

Effects of glutamine supplementation on innate immune response in rats with gut-derived sepsis

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The present study examined the effect of glutamine (Gln)-enriched diets before sepsis or Gln-containing total parenteral nutrition (TPN) after sepsis, or both, on the phagocytic activity and blood lymphocyte subpopulation in rats with gut-derived sepsis. Rats were assigned to a control group or one of four experimental groups. The control group and groups 1 and 2 were fed a semipurified diet; groups 3 and 4 had part of casein replaced by Gln. After feeding the diets for 10 d, sepsis was induced by caecal ligation and puncture (CLP); TPN was maintained for 3 d after CLP. The control group and groups 1 and 3 were infused with conventional TPN and groups 2 and 4 were supplemented with Gln in the TPN solution. All rats were killed 3 d after CLP or sham operation to examine their immune responses. The results showed that compared with the control group, the phagocytic activities of peritoneal macrophages were enhanced in groups 3 and 4, but not in groups 1 and 2. The proportion of CD3⁺ cells in group 1 was significantly lower ($P < 0.05$) than that of the control group, whereas no differences were observed among the control and Gln-supplemented groups. The CD4⁺ cell proportion was significantly lower ($P < 0.05$) in group 1 compared with the control group and groups 3 and 4. These findings suggest that Gln-enriched diets before CLP significantly enhanced peritoneal macrophage phagocytic activity, preserved CD4⁺ cells and maintained blood total T lymphocytes in gut-derived sepsis. However, parenteral Gln administration after caecal ligation and puncture had no favourable effects on modulating immune response in septic rats.

Glutamine: Gut-derived sepsis: Phagocytosis: Lymphocyte subpopulation

Glutamine (Gln) is the most abundant free amino acid in plasma and muscle and is an integral substrate in numerous metabolic functions (Smith & Wilmore, 1990; Souba, 1991). A variety of animal and human trials has demonstrated that Gln has immuno-enhancing properties (Calder, 1994; Heberer *et al.* 1996; Gismondo *et al.* 1998; Wilmore & Shabert, 1998). Previous reports have revealed that a relatively Gln-deficient state is created by the catabolic process, and Gln supplementation can correct this nutritional deficiency and hence improve outcomes (Hammerquist *et al.* 1989; Gianotti *et al.* 1995). Gln is required during catabolic processes to manifest optimal tissue responses to catabolism, inflammation and infection (Lacey & Wilmore, 1990).

Sepsis is a major cause of death in patients who have undergone surgery. Sepsis is initiated by bacteria and their related toxins. When bacterial toxins insult the body, profound alterations in both immune responses and organ functions occur (Zellweger *et al.* 1995). Previous studies have shown that when Gln-enriched total parenteral nutrition (TPN) was given to septic rats, they had better survival, improved N balance and increased protein synthesis, and

better protection of the morphology of the intestinal mucosa (Ardawi, 1992; Naka *et al.* 1996). Wischmeyer *et al.* (2001) demonstrated that Gln attenuated pro-inflammatory cytokine release and protected against end-organ damage in a rat model of endotoxin-induced septic shock. In addition, the study by Gianotti *et al.* (1995) showed that oral Gln supplementation decreases bacterial translocation in experimental gut-origin sepsis. Gln is an important fuel for lymphocytes and macrophages (Parry-Billings *et al.* 1990). Macrophages and neutrophils are involved in the early, non-specific host-defence responses and play an important role in the pathophysiology and/or protection against sepsis (Sawyer *et al.* 1989; Ringer & Zimmerman, 1992). Previous reports showed that during inflammatory states such as sepsis and injury, the consumption of Gln by circulating and immune cells increases (Askanazi *et al.* 1980; Roth *et al.* 1982; Hammerquist *et al.* 1989). Studies have revealed that supplemental Gln augments the *in vitro* bactericidal activity of neutrophils in burned or postoperative patients (Ogle *et al.* 1994; Furukawa *et al.* 2000). Parry-Billings *et al.* (1990) reported that depressed Gln concentrations were associated with reduced phagocytosis

Abbreviations: CLP, caecal ligation and puncture; Gln, glutamine; HBSS, Hanks' balanced salt solution; RPMI, Roswell Park Memorial Institute; TPN, total parenteral nutrition.

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by murine peritoneal macrophages. To our knowledge, there has not been any investigation of the effects of Gln on the innate immune responses in gut-derived sepsis. Therefore, our aim was to investigate the effect of Gln supplementation before, after, and both before and after sepsis, on the phagocytic activity of peritoneal macrophages; this is thought to be critical for killing translocated enteric bacteria in septic mice (sepsis induced by caecal ligation and puncture (CLP; Ringer & Zimmerman, 1992; Matsukawa *et al.* 2000). In addition, the proportion of systemic lymphocytes and cytokines released from splenocytes after mitogen stimulation was analysed to investigate the possible role of Gln on modulating immune response in a septic condition.

Materials and methods

Animals

Male Wistar rats aged 8 weeks and weighing 200–230 g were used in the present study. All rats were housed in temperature- and humidity-controlled rooms and were allowed free access to standard rat chow for 1 week before the experiment. The care of the animals followed the guidelines for the care and use of laboratory animals established by the Taiwan University Hospital, and protocols were approved by the Animal Care Committee.

Study protocol

Rats were randomly assigned to a control group or one of four experimental groups. The control group and experimental groups 1 and 2 were fed a common semipurified diet. Rats in experimental groups 3 and 4 were fed an identical diet except that part of the casein was replaced by Gln, which provided 25 % N (Table 1). After feeding rats the respective diets for 10 d, sepsis in the experimental groups was induced by CLP, whereas the control group

underwent a sham operation. CLP was performed according to the method of Wichterman *et al.* (1980). Briefly, rats were anaesthetized with intraperitoneal pentobarbital (50 mg/kg), and the abdomen was opened through a midline incision. The caecum was isolated, and a 3-0 silk ligature was placed around it, ligating the caecum just below the ileo-caecal valve. The caecum was then punctured twice with an 18-gauge needle and was replaced back into the abdomen. The abdominal wound was closed in layers. Immediately after the sham or CLP operation, all rats underwent placement of a catheter for TPN infusion. A silicon catheter (Dow Corning, Midland, MI, USA) was inserted into the right internal jugular vein. The distal end of the catheter was tunneled subcutaneously to the back of the neck, and exited through a coiled spring, which was attached to a swivel allowing free mobility of animals inside individual cages. TPN was administered at a rate of 2 ml/h the first day. Full-strength TPN (56–64 ml/d, according to the body weight) was given thereafter, and continued for a period of 3 d. The infusion speed was controlled by a Terufusion pump (model STC-503; Terumo, Tokyo, Japan). The TPN solution without fat was prepared in a laminar-flow hood. Sterilized fat emulsions were added to the TPN solution daily just before use. The TPN solution was infused for the entire day at room temperature. All animals were allowed to drink water freely, and no enteral nutrition was administered during the period of TPN. The control group and experimental groups 1 and 3 were infused with conventional TPN. Experimental groups 2 and 4 were supplemented with Gln, which provided 25 % total amino acid-N in the TPN solution. The TPN provided 1171 kJ (280 kcal)/kg body weight per d, and the energy (kJ (kcal)):N (g) ratio was 502 (120):1. The TPN solutions were isonitrogenous and identical in nutrient composition except for the difference in the amino acid content (Table 2). There were five groups of rats in this study: the control group,

Table 1. Composition of the semipurified diet (g/kg)*

Ingredients	Gln-supplemented	Without Gln
Casein	165	220
Gln	45	–
Maize starch	667	657
Soyabean oil	44	44
Vitamin mixture†	10	10
Salt mixture‡	35	35
Methyl-cellulose	30	30
Choline chloride	1	1
DL-Methionine	3	3

* Total N: Gln supplemented 34.4 g/kg; without Gln 34.4 g/kg.

† The vitamin mixture contained the following (mg/g): thiamin hydrochloride 0.600, riboflavin 0.600, pyridoxine hydrochloride 0.700, nicotinic acid 3.000, calcium pantothenate 1.600, D-biotin 0.020, cyanocobalamin 0.001, retinyl palmitate 1.600, DL- α -tocopheryl acetate 20.000, cholecalciferol 0.250, and menaquinone 0.005.

‡ The salt mixture contained the following (mg/g): calcium phosphate dibasic 500.00, sodium chloride 74.00, potassium sulfate 52.00, potassium citrate monohydrate 220.00, magnesium oxide 24.00, manganese carbonate 3.50, ferric citrate 6.00, zinc carbonate 1.60, cupric carbonate 0.30, potassium iodate 0.01, sodium selenite 0.01, chromium potassium sulfate 0.55

Table 2. Composition of the total parenteral nutrition solution (ml/l)

	Gln-supplemented	Without Gln
Glucose (500 g/l)	418	412
Fat emulsion (200 g/l)	50	50
Moriamin-SN (100 g/l)*	417	556
Gln	11‡	–
Infuvita†	8	8
NaCl (30 g/l)	35	35
KCl (70 g/l)	10	10
K ₃ PO ₄ (87 g/l)	10	10
Calcium gluconate	10	10
MgSO ₄	4	4
ZnSO ₄	2	2
Choline chloride	1‡	1‡

* From Chinese Pharmaceuticals, Taipei, Taiwan. Contents (g/l): Leu 12.50, Ile 5.60, Lys acetate 12.40, Met 3.50, Phe 9.35, Thr 6.50, Trp 1.30, Val 4.50, Ala 6.20, Arg 7.90, Asp 3.80, Cys 1.00, Glu 6.50, His 6.00, Pro 3.30, Ser 2.20, Tyr 0.35, aminoacetic acid (Gly) 15.70.

† From Yu-Liang Pharmaceuticals, Taoyuan, Taiwan. Contents (per ml) ascorbic acid 20 mg, vitamin A 660 IU, ergocalciferol 0.025 μ g, thiamin hydrochloride 0.6 mg, riboflavin 0.72 mg, niacinamide 8 mg, pyridoxine hydrochloride 0.8 mg, D-panthenol 3 mg, DL- α -tocopheryl acetate 2 mg, biotin 12 μ g, folic acid 80 μ g, cyanocobalamin 1 μ g.

‡ g/l.

not Gln-supplemented before or after the sham operation; group 1, not Gln-supplemented before or after CLP (-/-); group 2, a semipurified diet given before and Gln-containing TPN after CLP (-/+); group 3, a Gln-enriched diet given before and conventional TPN after CLP (+/-); group 4, a Gln-enriched diet given before and Gln-containing TPN after CLP (+/+).

Measurements and analytical procedures

TPN was given until the time of killing on day 3 after CLP. The surviving rats were weighed and anaesthetized. A middle abdominal incision was made, and 10 ml PBS was injected intraperitoneally to elute the peritoneal cells. After harvesting the peritoneal lavage fluid, rats were killed by drawing arterial blood from the abdominal aorta. Fresh peritoneal lavage fluid was used for macrophage cell counts and phagocytic activity analysis. Heparinized whole blood was used for analysing the lymphocyte subpopulation and neutrophil phagocytosis. Plasma was centrifuged from the remaining blood; it was stored at -80°C for analysis of amino acid concentrations.

Plasma amino acid analysis

Blood samples were collected in tubes containing heparin and were centrifuged to separate the plasma. All plasma samples were stored at -70°C until assay. After thawing, plasma samples were deproteinized with salicylic acid (50 g/l); plasma amino acids were analysed by standard ion-exchange chromatography with ninhydrin detection (model 6300; Beckman Instruments, Palo Alto, CA, USA) (Smith & Panico, 1985).

Lymphocyte subpopulation distribution

Fluorescein-conjugated mouse anti-rat CD3 (Serotec, Oxford, UK) and phycoerythrin-conjugated mouse anti-rat CD45Ra (Serotec) mAbs were used to distinguish T cells and B cells, while fluorescein-conjugated mouse anti-rat CD8 and phycoerythrin-conjugated mouse anti-rat CD4 (Serotec) mAbs were used to determine the proportions of T helper cells and cytotoxic T cells respectively. Fresh blood (100 μl) was incubated with fluorescein-labelled mAbs for 15 min at 4°C . After this, erythrocytes were lysed with lysing buffer (Serotec). Fluorescence data were collected on 5×10^4 viable cells and analysed by flow cytometry (Coulter; Miami, FL, USA).

Phagocytosis assay of blood polymorphonuclear neutrophils

A flow cytometric phagocytosis test was used to evaluate the phagocytic activity of blood polymorphonuclear cells (Böhmer *et al.* 1992; Schiffrin *et al.* 1995). Heparinized whole blood (100 μl) was pipetted onto the bottom of a 12×75 mm Falcon polystyrene tube (Becton Dickinson, Oxford, CA, USA), which was then placed in an iced-water bath. Pre-cooled opsonized fluorescein isothiocyanate (FITC)-labelled *Escherichia coli* (20 μl ; Molecular Probes, Eugene, OR, USA) was added to each tube.

Control tubes remained on iced water, and assay samples were incubated for precisely 10 min at 37°C in a shaking water-bath. After incubation, samples were immediately placed in iced water, and 100 μl pre-cooled Trypan Blue (Sigma, St Louis, MO, USA) solution (0.25 mg/ml citrate salt buffer, pH 4.4) was added to quench the fluorescence of the bacteria merely adhering to the surface of the phagocytized cells. Cells were washed twice in Hanks' balanced salt solution (HBSS), and erythrocytes were lysed by the addition of fluorescence-activated cell sorter (FACS) lysing solution (Becton Dickinson). After an additional wash in HBSS, 100 μl propidium iodide solution (1 $\mu\text{g}/\text{ml}$ HBSS) was added to stain the nuclear DNA 10 min before the flow cytometric analysis. Phagocytosis analysis was performed by a flow cytometer (Coulter, Miami, FL, USA). In each sample, 10^4 cells were measured. A live gate was set on the red (propidium iodide) fluorescence histogram during acquisition to include only those cells with a DNA content at least equal to human diploid cells. The number of cells with phagocytic activity did not exceed 6% at 0°C . The phagocytic activity of blood polymorphonuclear cells was presented as the percentage of phagocytizing cells in the test samples.

Quantitative phagocytosis assay of peritoneal macrophages

Because the isolated peritoneal macrophages tend to aggregate and adhere to the culture plates and the adherent macrophages have stronger phagocytic activity than those suspended in solution, we used a Vybrant™ phagocytosis assay kit (Molecular Probes, Eugene, OR, USA) rather than the flow cytometric method to evaluate the phagocytic activity of peritoneal macrophages. Peritoneal macrophages were harvested from peritoneal lavage fluid. The pellets were washed three times with HBSS. The cell number was adjusted to 10^6 cells/ml with Rosswell Park Memorial Institute (RPMI)-1640 supplemented with fetal bovine serum (50 ml/l) and an adequate amount of antibiotics. After distributing 100 μl diluted cells into each well of a ninety-six-well microplate, the plate was transferred to a CO_2 incubator at 37°C for 1 h to allow the cells to adhere to the microplate surface. The RPMI solution was removed from all microplate wells by vacuum aspiration, and then 100 μl of the prepared FITC-labelled *E. coli* was added to each well for 2 h. Labelled bacteria were removed by vacuum aspiration, and 100 μl Trypan Blue suspension was added to all wells within 1 min. The excess Trypan Blue was immediately aspirated, and the experimental and control wells (without peritoneal macrophages) were read in the fluorescence plate reader using about 480 nm for excitation and about 520 nm for emission.

In vitro cultures of splenocytes

Splenocytes were obtained by mechanical disruption of the spleen with a spatula on a stainless-steel mesh. Cell suspensions were passed through a sterile nylon mesh to remove debris. Erythrocytes were lysed using sterile distilled water for 15 s and immediately neutralized to isotonic cell suspensions. After washing with PBS three times

(300 g for 5 min), splenocytes were resuspended in RPMI-1640 with antibiotics and fetal calf serum. The number of isolated splenocytes was determined by a haemocytometer count using the Trypan Blue dye exclusion method.

Cytokine assay

Phytohaemagglutinin (200 ng/ml; Sigma, St Louis, MO, USA) was used to stimulate cytokine production by isolated splenocytes in culture. Triplicate wells of ninety-six-well flat-bottomed microtitre plates (Falcon; Becton Dickinson) were seeded with splenocytes (2.5×10^6 cells/ml RPMI-1640) and mitogen. The control well contained cells plus an equal volume of medium. After incubating phytohaemagglutinin for 24 h at 37°C in a CO₂ incubator, supernatant fractions were centrifuged and stored at -70°C until being analysed for cytokine. IL-2, IL-4, IL-10 and interferon- γ concentrations in the supernatant fractions of phytohaemagglutinin-stimulated splenocytes were determined by commercially available ELISA kits (Amersham Pharmacia Biotech, Bucks., UK).

Statistical analyses

Results are expressed as the mean values and standard deviations. Differences among groups were analysed by one-way ANOVA using Fisher's test. A *P* value of <0.05 was considered statistically significant.

Results

There were no differences in initial body weights, body weights after feeding the experimental diets for 10 d and body weights after TPN administration for 3 d among the five groups (results not shown). The Gln-supplemented groups had significantly higher plasma Gln concentrations (group 2 498.0 (SD 50.4), group 3 473.1 (SD 50.1), group 4 481.7 (SD 55.0) nmol/ml) than did group 1 (378.9 (SD 42.9) nmol/ml); there were no significant differences from the control group value (483.9 (SD 61.5) nmol/ml).

CLP may induce the migration of macrophages to the peritoneal cavity: the cell numbers of peritoneal

Table 3. Number of macrophages in peritoneal lavage fluid and phagocytic activity of peritoneal macrophages§

(Mean values and standard deviations for rats per group)

	<i>n</i>	Macrophages ($\times 10^6$ cells/ml)		Phagocytic activity	
		Mean	SD		
Control	9	1.72	1.02	220.9	175.0
Group 1 (-/-)	11	5.44*	1.09	630.5	530.3
Group 2 (-/+)	9	3.22	1.4	907.0	504.3
Group 3 (+/-)	9	6.30*‡	4.17	1830.6*†	1492.6
Group 4 (+/+)	11	4.13	2.77	2086.1*†‡	1374.1

Mean values were significantly different from those of the control group: **P*<0.05.

Mean values were significantly different from those of group 1: †*P*<0.05.

Mean values were significantly different from those of group 2: ‡*P*<0.05.

§ For details of diets, groups, treatments and procedures, see Tables 1 and 2 p. 424.

|| Fluorescein on excitation 485 nm, emission 535 nm; for details, see p. 425.

macrophages were similar in groups 2 (-/+) and 4 (+/+) and the control group. Groups 1 (-/-) and 3 (+/-) had higher peritoneal macrophage numbers than the control group. The phagocytic activity of peritoneal macrophages was significantly higher (*P*<0.05) in group 4 (+/+) than in other groups, except group 3 (+/-). Group 3 had higher phagocytic activity than in the control group and groups 1 (-/-), whereas there were no differences among the control group, group 1 (-/-) and 2 (-/+) (Table 3). However, there were no differences in the phagocytic activities of blood polymorphonuclear cells among all groups by flow cytometric phagocytosis analysis (control 55.1 (SD 27.0), group 1 (-/-) 55.1 (SD 27.0), group 2 (-/+) 52.6 (SD 19.7), group 3 (+/-) 57.1 (SD 24.6), group 4 (+/+) 53.1 (SD 25.7) %).

The proportions of CD3⁺ cells in the blood in group 1 (-/-) was significantly lower than that of the control group, whereas no differences were observed among the control and Gln-supplemented groups. The CD4⁺ cell proportion was significantly lower (*P*<0.05) in group 1 than those of the control group or groups 3 (+/-) and 4 (+/+). There were no differences in the proportions of blood CD8⁺ cells among all groups. No differences were observed in CD45Ra⁺ proportion among the control and experimental groups except for group 4 (+/+). Group 4 had a higher proportion of CD45Ra⁺ than the control group (Fig. 1).

There were no differences in IL-2, IL-10 and interferon- γ production among the five groups (Table 4) and IL-4 was not detectable when splenocytes were stimulated with mitogen.

The survival rates were higher in the control group (100%) than the experimental groups. No differences in survival rates were observed among the four experimental groups 3 d after CLP (group 1 (-/-) 64.7, group 2 (-/+) 69.2, group 3 (+/-) 71.4, group 4 (+/+) 68.8 %).

Discussion

In the present study, 25% total N in the diet or TPN solution was supplied by Gln. This amount of Gln was

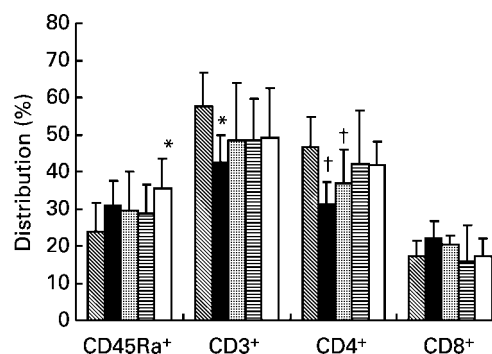


Fig. 1. Distributions of blood CD45Ra⁺, CD3⁺, CD4⁺, and CD8⁺ lymphocytes among the control and experimental groups 3 d after caecal ligation and puncture. ▨, Control group (*n* 9); ■, group 1 (-/-; *n* 11); □, group 2 (-/+; *n* 9); ▤, group 3 (+/-; *n* 9); ▥, group 4 (+/+; *n* 11). For details of diets, groups, treatments and procedures, see Tables 1 and 2 and p. 424. Values are means with standard deviations shown by vertical bars. Mean values were significantly different from those of the control group: **P*<0.05. Mean values were significantly different from those of groups 3 and 4: †*P*<0.05.

Table 4. The concentrations of IL-2, IL-10 and interferon (IFN)- γ (pg/ml) released by splenocytes after phytohaemagglutinin stimulation for 24 h[†]

(Mean values and standard deviations for rats per group)

	n	IL-2		IL-10		IFN- γ	
		Mean	SD	Mean	SD	Mean	SD
Control	9	14.2	11.3	91.2	50.9	5.2	1.1
Group 1 (-/-)	11	23.7	5.8	165.1	138.9	8.4	6.0
Group 2 (-/+)	9	22.5	17.9	169.8	108.5	8.2	2.0
Group 3 (+/-)	9	20.9	13.2	192.6	193.1	9.7	7.7
Group 4 (+/+)	11	19.4	12.4	129.4	175.0	6.5	2.8

* For details of diets, groups, treatments and procedures, see Tables 1 and 2 and p. 424.

[†] No significant differences were seen among the groups.

previously found to enhance the immune response in healthy rodents (Kew *et al.* 1999; Wells *et al.* 1999). We provided oral Gln supplementation for 10 d before sepsis was induced. This model mimics the septic complications in patients with abdominal surgery, in whom preventive use of a Gln-supplemented enteral diet may be recommended. TPN was administered after CLP, because sepsis has been shown to cause histological damage and adversely affect the barrier and metabolic functions of the small intestine (Navaratnam *et al.* 1990; Gardiner & Barbul, 1993). A study by Gardiner *et al.* (1995) showed that sepsis induced by CLP resulted in impaired intestinal amino acid uptake, so the parenteral rather than the enteral route of Gln therapy may have benefits for survival from septic insult. We used CLP as a sepsis model, because CLP is clinically relevant and is considered a simple and reproducible model of gut-derived sepsis in rats (Wichterman *et al.* 1980).

Results of phagocytic activity of peritoneal macrophages showed that a Gln-enriched diet before sepsis had beneficial effects on enhancing phagocytic activity. This result was consistent with *in vitro* studies, which showed that Gln augments phagocytosis by neutrophils from burned and postoperative patients (Ogle *et al.* 1994; Furukawa *et al.* 2000). An *in vivo* study by Shou & Daly (1994) also showed that a Gln-enriched diet preserved peritoneal macrophage superoxide production and bacteria killing in a diet-induced bacteria translocation rat model. Since the reports demonstrating that Gln enhances phagocytosis were *in vitro* studies, or *in vivo* studies not involving sepsis, the present study is the first to show that Gln-enriched diet before sepsis significantly enhanced peritoneal macrophage phagocytic activity in a gut-derived sepsis model. In the present study, we found that parenterally infused Gln after CLP had no favourable effect on peritoneal phagocytosis. The lack of effect of parenteral Gln might be linked to the very short period of administration. Polymorphonucleocytes are potent inflammatory cells, and both the total number and percentage of circulating polymorphonucleocytes can be increased by acute infection and endotoxin (Sawyer *et al.* 1989). The phagocytic activity of blood polymorphonuclear cells did not differ among all groups in the present study. Since the peritoneal cavity is the primary site of injury, it is possible that

Gln stimulated phagocytic activity at the site of bacterial invasion. The effect of Gln on phagocytic cells in the systemic circulation was not obvious.

In order to understand the effect of Gln on the distribution of systemic total B cells (CD45Ra⁺), total T cells (CD3⁺), helper T (CD4⁺), and cytotoxic T cells (CD8⁺) in septic rats, the subpopulations of lymphocytes were evaluated. The current work demonstrated that CLP with conventional TPN (-/-) resulted in lower numbers of total T and CD4⁺ cells. Oral or parenteral Gln supplementation maintained total T lymphocytes, and a Gln-enriched diet before CLP (+/-, +/+) preserved CD4⁺ cells. These results are similar to previous reports. A study by Kew *et al.* (1999) showed that supplemental dietary Gln resulted in increased proportions of CD4⁺ and CD8⁺ cells in the spleen of normal mice. Alverdy *et al.* (1992) also reported that Gln-enriched TPN maintained B and T cell populations in gut lamina propria of normal rats at levels similar to chow-fed animals. In the present study, we found that Gln supplementation both before and after CLP significantly increased systemic B cell distribution. A study by Kudsk *et al.* (2000) revealed that, compared with conventional TPN, normal mice with Gln-enriched TPN had improved respiratory and intestinal IgA levels. Whether Gln supplementation both before and after CLP enhances humoral immunity in septic conditions requires further investigation.

Cytokines are peptides produced by cells of the immune system that act as mediators of the immune response. According to our previous observations, IL-2 and interferon- γ levels in plasma and peritoneal lavage fluid were not detectable in this septic model (Yeh *et al.* 2001). Therefore, we analysed the production of cytokines including IL-2, IL-4, IL-10 and interferon- γ by splenocytes after mitogen stimulation to investigate the effect of Gln supplementation on the systemic immune response. IL-2 and interferon- γ are produced by Th1 lymphocytes. Th1 cytokines enhance cell-mediated immunity. Th2 cytokines, including IL-4 and IL-10, enhance humoral immunity. The effects of Th1 or Th2 lymphocytes are counter-regulatory (Parronchi *et al.* 1992; DiPiro, 1997). The findings showed that IL-4 levels were not detectable, and IL-2, IL-10 and interferon- γ did not differ among groups after mitogen stimulation. This finding may indicate that Gln had no effect on modulating the production of cytokines under the present experimental conditions. Similarly, Kew *et al.* (1999) reported that Gln supplementation did not influence IL-4, IL-10 or interferon- γ production. However, IL-2 production was increased in mouse splenocytes when Gln was administered. An *in vitro* study by Rohde *et al.* (1996) showed that Gln enhanced the production of IL-2 and interferon- γ by human blood mononuclear cells. Since in these studies the samples used for evaluating the effect of Gln on cytokine production were derived from healthy volunteers and normal mice, very different conditions from the present study and consequently possibly leading to different immune responses. Considering that *in vitro* splenocyte stimulation might not reflect actual *in vivo* situations, quantitation of cytokine mRNA expression in the spleen is now under investigation in our laboratory.

In summary, the present study showed for the first time that enteral Gln-enriched diets before CLP significantly enhanced peritoneal macrophage phagocytic activity, preserved CD4⁺ cells and maintained blood total T lymphocytes in a gut-derived sepsis model. Gln administered both before and after CLP increased the systemic B cell distribution. However, parenteral Gln administration after CLP had no favourable effects on modulating immune response in septic rats. In addition, no significant benefit on survival was observed in Gln-supplemented rats with gut-derived sepsis. We speculate that Gln predominantly augments the immune response at the site of the injurious stimulus, and that the effect of Gln on the systemic immune response might not be potent enough to evoke a survival benefit under the present experimental condition. Since survival was only observed for 3 d in the present study, determining whether Gln supplementation improves survival over a longer period requires further investigation.

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