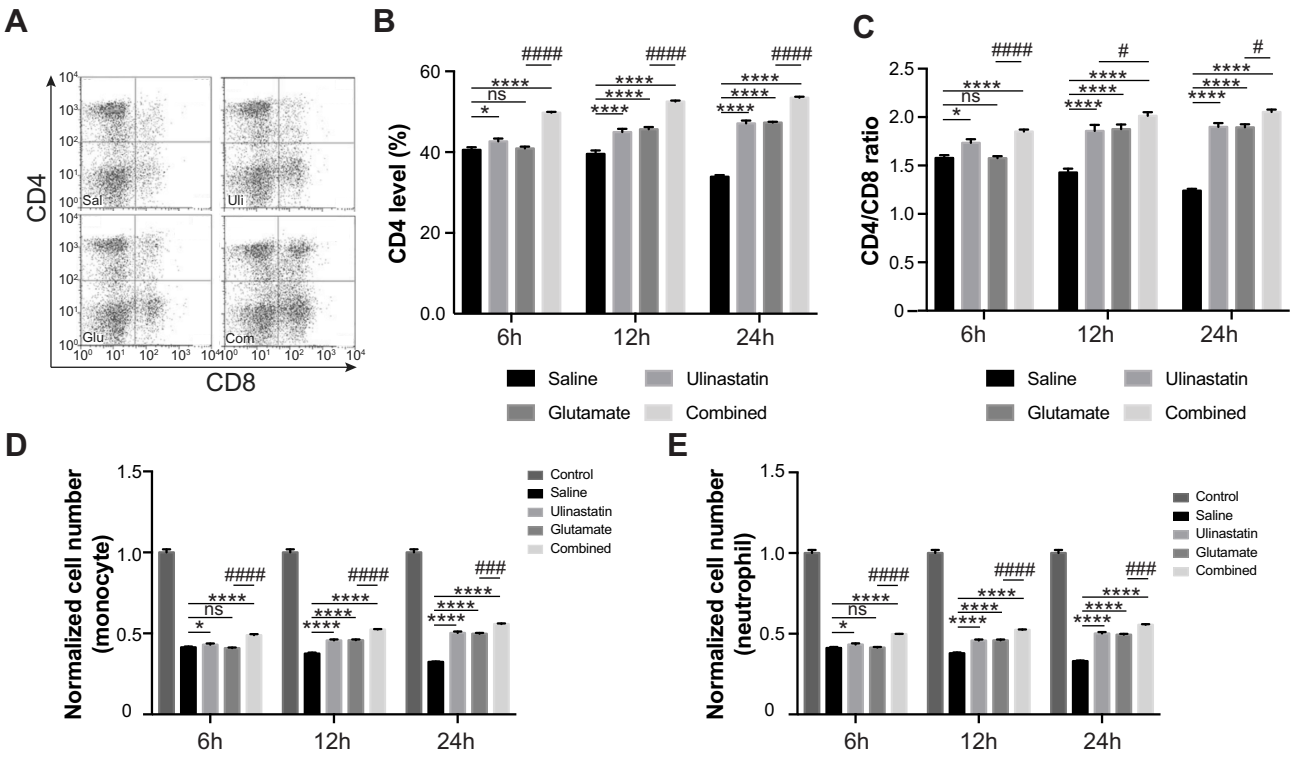

Figure 2 CD4 levels, CD4/CD8 ratios, and the numbers of monocytes and neutrophils in rats following CLP surgery and drug treatments.
Notes: (**A**) Representative flow cytometry dot plots in different groups of rats 24 h following CLP surgery (n = 15 in each group). (**B**) CD4 levels in different groups of rats at different time points following CLP surgery (n = 15 in each group). (**C**) CD4/CD8 ratios in different groups of rats at different time points following CLP surgery (n = 15 in each group). (**D**) The number of monocytes in different groups of rats at different time points following CLP surgery (n = 15 in each group). (**E**) The number of neutrophils in different groups of rats at different time points following CLP surgery (n = 15 in each group). Data are presented as mean ± SEM. ns, not significant; $^{*}P < 0.05$; $^{****}P < 0.0001$; $^{\#}P < 0.05$; $^{\#\#\#\#}P < 0.001$; $^{\#\#\#\#}P < 0.0001$.

together, these results show that ulinastatin and glutamine treatments strongly increase the immunity against sepsis and thus improve the outcome.

Discussion

In the study, we show that glutamine and ulinastatin treatments can inhibit the inflammation process but strengthen the immune system during sepsis, resulting in improved outcome with alleviated symptoms. Mechanistically, we found that glutamine and ulinastatin decrease PCT, IL-6, TNFα, and IL-1β levels but up-regulate CD4, CD4/CD8 ratio, and the numbers of monocytes and neutrophils. Our work reveals that the combination of glutamine and ulinastatin greatly protects from sepsis and therefore could be used as a future therapy for sepsis.

The development and progression of sepsis is extremely complicated and many factors are involved.[2] Following infection, septicemia pathogens activate the immune system, which can cause ischemia and hypoxia of cells and tissue damage.[6] Such excessive immune responses can further trigger inflammatory response syndrome and even cause multiple organ dysfunction.[33] The body undergoes anti-inflammatory and pro-inflammatory dynamic responses through related molecular patterns and various signal pathways, such as NOD-like receptor signaling pathway and activation of related immune cells, leading to immune disorders and a series of pathophysiological reactions.[34,35] In view of this, control of the inflammatory responses is very critical and clinically treatment with antibiotics always starts immediately. Ulinastatin is a multivalent Kunitz-type serine protease inhibitor and has been shown to possess anti-inflammatory effects that can suppress the expression of multiple inflammation cytokines including TNF-α, IL-6, IL-8.[36–38] Here, we showed that intravenous injection of ulinastatin attenuated inflammation induced by CLP surgery and thus ameliorated the symptoms.

With the progression of sepsis, the immune system gets inhibited as a large number of lymphocytes become apoptotic and the function of macrophages is lost. Moreover, anti-inflammatory factors like IL-6 and IL-10 are released sharply in the body, causing excessive inflammatory reactions and thereby suppressing the immune system.[39–41] Therefore, increasing the immunity is very important for sepsis therapy.

Glutamine, as the most abundant free amino acid, is utilized as an energy source for cells of the immune system and is essential to support optimal lymphocyte proliferation. Low plasma glutamine concentration could contribute to the immunosuppression and therefore glutamine has been used to maintain the immune function in patients with various diseases. In this study, we also found that glutamine treatment could greatly enhance the immune function in sepsis. The percentage of CD4 positive cells and CD4/CD8 ratio was largely increased following glutamine treatment during sepsis. Further, the number of monocytes and neutrophils were up-regulated as well.

Interestingly, we found that the combination of ulinastatin and glutamine treatments has better effects compared to ulinastatin alone or glutamine alone. In the combined group, the rats had the most minor symptoms. Therefore, the combination of both drugs has synergic effects.

Conclusion

In summary, our work demonstrates that the combination of ulinastatin and glutamine can largely improve the outcomes of sepsis by suppressing inflammation and strengthening the immune system. This provides a therapeutic avenue for sepsis treatment in the future.

Abbreviations

CD 4/8, cluster of differentiation 4/8; CLP, cecal ligation and puncture; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell scanner; IL- 6/8/10/1β, Interleukin 6/8/10/1β; PCT, procalcitonin; TNF-α, tumor Necrosis Factor-alpha.

Ethics Approval

All procedures were approved by Inner Mongolia Medical University Committee of Animal Care and Use.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests related to this study.

References

1. Rello J, Valenzuela-Sanchez F, Ruiz-Rodriguez M, Moyano S. Sepsis: a review of advances in management. *Adv Ther*. 2017;34(11):2393–2411. doi:10.1007/s12325-017-0622-8
2. Taeb AM, Hooper MH, Marik PE. Sepsis: current definition, pathophysiology, diagnosis, and management. *Nutr Clin Pract*. 2017;32(3):296–308. doi:10.1177/0884533617695243
3. Gotts JE, Matthay MA. Sepsis: pathophysiology and clinical management. *BMJ*. 2016;353:i1585. doi:10.1136/bmj.i1585
4. Cohen J, Vincent JL, Adhikari NK, et al. Sepsis: a roadmap for future research. *Lancet Infect Dis*. 2015;15(5):581–614. doi:10.1016/S1473-3099(15)70112-X
5. Stearns-Kurosawa DJ, Osuchowski MF, Valentine C, Kurosawa S, Remick DG. The pathogenesis of sepsis. *Annu Rev Pathol*. 2011;6:19–48. doi:10.1146/annurev-pathol-011110-130327
6. Delano MJ, Ward PA. The immune system's role in sepsis progression, resolution, and long-term outcome. *Immunol Rev*. 2016;274(1):330–353.
7. Brosnan JT, Brosnan ME. Glutamate: a truly functional amino acid. *Amino Acids*. 2013;45(3):413–418. doi:10.1007/s00726-012-1280-4
8. Walker MC, van der Donk WA. The many roles of glutamate in metabolism. *J Ind Microbiol Biotechnol*. 2016;43(2–3):419–430. doi:10.1007/s10295-015-1665-y
9. Du J, Li XH, Li YJ. Glutamate in peripheral organs: biology and pharmacology. *Eur J Pharmacol*. 2016;784:42–48. doi:10.1016/j.ejphar.2016.05.009
10. Pacheco R, Oliva H, Martinez-Navio JM, et al. Glutamate released by dendritic cells as a novel modulator of T cell activation. *J Immunol*. 2006;177(10):6695–6704. doi:10.4049/jimmunol.177.10.6695
11. Rohde T, MacLean DA, Klarlund Pedersen B. Glutamine, lymphocyte proliferation and cytokine production. *Scand J Immunol*. 1996;44(6):648–650. doi:10.1046/j.1365-3083.1996.d01-352.x
12. Xue H, Field CJ. New role of glutamate as an immunoregulator via glutamate receptors and transporters. *Front Biosci (Schol Ed)*. 2011;3:1007–1020. doi:10.2741/s205
13. Palmieri EM, Menga A, Martin-Perez R, et al. Pharmacologic or genetic targeting of glutamine synthetase skews macrophages toward an M1-like phenotype and inhibits tumor metastasis. *Cell Rep*. 2017;20(7):1654–1666. doi:10.1016/j.celrep.2017.07.054
14. Jiang J, Yin L, Li JY, et al. Glutamate attenuates lipopolysaccharide-induced oxidative damage and mRNA expression changes of tight junction and defensin proteins, inflammatory and apoptosis response signaling molecules in the intestine of fish. *Fish Shellfish Immunol*. 2017;70:473–484. doi:10.1016/j.fsi.2017.09.035
15. Jiao N, Wu Z, Ji Y, Wang B, Dai Z, Wu G. L-glutamate enhances barrier and antioxidative functions in intestinal porcine epithelial cells. *J Nutr*. 2015;145(10):2258–2264. doi:10.3945/jn.115.217661
16. Andersson LE, Shcherbina L, Al-Majdoub M, et al. Glutamine-elicited secretion of glucagon-like peptide 1 is governed by an activated glutamate dehydrogenase. *Diabetes*. 2018;67(3):372–384. doi:10.2337/db16-1441

17. Maechler P. Glutamate pathways of the beta-cell and the control of insulin secretion. *Diabetes Res Clin Pract*. 2017;131:149–153. doi:10.1016/j.diabres.2017.07.009
18. Jain P, Khanna NK. Evaluation of anti-inflammatory and analgesic properties of L-glutamine. *Agents Actions*. 1981;11(3):243–249. doi:10.1007/BF01967621
19. Cruzat V, Macedo Rogero M, Noel Keane K, Curi R, Newsholme P. Glutamine: metabolism and immune function, supplementation and clinical translation. *Nutrients*. 2018;10:11. doi:10.3390/nu10111564
20. Atal SS, Atal S. Ulinastatin - a newer potential therapeutic option for multiple organ dysfunction syndrome. *J Basic Clin Physiol Pharmacol*. 2016;27(2):91–99. doi:10.1515/jbcpp-2015-0003
21. Wang WK, Lu QH, Wang X, et al. Ulinastatin attenuates diabetes-induced cardiac dysfunction by the inhibition of inflammation and apoptosis. *Exp Ther Med*. 2017;14(3):2497–2504. doi:10.3892/etm.2017.4824
22. Hu CL, Xia JM, Cai J, et al. Ulinastatin attenuates oxidation, inflammation and neural apoptosis in the cerebral cortex of adult rats with ventricular fibrillation after cardiopulmonary resuscitation. *Clinics (Sao Paulo)*. 2013;68(9):1231–1238. doi:10.6061/clinics
23. Bingyang J, Jinping L, Mingzheng L, Guyan W, Zhengyi F. Effects of urinary protease inhibitor on inflammatory response during on-pump coronary revascularisation. Effect of ulinastatin on inflammatory response. *J Cardiovasc Surg (Torino)*. 2007;48(4):497–503.
24. Li W, Qiu X, Jiang H, Zhi Y, Fu J, Liu J. Ulinastatin inhibits the inflammation of LPS-induced acute lung injury in mice via regulation of AMPK/NF-kappaB pathway. *Int Immunopharmacol*. 2015;29(2):560–567. doi:10.1016/j.intimp.2015.09.028
25. Lagoo JY, D'Souza MC, Kartha A, Kutappa AM. Role of ulinastatin, a trypsin inhibitor, in severe acute pancreatitis in critical care setting: A retrospective analysis. *J Crit Care*. 2018;45:27–32. doi:10.1016/j.jcrc.2018.01.021
26. Toscano MG, Ganea D, Gamero AM. Cecal ligation puncture procedure. *J Vis Exp*. 2011;(51):pii:2860. doi:10.3791/2860
27. de Oliveira GP, Silva JD, de Araujo CC, et al. Intravenous glutamine administration reduces lung and distal organ injury in malnourished rats with sepsis. *Shock*. 2014;41(3):222–232. doi:10.1097/SHK.0000000000000102
28. Rowland T, Hilliard H, Barlow G. Procalcitonin: potential role in diagnosis and management of sepsis. *Adv Clin Chem*. 2015;68:71–86.
29. Wacker C, Prkno A, Brunkhorst FM, Schlattmann P. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2013;13(5):426–435. doi:10.1016/S1473-3099(12)70323-7
30. Laidlaw BJ, Craft JE, Kaech SM. The multifaceted role of CD4(+) T cells in CD8(+) T cell memory. *Nat Rev Immunol*. 2016;16(2):102–111. doi:10.1038/nri.2015.10
31. Bruno G, Saracino A, Monno L, Angarano G. The revival of an "old" marker: CD4/CD8 ratio. *AIDS Rev*. 2017;19(2):81–88.
32. McBride JA, Striker R. Imbalance in the game of T cells: what can the CD4/CD8 T-cell ratio tell us about HIV and health? *PLoS Pathog*. 2017;13(11):e1006624. doi:10.1371/journal.ppat.1006624
33. Pool R, Gomez H, Kellum JA. Mechanisms of organ dysfunction in sepsis. *Crit Care Clin*. 2018;34(1):63–80. doi:10.1016/j.ccc.2017.08.003
34. Saxena M, Yeretssian G. NOD-like receptors: master regulators of inflammation and cancer. *Front Immunol*. 2014;5:327. doi:10.3389/fimmu.2014.00327
35. Franchi L, Warner N, Viani K, Nunez G. Function of Nod-like receptors in microbial recognition and host defense. *Immunol Rev*. 2009;227(1):106–128. doi:10.1111/imr.2008.227.issue-1
36. Li Y, Zhao L, Fu H, Wu Y, Wang T. Ulinastatin suppresses lipopolysaccharide induced neuro-inflammation through the downregulation of nuclear factor-kappaB in SD rat hippocampal astrocyte. *Biochem Biophys Res Commun*. 2015;458(4):763–770. doi:10.1016/j.bbrc.2015.01.155
37. Wang LZ, Luo MY, Zhang JS, Ge FG, Chen JL, Zheng CQ. Effect of ulinastatin on serum inflammatory factors in Asian patients with acute pancreatitis before and after treatment: a meta-analysis. *Int J Clin Pharmacol Ther*. 2016;54(11):890–898. doi:10.5414/CP202454
38. Jiang L, Yang L, Zhang M, et al. Beneficial effects of ulinastatin on gut barrier function in sepsis. *Indian J Med Res*. 2013;138(6):904–911.
39. Hutchins NA, Unsinger J, Hotchkiss RS, Ayala A. The new normal: immunomodulatory agents against sepsis immune suppression. *Trends Mol Med*. 2014;20(4):224–233. doi:10.1016/j.molmed.2014.01.002
40. Conway-Morris A, Wilson J, Shankar-Hari M. Immune activation in sepsis. *Crit Care Clin*. 2018;34(1):29–42. doi:10.1016/j.ccc.2017.08.002
41. Chaudhry H, Zhou J, Zhong Y, et al. Role of cytokines as a double-edged sword in sepsis. *In Vivo*. 2013;27(6):669–684.