Original Article

Glutamine treatment decreases plasma and lymph cytotoxicity during sepsis in rats

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Glutamine (Gln) is considered as a conditionally essential amino acid. Pharmacological supplementation of Gln helps to maintain the intestinal mucosal barrier, modulate cytokine production, and prevent organ injury during sepsis. Our previous study demonstrated the different effects of Gln on macrophage cytokine production in vitro or in vivo. The purpose of this study was to investigate the potential mechanism of Gln treatment to protect cells and modulate inflammation during sepsis in vivo. The results showed that administration of Gln significantly attenuated plasma-induced macrophage cytokine production and endothelial cell necrosis after cecal ligation and puncture in rats. In addition, it preserved human umbilical vein endothelial cell (HUVEC) viability and migration ability. Gln treatment also reduced lymph cytotoxicity by restoring macrophage tumor necrosis factor-α production, maintaining HUVEC viability, and decreasing endothelial cell necrosis. Mesenteric lymph duct ligation did not alleviate plasma cytotoxicity. Plasma lipopolysaccharide and β-lactate levels were suppressed after Gln treatment. Taken together, these results indicated that Gln administration can protect cells by attenuating the cytotoxicity of plasma and mesenteric lymph during sepsis.

Keywords sepsis; glutamine; plasma; lymph; cytotoxicity

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Introduction

Sepsis is a major cause of admission to intensive care and has become the leading cause of non-coronary intensive care unit deaths [1]. Glutamine (Gln) is considered as a conditionally essential amino acid, particularly in catabolic states [2]. A significant decrease in plasma Gln has been observed in sepsis [3]. Pharmacological supplementation of Gln helps to maintain the intestinal mucosal barrier [4], modulate cytokine production [5,6], and prevent organ injury [2], but the exact protective mechanism remains to be explored. Previous studies suggested that early administration of Gln may attenuate inflammation and protect against a variety of cell/tissue injuries after surgery through the induction of heat shock proteins (HSPs) [4,7,8]. Specifically, the release of interleukin (IL)-6 and tumor necrosis factor (TNF)-α was attenuated at 6 h post-injury, and was thought to be due to Gln-mediated inhibition of nuclear binding/activation of nuclear factor κB (NF-κB) [9]. Recently, we reported that Gln treatment directly augmented macrophage TNF-α production in vitro, but decreased TNF-α release in vivo, even though the expression of HSP72 was increased in both cases [5]. This suggested that the protective effect of Gln in vivo was not completely attributable to HSPs expression. Another report suggests that dietary Gln administration results in higher inflammatory cytokine production and greater neutrophil recruitment during the early stage of acute lung injury [10]. Therefore, the exact protective mechanism of Gln treatment in sepsis is worthy of further research.

The gut is a proposed common link between injurious events and the resultant systemic effects. Gut injury may initiate an inflammatory cascade, and may ultimately result in distant organ injury. It is thought that the essential protective mechanism for Gln is at the intestinal level, as Gln plays a role in preventing apoptosis in human colonic epithelial cells following stress or injury [11], and enhancing peroxisome proliferator-activated receptor-γ DNA binding in the post-ischemic small bowel [12]. However, the mechanism of Gln-induced suppression of remote tissue inflammation has not been well described. By the 1990s, the concept of bacterial translocation and the gut hypothesis of multiple organ dysfunction (MODS) had become accepted [13]. As conflicting clinical data were published [14], the gut lymph hypothesis of MODS was proposed, which suggested that gut injury leads to systemic inflammation and distant organ injury via gut-derived factors contained primarily in the mesenteric lymph and not the portal circulation [13]. Recently, plasma from septic shock patients was shown to increase the reactive oxygen species levels in naive human umbilical vein endothelial cells (HUVECs),
and the extent of reactive oxygen species formation correlated with mortality and the severity of septic shock [15,16]. Thus, Gln treatment may attenuate systemic inflammation and preserve remote cell/tissue viability by reducing the plasma or lymph cytotoxicity during sepsis.

In the present study, we tested the effects of Gln injection on plasma and lymph cytotoxicity by measuring its ability to stimulate macrophage cytokine production and damage the endothelial cells. We employed a cecal ligation and puncture (CLP) model to mimic the clinical situation of sepsis. This model allows the cannulation of the mesenteric lymphatics and collection of pure intestinal lymph for in vitro analysis of its biological effects, or ligation of the efferent mesenteric lymphatics to distinguish the respective roles of plasma and lymph. To determine whether the removal of intestinal lymph can alleviate plasma toxicity, the role of mesenteric lymph duct ligation (LDL) on plasma cytotoxicity was investigated.

Materials and Methods

Animals
Adult male Sprague–Dawley rats weighting 200–250 g were purchased from the Laboratory Animal Science Department of Fudan University (Shanghai, China). Animals had free access to food and water, and were housed in a temperature- and humidity-controlled room on a 12-h light/dark cycle. The animal experiments were approved by the Ethical Committee of Animal Research at the Medical College of Shanghai Jiaotong University (Shanghai, China).

CLP procedure
A CLP model was used to induce sepsis as described previously [17]. Briefly, rats were anesthetized with 50 mg/kg body weight of pentobarbital intraperitoneally. Then, a 2 cm incision was made in the abdominal wall and the cecum was ligated just below the ileocecal valve. The cecum was punctured twice using a sterile 20-gauge needle, and a small amount of fecal material was extruded into the peritoneal cavity. Sham group animals underwent the same procedure without ligation and puncture of the cecum. The abdomen was then closed and all animals received 20 ml/kg normal saline fluid for resuscitation post-procedure. Either 0.75 g/kg Gln (GLN/LC, n = 6) or saline (SHAM/LC and CLP/LC, n = 6, respectively) was administered immediately after surgery via single tail-vein injection. The mesenteric lymph was continuously collected in heparin, iced tuberculin syringes at 1 h intervals over 6 h. The animal’s temperature was maintained >36.3°C with the use of heating pads or lamps as necessary. The collected lymph were centrifuged at 3000 g for 5 min to remove any formed elements and stored at −80°C until use. Lymph samples collected between the fifth and sixth hour after mesenteric lymph duct cannulation were used in this study.

Collection of mesenteric lymph
Mesenteric lymph was collected as previously described [18] from animals undergoing the CLP procedure. The main mesenteric lymph duct was identified and cannulated with silastic tubing and the catheter was secured in place with glue. The abdomen was then closed and all animals received 20 ml/kg normal saline fluid for resuscitation post-procedure. Either 0.75 g/kg Gln (GLN/LC, n = 6) or saline (SHAM/LC and CLP/LC, n = 6, respectively) was administered immediately after surgery via single tail-vein injection. The mesenteric lymph was continuously collected in heparin, iced tuberculin syringes at 1 h intervals over 6 h. The animal’s temperature was maintained >36.3°C with the use of heating pads or lamps as necessary. The collected lymph were centrifuged at 3000 g for 5 min to remove any formed elements and stored at −80°C until analysis.

Lymph duct ligation
Animals receiving CLP underwent mesenteric LDL (CLP + LDL, n = 6) or flipping of the intestine only (CLP – LDL, n = 6) as previously described [19]. A sham group of animals received laparotomy and intestine flipping (SHAM – LDL, n = 6). The abdomen was then closed and all animals received 20 ml/kg normal saline fluid for resuscitation post-procedure. Animals were then returned to their cages and allowed food and water. Blood was drawn by cardiac puncture under anesthesia at 6 h after surgery, and then centrifuged at 3000 g for 5 min at 4°C. Plasma was stored at −80°C until used in the assay.

Cytokine induction of RAW264.7 cells with plasma or lymph
A murine peritoneal macrophage cell line (RAW264.7) was obtained from American Type Culture Collection (Rockville, USA). Cells were seeded at $5 \times 10^5$ cells per well in 24-well plates and grown in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Grand Island, USA) containing Gln for 24 h. The supernatant was then removed and plasma (250 μl) or lymph (100 μl) from the different experimental groups was added to each well. Gln-free DMEM containing lipopolysaccharide (LPS; *Escherichia coli* O55:B5, Sigma-Aldrich, St Louis, USA) at a final concentration of 100ng/ml was added to make the final volume of 500 μl per well. The cells then were incubated for 4 h at 37°C. At the end of the incubation, the cells and supernatants were harvested and frozen at −80°C until used for HSP72 or cytokine analysis.

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Cytokine enzyme-linked immunosorbent assay
The TNF-α and IL-6 levels in supernatant or lymph collected as described above were determined by enzyme-linked immunosorbent assay (ELISA) with commercially available kits (R&D Systems, Minneapolis, USA) according to the manufacturer’s instructions.

HUVEC viability assay
HUVECs (Kaiji, Nanjing, China) were seeded at 5 × 10³ cells per well in 96-well plates and grown in DMEM for 24 h. HUVEC viability was measured using the Cell Counting Kit (Dojindo Laboratories, Kumamoto, Japan), which is based on the conversion of water-soluble tetrazolium salt (WST-8) to a water-soluble formazan dye upon reduction in the presence of a electron carrier by dehydrogenase [20]. Gln-free DMEM, or medium containing 50% plasma or 20% lymph was added to each well to make the final volume of 100 µl. HUVECs were incubated for 4 h, and then 10 µl of WST-8 solution was added for 4 h incubation at 37°C. Absorbance was read at 450 nm on a microplate reader. HUVEC viability was expressed as an odds ratio value. All samples were analyzed in triplicate.

HUVEC necrosis assay
Cellular necrosis was evaluated by the Hoechst 33258/pro-pidium iodide staining technique described previously [21]. HUVECs were seeded at 5 × 10³ cells per well in 96-well plates and exposed to Gln-free DMEM containing 50% plasma, or 20% lymph for 8 h. After two washes with phosphate-buffered saline (PBS), Hoechst 33342, and propidium iodine (Sigma-Aldrich) dissolved in PBS were added to each well at a final concentration of 10 and 8 µg/ml, respectively. Cells were stained for 10 min at 37°C, and examined on an inverted fluorescence microscope at ×100 magnification. The necrotic cells that emitted red fluorescence and cells that emitted blue fluorescence were counted per one high power field (×400) and presented as a percentage value. Counting was performed in five independent fields.

Wound healing assay
Confluent HUVEC monolayers in 24-well plates were ‘scratch’ wounded using a universal blue pipette tip and rinsed twice with PBS. Gln-free DMEM containing 15% plasma was added to each well. HUVECs were incubated at 37°C, and the width of the wound was measured at indicated time points (0, 12, and 24 h) using an IX70 microscope (Olympus, Tokyo, Japan). The image was analyzed with NIH Image 1.57 software. Three random measurements of wound width were taken for each well at each time point and the mean value of three data represented the migration ability of the cells.

Western blot analysis of HSP72 protein
Cells were washed, centrifuged, and resuspended in 100 µl of lysis homogenization buffer (10 mM of Tris buffer, pH 7.4; 5 mM of MgCl₂, 1 mM of phenylmethylsulfonyl fluoride, 50 U/ml of deoxyribonuclease and ribonuclease, and 10 µg/ml each of leupeptin, aprotinin, and pepstatin). Protein concentration was determined by bicinchoninic acid protein assay. Twenty micrograms of each sample was analyzed by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane using a polyvinylidene difluoride membrane using a specific D-lactic dehydrogenase enzyme coupled to gel electrophoresis and transferred to a polyvinylidene difluoride membrane using 1× Towbin buffer (25 mM Tris buffer, pH 8.8; 192 mM of glycerine with 15% v/v methanol). Non-specific binding was blocked with 5% w/v Blotto non-fat dry milk in PBS with 0.2% v/v Nonidet P-40. For HSP72 detection, blots were incubated with specific mouse anti-HSP72 monoclonal antibody (Stressgen, Victoria, Canada) or anti-β-actin polyclonal antibody (Cell Signaling Technology, Beverly, USA). Blots were then washed, incubated with horseradish peroxidase-conjugated secondary anti-mouse antibody, developed with an enhanced chemiluminescence system (Pierce Biotechnology, Rockford, USA), and analyzed by densitometry.

Plasma Glu assay
The Glu level in plasma was determined by enzymatic degradation using commercially available kits (Jiancheng Bioengineering Ins., Nanjing, China). Plasma (0.2 ml) was deproteinized with an equal volume of perchloric acid (0.2 M), centrifuged at 3000 g for 10 min at 4°C, and the supernatant was removed. To 0.25 ml supernatant, 0.2 ml acetate buffer (0.5 M), 0.1 ml glutaminase (10 U/ml), 0.45 ml H₂O were added, and incubated at 37°C for 1 h. To 0.5 ml mixture, 1 ml Tris-EDTA-hydrazine buffer (2 mM), 0.1 ml NAD solution (30 mM), 0.01 ml ADP solution (100 mM), and 0.39 ml H₂O were mixed and the background absorbance was read at 340 nm. After addition of 0.02 ml glutamic dehydrogenase (1200 U/ml) and incubation for 40 min at room temperature, the absorbance was read at 340 nm and the background absorbance was subtracted from this reading to determine the net absorbance. The Glu concentration was then calculated using the linear regression standard data.

Plasma d-lactate assay
The assay is based on the enzymatic oxidation of d-lactate with a specific d-lactic dehydrogenase enzyme coupled to reduction of NAD⁺ with the spectrophotometric measurement of NADH at 340 nm [22]. Plasma (0.4 ml) was deproteinized with 0.04 ml perchloric acid (5.8 M) with mixing on a vortex for 20 s and incubation on ice for 10 min. The denatured protein solution was centrifuged at 3000 g for 10 min at 4°C and the supernatant harvested. To 0.2 ml of supernatant, 0.02 ml KOH (11.6 M) was added
with mixing for 20 s. After 10 min standing in ice bath, the precipitant KClO4 was removed by centrifugation at 3000 g for 10 min. To 0.125 ml supernatant, 0.375 ml of NAD+–glycine-hydrazine solution and 0.025 ml D-lactate dehydrogenase (600 U/ml) were added. After 90 min incubation in a water bath at 25°C, the absorbance at 340 nm was measured, and the concentration of D-lactate in plasma was determined from the linear regression standard curve data.

**Plasma LPS assay**

A specific kinetic limulus amebocyte lysate (LAL) kit from Bokang Biological Ltd (Zhanjiang, China) was used to measure LPS levels. Plasma samples were diluted 1:10 with sterile nanopure water, mixed by vortexing, and placed in a 75°C water bath for 10 min. Standards and samples were incubated with LAL for 10 min at 37°C followed by 6 min incubation with the colorimetric substrate. The reaction was stopped by the addition of 25% acetic acid, and the absorbance at 405 nm was read using a microplate reader.

**Statistical analysis**

Data are presented as mean ± standard deviation. Analysis of variance with Tukey’s test was used for statistical analysis where applicable. *P*, 0.05 was considered significant.

**Results**

**Administration of Gln attenuates plasma cytotoxicity**

To investigate the effect of Gln treatment on plasma cytotoxicity, cytokine production in RAW264.7 cells incubated with plasma from septic rats was measured. Plasma from the CLP group induced 3-fold increase of TNF-α production in RAW264.7 cells compared with the SHAM control. Gln treatment attenuated plasma-induced TNF-α production to the level of the SHAM group [Fig. 1(A)]. Similarly, plasma isolated from the CLP group doubled IL-6 production in RAW264.7 cells, and treatment of Gln reduced it to initial levels [Fig. 1(B)]. Plasma from the CLP group decreased cellular viability by 65%, as measured by absorbance at 450 nm. However, treatment with Gln almost completely restored the viability of HUVECs (Fig. 2). Necrosis of HUVECs induced by septic plasma was analyzed by Hoechst 33258/propidium iodide staining. The results showed plasma of CLP group induced an increase of more than six times in cell necrosis rate, which was significantly reduced after Gln treatment (Fig. 3). The migration ability of HUVECs was significantly decreased by the plasma from the CLP group at 12 and 24 h of incubation. However, Gln treatment restored the ability of HUVECs to migrate (Fig. 4, Table 1). Collectively, all these results support the idea that Gln administration attenuates plasma cytotoxicity.

**Administration of Gln attenuates lymph cytotoxicity**

Rather than stimulating cytokine production, the lymph from CLP/LC group down-regulated TNF-α and IL-6 production in RAW264.7 cells by 44% and 53%, respectively, indicating that cellular function was seriously harmed. Gln treatment partly attenuated lymph cytotoxicity by restoring cellular TNF-α production, but not IL-6 [Fig. 5(A,B)]. The lymph from the CLP/LC group decreased cell viability or increased HUVEC necrosis, which could be attenuated by Gln treatment (Figs. 6 and 7). These results suggest that Gln administration attenuates lymph cytotoxicity.

TNF-α and IL-6 levels of lymph in CLP/LC group were increased compared with sham-operated rats, which were not affected by Gln injection (Table 2), implying that the effect of Gln treatment on lymph cytotoxicity may not be attributed to decreasing lymph cytokine levels.

**Mesenteric LDL does not affect plasma cytotoxicity**

The plasma isolated from the CLP–LDL group induced a 3-fold increase of TNF-α production in RAW264.7 cells.
However, mesenteric LDL did not decrease plasma-induced TNF-α production (Fig. 8). Plasma of CLP–LDL group reduced cell viability as measured by absorbance at 450 nm, which could not been attenuated by mesenteric LDL (Fig. 9). These results suggest that the increase of cytotoxicity in plasma is not due to toxin in lymph after CLP.

**Administration of Gln does not affect plasma-induced HSP72 expression**

To further explore the involvement of HSPs in protective effect of Gln treatment, we analyzed Gln concentration in plasma or lymph from different groups and the HSP72 expression in target cells. The results showed no differences in plasma or lymph Gln concentration (Tables 2 and 3). And no differences in HSP72 expression were found among three groups [Fig. 10(A,B)].

**Administration of Gln suppresses plasma D-lactate and LPS levels**

To reflect the alterations in intestinal barrier function, we measured plasma D-lactate and LPS levels 6 h after surgery. Consistent with the plasma cytotoxicity variation, plasma D-lactate and LPS levels augmented significantly in...
the CLP group compared with sham-operated rats, indicating increase of intestinal permeability. Animals treated with Gln had markedly reduced plasma \(\beta\)-lactate and LPS concentrations (Table 3).

**Discussion**

The role of Gln in regulating inflammation is currently an area of avid study. The mechanism for its anti-inflammatory effect has not been fully explored but is believed to involve inducing HSP expression [23–25]. Previous studies demonstrated that Gln can enhance HSP expression in target cells and tissues [7], attenuate inflammatory cytokine production, and NF-κB activation [6]. However, Gln inhibited inflammatory responses during sepsis in vivo, while stimulating cytokine release in macrophages, neutrophils, and lymphocytes in vitro [5,26]. Therefore, further elucidation of the mechanisms involved is required to explain the protective effect of Gln in sepsis.

The gut is important in the development of organ failure after initial injuries such as major trauma and hemorrhagic shock, which may disturb the splanchnic blood flow and lead to increased gut permeability. The increase in intestinal permeability can result in a higher incidence of bacteria and toxin translocation from the intestinal lumen to the systemic circulation, causing infectious complications and systemic inflammation [27]. Recent reports demonstrated that toxic products produced by the gut during shock are carried by the mesenteric lymph into the systemic circulation, resulting in multiple organ failure [13,28]. Gln is the preferred respiratory fuel for immune cells and various organs such as the intestine [26], and therefore it may attenuate inflammation by preserving intestinal epithelial cells, decreasing intestinal permeability, and preventing toxic substances entering into the circulation during sepsis.

The results of this study showed that plasma and mesenteric lymph from septic rats could affect macrophage function and endothelial cell viability, which were reversed by

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**Table 1** Gln preserves HUVEC migration ability after CLP

<table>
<thead>
<tr>
<th>Group</th>
<th>Migration (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>SHAM</td>
<td>1.27 ± 0.06</td>
</tr>
<tr>
<td>CLP</td>
<td>1.21 ± 0.14</td>
</tr>
<tr>
<td>GLN</td>
<td>1.29 ± 0.10</td>
</tr>
</tbody>
</table>

**P < 0.01 vs. SHAM group; @ P < 0.05 vs. CLP group; ## P < 0.01 vs. CLP group.
Glutamine treatment decreases plasma and lymph cytotoxicity

Glutamine treatment. This suggests that the anti-inflammatory mechanism of Gln is partly due to a reduction in the cytotoxicity of plasma and lymph, thereby inhibiting systemic inflammation. A large number of components are responsible for plasma and lymph cytotoxicity, such as cytokines, LPS levels, and other toxic substances [15,29]. Rather than evaluate the cytotoxicity by testing each different component that may affect it, we measured the capacity of plasma and lymph as a whole to induce cytokine production in RAW264.7 cells and to injure HUVECs. To address whether disparity in plasma cytotoxicity between groups was caused by LPS, 100 ng/ml LPS was added into the RAW264.7 cell culture medium, which was remarkably higher than plasma LPS concentration. Under these conditions, the LPS concentration present in all groups was similar but the production of cytokines was still significantly different between experimental groups. This suggests that LPS may not be critical for the diversity of plasma and lymph cytotoxicity between groups. Gln may attenuate inflammation and protect cells by inducing cellular HSP expression [30]. However, both the Gln concentration in plasma and lymph and the HSP72 expression in target cells showed no differences between groups. Thus, the effect of Gln treatment on the cytotoxicity of plasma and lymph could not be attributed to HSP expression in this study.

D-lactate is a product of bacterial fermentation and has been proposed as a sensitive and reliable marker for translocation in gut failure [31]. Plasma D-lactate and LPS concentrations are used for evaluating intestinal permeability [32,33]. In this study, the change of plasma D-lactate and LPS levels implied that the protective effect of Gln treatment might be partly due to decreasing intestinal permeability. As a fuel source for epithelial cells, Gln prevents the atrophy of the mucosa and maintains its absorptive capacity [27]. Gln may also enhance HSP expression and decrease apoptosis of gut epithelial cells [24]. It has been shown that intravenous Gln infusion partially maintains the

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Table 2 Effect of Gln administration on lymph cytokine and Gln levels

<table>
<thead>
<tr>
<th>Group</th>
<th>TNF-α (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
<th>Gln (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM/LC</td>
<td>97 ± 23</td>
<td>152 ± 38</td>
<td>1.72 ± 0.69</td>
</tr>
<tr>
<td>CLP/LC</td>
<td>133 ± 22*</td>
<td>357 ± 160*</td>
<td>1.59 ± 0.36</td>
</tr>
<tr>
<td>GLN/LC</td>
<td>146 ± 24</td>
<td>286 ± 102</td>
<td>1.90 ± 0.21</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. SHAM/LC group.

Table 3 Effect of Gln administration on plasma LPS, D-lactate, and Gln levels

<table>
<thead>
<tr>
<th>Group</th>
<th>LPS (EU/ml)</th>
<th>D-lactate (μg/ml)</th>
<th>Gln (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>1.2 ± 0.5</td>
<td>2.13 ± 0.23</td>
<td>0.62 ± 0.16</td>
</tr>
<tr>
<td>CLP</td>
<td>6.1 ± 1.1**</td>
<td>5.08 ± 0.47**</td>
<td>0.5 ± 0.16</td>
</tr>
<tr>
<td>GLN</td>
<td>2.3 ± 1.0##</td>
<td>2.04 ± 0.13##</td>
<td>0.55 ± 0.13</td>
</tr>
</tbody>
</table>

**P < 0.01 vs. SHAM group; ##P < 0.01 vs. CLP group.

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Figure 8 Mesenteric LDL does not affect plasma-induced TNF-α production

RAW264.7 cells were incubated for 4 h with 50% plasma from septic rats of different experimental groups and then the supernatants were harvested. Levels of TNF-α in the supernatants were determined by ELISA using commercially available kits. **P < 0.01 vs. SHAM–LDL group.

Figure 9 Mesenteric LDL does not affect plasma cytotoxicity to HUVEC viability

HUVECs were incubated with 50% plasma from septic rats of different experimental groups for 8 h. The cell viability was measured by reading the absorbance at 450 nm. *P < 0.05 vs. SHAM–LDL group.

Figure 10 Gln administration does not affect plasma-induced HSP72 expression in RAW264.7 cells or HUVECs

RAW264.7 cells (A) and HUVEC (B) were incubated with 50% plasma from septic rats of different experimental groups for 4 h. HSP72 expression was measured by western blot analysis.
intestinal glutathione levels and reduces cellular membrane lipid peroxidation [34].

To elucidate the major route by which gut-derived toxins reach the systemic circulation, the mesenteric lymph duct was selectively ligated, and we observed that the cytotoxicity of plasma was not attenuated by mesenteric LDL. Thus, plasma cytotoxicity was not caused by toxic substances in the lymph in this model. Previous studies indicated that toxic factors are present in the mesenteric lymph, but not the portal plasma, of rats with intestinal injury induced by hemorrhagic shock or burns [18]. The difference in the results between the studies may be due to variations in the animal models. Compared with hemorrhage shock or burn, the CLP model induces a more violent abdominal inflammation and edema, and may result in severe gut barrier dysfunction. It has been demonstrated experimentally that portal bacteremia or endotoxemia may occur if the magnitude of the injury is sufficiently great [35].

The anti-inflammatory effect of Gln remains the focus of a great deal of debate and research [36]. Our study demonstrated for the first time, a beneficial effect of Gln treatment on the cytotoxicity of plasma and lymph, and proposed a link between gut protection and systemic inflammation inhibition by Gln treatment. This mechanism can partly explain the different effects of Gln in vivo from that observed in vitro [5], and the different roles of Gln during sepsis with or without gut injury [8,10,17]. Since the protective effect of Gln treatment was mediated by decreasing gut permeability and suppressing plasma and lymph cytotoxicity, clinical Gln treatment would be beneficial in patients with gut injury rather than those without it in order to offset potential side effects [5,10,37].

A limitation of the present study is that we measured plasma D-lactate and LPS levels to evaluate the intestinal permeability. They are not adequate or a direct index of intestinal barrier function, which can be affected by many factors [38]. In addition, plasma and lymph as a whole were used in this experiment and thus the specific toxin factors or cytokines responsible for plasma or lymph cytotoxicity remain unknown.

In conclusion, our results demonstrate that Gln treatment inhibited inflammation and preserved cellular function by attenuating the cytotoxicity of plasma and mesenteric lymph, and this effect appeared to be associated with variations in the gut permeability. Further studies are required to explore the mechanisms of cytotoxicity change, and how the plasma or lymph affects target cells during sepsis.

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