Glutamine Supplementation Enhances Peritoneal Macrophage Phagocytic Activity in Rats with Gut-derived Sepsis

Sung-Ling Yeh, PhD[*], Yu-Ni Lai, BS[*], Ming-Tsan Lin, MD[+], Huey-Fang Shang, PhD[†], Wei-Jao Chen, MD[+]

[*]Institute of Nutrition and Health Sciences, [†]Department of Microbiology and Immunology, Taipei Medical University, Taipei, Taiwan, ROC

[+] Department of Surgery, College of Medicine, National Taiwan University, Taipei, Taiwan, ROC

Running title: Effect of glutamine on phagocytosis in sepsis

Corresponding author:
Ming-Tsan Lin, MD
Department of Surgery
National Taiwan University Hospital
7 Chung-Shan S. Road
Taipei, Taiwan 100
Republic of China
Tel: 8862-23123456, ext. 5281
Fax: 8862-23412969
E-mail: linmt@ha.mc.ntu.edu.tw

ABSTRACT

***Background*:** Previous reports have shown that glutamine (Gln) augments the in vitro bactericidal activity of neutrophils, and is required for macrophage phagocytosis. Few studies have addressed the importance of Gln supplementation on macrophage and neutrophil function after sepsis. This study examined the effect of Gln-enriched diets before and Gln-containing total parenteral nutrition (TPN) after sepsis or both on the phagocytic activity of peritoneal macrophages and blood polymorphonuclear cells in rats with gut-derived sepsis.

Methods: Rats were assigned to the control and 4 experimental groups. The control and groups 1 and 2 were fed a semipurified diet, while groups 3 and 4 had part of the casein replaced with 25% of total nitrogen as Gln. After feeding the experimental diets for 10 d, sepsis was induced by cecal ligation and puncture (CLP), whereas the control groupunderwent sham operation; at the same time, an internal jugular vein was cannulated. All rats were maintained on TPN for 3 d. The control and groups 1 and 3 were infused with conventional TPN, while groups 2 and 4 were supplemented with Gln, replacing 25% of total nitrogen in the TPN solution. All rats were sacrificed 3 d after sham operation or CLP to examine their immune responses.

Results: The distributions of blood CD3+ in the group 1were significantly lower than the control group, whereas no differences were observed among the control and Gln supplemented groups. CD4+ distribution was significantly lower in the group 1 compared with the control and groups 3 and 4. The group 4 had higher CD45Ra+ distribution than the control group. Compared with the control, the phagocytic activity of peritoneal macrophages was enhanced in groups 3 and 4 but not in the other 2 groups. There were no significant differences in the phagocytic activities of

blood polymorphonuclear cells and survival rates among the 4 experimental groups. ***Conclusions:*** Enteral Gln-enriched diets before CLP significantly enhances peritoneal macrophage phagocytic activity, preserved CD4+ cells and maintained total T lymphocytes in blood. Gln administered both before and after CLP seemed to have a synergistic effect on enhancing peritoneal macrophage phagocytic activity and systemic B cell distribution. However, Gln administration had no favorable effects on systemic phagocytic activity and survival in rats with gut-derived sepsis.

INTRODUCTION

Glutamine (Gln) is the most abundant free amino acid in plasma and muscle and is an integral substrate in numerous metabolic function.[1,2] A variety of animal and human trials have been published demonstrating that Gln has immunoenhancing properties (3-6). Previous reports have revealed that a relative Gln deficiency state has been created by the catabolic process, and Gln supplementation have corrected the nutritional deficiency and hence improved outcome. Gln is required during a catabolic process to manifest optimal tissue responses to catabolism, inflammation and infection (7).

Sepsis is a cause of death in patients with major 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 may occur.[8] Studies showed that during inflammatory states such as sepsis and injury, the consumption of Gln by circulating and immunologic cell increases (9-11). Gln is an important fuel for lymphocytes and macrophages (12). Studies revealed that supplemental Gln augments the in vitro bactericidal activity of neutrophils in burned or postoperative patients (13-14). Parry-Billings et al.[12] reported that depressed Gln concentrations

were associated with reduced phagocytosis by peritoneal macrophages in normal mice. An in vitro study by Wallace and Keast[15] showed that Gln is required for phagocytosis of opsonized sheep erythrocytes in macrophage culture. In an in vivo study, a Gln-enriched diet significantly increased superoxide production, and *Candida albicans* killing by peritoneal macrophage (16). Phagocytic cells are an essential arm of the host defense against microbes. Macrophages and neutrophils are involved in the early, nonspecific host-defense responses and play an important role in the pathophysiology and/or protection against sepsis.[17,18] Most of the reports demonstrated that Gln augments phagocytosis were in vitro studies, and samples used for evaluating the effect of Gln were derived from healthy or conditions other than sepsis. To our knowledge, the effects of Gln on phagocytic activity in gut-origin sepsis have not been previously studied. Therefore, our aim was to investigate the effect of Gln supplementation before and after sepsis and both before and after on the ability of the host defense to kill translocated enteric bacteria in the peritoneal cavity and organisms that invade the systemic circulation. Also, the distribution of systemic lymphocyte subpopulations were analyzed to investigate the effect of Gln on the phenotype of blood lymphocytes in septic rats.

MATERIALS AND METHODS

Animals

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

approved by that committee.

Study protocol

All rats were randomly assigned to the control and 4 experimental groups. The control group and experimental groups 1 and 2 were fed a common semipurified diet. Rats in the experimental groups 3 and 4 were fed an identical diet except that part of the casein was replaced by Gln, which provided 25% of nitrogen (Table 1). After feeding rats the respective diets for 10 days, sepsis in the experimental groups was induced by CLP, whereas the control group underwent sham operation. CLP was performed according to the method of Wichterman et al. [19] Briefly, rats were anesthetized with intraperitoneal pentobarbital (50 mg/kg), and the abdomen was opened through a midline incision. The cecum was isolated, and a 3-0 silk ligature was placed around it, ligating the cecum just below the ileocecal valve. The cecum 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 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. Two mL per h was

administered on the first day. Full-strength TPN 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 the experimental groups 1 and 3 were infused with conventional TPN. Experimental groups 2 and 4 were supplemented with Gln, which replaced 25% of total amino acid nitrogen in the TPN solution. TPN provided 280 kcal/kg body weight, and the kcal/nitrogen ratio was 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 5 groups in this study: normal control group, Gln was not supplemented before and after sham operation; group 1, Gln was not supplemented before and after CLP (-/-); group 2, semipurified diet before and Gln-containing TPN after CLP (-/+); group 3, Gln-enriched diet before and conventional TPN after CLP (+/-); and group 4, Gln-enriched diet before and Gln-containing TPN after CLP (+/+).

Measurements and analytical procedures

TPN was continued until the time of sacrifice at day 3 after CLP and surviving

rats were weighed and anesthetized. A middle abdominal incision was made, and 10 ml of phosphate-buffered saline (PBS) was intraperitoneally injected to elute the peritoneal cells. After harvesting the peritoneal lavage fluid (PLF), the rats were sacrificed by drawing arterial blood from the aorta of the abdomen. Fresh PLF was used for macrophage cell counts and phagocytic activity analysis. Blood samples were collected in tubes containing heparin. Whole blood was used for analyzing lymphocyte subpopulation distribution and neutrophil phagocytosis. Plasma was centrifuged from the remaining blood, and was stored at -80℃ for analysis of amino acid concentrations.

Plasma amino acid analysis

Plasma amino acids were analyzed by standard ninhydrin technology (Beckman Instruments, model 6300, Palo Alto, CA), after deproteinization of the plasma with 5% salicylic acid.[20]

Lymphocytes subpopulation distribution

Flow cytometry was used to determine the proportions of CD45Ra, CD3, CD4, and CD8 in fresh blood. One hundred microliters of blood was incubated for 15 min at 4 ^{o}C containing fluorescein-conjugated mouse anti-rat CD3 (Serotec, Oxford, UK) and phycoerythrin-conjugated mouse anti-rat CD45Ra (Serotec) to distinguish T cells and B cells. Fluorescein-conjugated mouse anti-rat CD8 and phycoerythrin-conjugated mouse anti-rat CD4 (Serotec) to identify T helper cells and cytotoxic T cells, respectively. After this, red blood cells were lysed with lysing buffer (Serotec). Fluorescence data were collected on 5 x 10^4 viable cells and analyzed by flow cytometry (Coulter, Miami, FL, USA).

Phagocytosis assay of blood polymorphonuclear neutrophils (PMNs):[21,22]

A flow cytometric phagocytosis test was used to evaluate the phagocytic

activity of blood PMNs. One hundred microliters of heparinized whole blood was aliquoted on the bottom of a 12 x 75-mm Falcon polystyrene tube (Becton Dickinson) and placed in an ice water bath. Twenty microliters of precooled opsonized FITC-labeled *E. coli* (Molecular Probes, Eugene, Oregon, USA.) was added to each tube. Control tubes remained on ice, and assay samples were incubated for precisely 10 min at 37 ℃ in a shaking water bath. After incubation, samples were immediately placed in ice water, and 100 μL of a precooled trypan blue (Sigma, St. Louis, MO, USA) solution (0.25 mg/ml in citrate salt buffer; pH 4.4) was added to quench the fluorescence of the bacteria merely adhering to the surface of the phagocytizing cells. Cells were washed twice in Hanks buffered saline (HBSS), and erythrocytes were lysed by the addition of FACS lysing solution (Becton Dickinson). After an additional wash in HBSS, 100 μL of propidium iodide (PI) solution (1μg/mL in HBSS) was added to stain the nuclear DNA 10 min before the flow cytometric analysis. Flow cytometry was performed on a FACSCalibur^{TM} flow cytometer (Becton Dickinson) equipped with a 488-nm argon laser. A live gate was set on the red (PI) 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 ^{o}C.

Quantitative and phagocytosis assay of peritoneal macrophages

Peritoneal macrophages were centrifuged from PLF. The pallets were washed 3 times with HBSS. After dilution with equal volume of Turk's solution, the cell concentration was counted. The cell number was adjusted to 10^6 cells/ml with RPMI-1640 supplemented with 5% fetal bovine serum and an adequate amount of antibiotics. After distributing 100 μl of diluted solutions into each well on a 96-well microplates it was transferred to a 37 ℃ CO_2 incubator for 1 h to allow the cells to

adhere to the microplate surface. A Vybrant ^{TM} Phagocytosis Assay kit (Molecular Probes) was used to evaluate the phagocytic activity of peritoneal macrophages. The RPMI solution was removed from all microplate wells by vacuum aspiration, and then 100 μl of the prepared FITC-labeled *E. coli* was added to each well for 2 h. Labeled bacteria were removed by vacuum aspiration, and 100 μl of 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 ~480 nm for excitation and ~520 nm for emission.

In vitro cultures of splenocytes. Splenocytes were obtained by mechanical disruption of the spleen with a spatula on a stainless mesh. Cell suspensions were passed through a sterile nylon mesh to remove debris. RBCs were lysed by sterile distilled water for 15 s, and immediately neutralized to isotonic cell suspensions. After washing with PBS 3 times (300 x*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 hemacytometer count using the trypan blue dye exclusion method.

Cytokine assay. Phytohemagglutinin (PHA, 200 ng/mL; Sigma) were used to stimulate cytokine production by isolated splenocytes lymphocytes in culture. Triplicate wells of 96-well flat-bottomed microtiter plates (Falcon, Becton Dickinson, Oxford, CA, USA) were seeded with splenocytes (2.5 x 10^6 cells/mL in RPMI-1640) and mitogen. The control well contained cells plus an equal volume of medium. After incubating PHA or LPS for 24 h at 37 ^{o}C in a CO_2 incubator, supernatants were centrifuged and stored at –70 ^{o}C until being analyzed for cytokine. Interleukin

(IL)-2, IL-4, IL-10, and interferon (IFN)-γ concentrations in splenocyte supernatants were determined by commercially available enzyme-linked immunosorbent assay (ELISA) kits (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Statistical Analysis

Data are expressed as the mean ± SD. Differences among groups were analyzed by two-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 and body weights after feeding the experimental diets for 10 days, and after TPN administration for 3 days among the 5 groups (data not shown). The Gln-supplemented groups (group 2, 498.0 ± 50.4; group 3, 473.1 ± 50.1; group 4, 481.7 ± 55.0 nmol/mL) had significantly higher plasma Gln levels than did group 1 (378.9 ± 42.9 nmol/mL), and had no differences from the control group (483.9 ± 61.5 nmol/mL).

Groups 1 and 3 had higher peritoneal macrophage numbers than the control group. The cell numbers of peritoneal macrophage were comparable among groups 2, 4 and the control group (Fig 1). Phagocytic activities were significantly higher in groups 3 (+/-) and 4 (+/+) than in the control group and groups 1 (-/-), whereas there were no differences among group 2 and the control group. There were no differences in the phagocytic activities of blood PMNs among all groups (Fig 2).

The distributions of CD3+ in the group 1 (-/-) were significantly lower than the control group, whereas no differences were observed among the control and Gln supplemented groups. CD4+ distribution was significantly lower in the group 1

compared with the control or groups 3 and 4. There were no differences in the distributions of blood CD8+ cells among the all groups. The group 4 had higher CD45Ra+ distribution than the control group. No differences were observed in CD45Ra+ distribution among the control and the experimental groups except group 4 (Fig 3).

There were no differences in the IL-2, IL-10 and IFN-γ levels among the 5 groups, and IL-4 was not detectable when splenocytes were stimulated with mitogen (Fig 4).

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

DISCUSSION

In this study, 25% of total nitrogen in the TPN solution was supplied by Gln. This amount of Gln was previously found to enhance the immune response in rodents.[23,24] We provided oral Gln supplementation for 10 days before sepsis induction. 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 histologic damage, and adversely affect the barrier and metabolic functions of the small intestine[25,26] A study by Gardiner et al.[27] showed that sepsis induced by CLP resulted in impaired intestinal amino acid uptake, and 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 to be a simple and reproducible model of gut-derived sepsis in rats (19).

Results of phagocytic activity of peritoneal macrophages showed that Gln-enriched diet before sepsis had beneficial effects on enhancing phagocytic activity. Gln administered both before and after CLP seemed to have a synergistic effect on phagocytic activity. A study by Furukawa et al (14) demonstrated that Gln-enriched enteral diet enhances bacterial clearance in a bacterial peritonitis model, possibly by promoting peritoneal macrophage phagocytosis. We observed that the enhancing effect of peritoneal phagocytosis was not obvious when Gln was administered intravenously after CLP. This finding may indicate that although parenteral Gln infusion maintained plasma Gln levels after CLP, the effect of Gln on phagocytic activity of peritoneal macrophages is limited. PMNs are potent inflammatory cells, and both the total number and percentage of circulating PMNs can be induced by acute infection and endotoxin.[18] PMNs efficiently engulf a wide variety of microbes. In this study, the phagocytic activity of blood PMNs cells did not differ among the all groups. Since the peritoneal cavity is the primary site of injury, it is possible that Gln augments phagocytic activity at the location 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 a lower total T and CD4+ cells. Gln supplementation maintained total T lymphocyte, and Gln-enriched diet before CLP (+/-, +/+) preserved CD4+ cells. Gln supplemented both before and after CLP seemed to have synergistic effect on enhancing systemic B cell distribution. This work is similar to the previous reports. A study by Kew et al (23) showed that supplemental dietary Gln resulted in a increased proportions of CD4+ and CD8+ cells

in the spleen of normal mice( ). Alverdy et al (28) also reported that Gln-enriched TPN maintained B and T cells population in gut laminar propria at levels similar to chow-fed animals.

Cytokines are peptides produced by cell of the immune system that act as a mediator of the immune response. According to our previous observation, plasma IL-2, IL-4 and IFN-γ levels were not detectable in septic condition. Therefore, we analyze the production of cytokines including IL-2, IL-4, IL-10, and IFN-γ by splenocytes to investigate the effect of Gln supplementation on systemic immune response. IL-2 and IFN-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 (28,29). The finding showed that IL-4 levels were not detectable and IL-2, IL-10, and IFN-γ did not differ among groups after mitogen stimulation. The finding may indicate that Gln had no effect on modulating the production of the cytokines under the present experimental condition. This result was consistent with the report by Kew et al (23) that Gln supplementation did not influence IL-4, IL-10, and IFN-γ production by spleen lymphocytes. However, study by Kew et al (23) showed that IL-2 production was increased in spenocytes when Gln was administered. Also, an in vitro study by Rohde et al (31) showed that Gln enhanced the production of IL-2 and IFN-γ by blood mononuclear cells. Since the samples used for evaluating the effect of Gln on cytokine production were derived from healthy volunteers and normal mice, response to the stressed metabolic condition observed in this study may differ, and may consequently lead to different immune responses. Considering that the in vitro splenocyte stimulation may not totally reflect actual in vivo situations, quantitation of cytokine mRNA expression in spleen is now under investigation in our

laboratory.

In summary, this study shows that enteral Gln-enriched diets before CLP significantly enhances peritoneal macrophage phagocytic activity, preserved CD4+ cells and maintained blood total T lymphocytes. Gln administered both before and after CLP seemed to have a synergistic effect on enhancing peritoneal macrophage phagocytic activity and systemic B cell distribution. However, Gln administration had no favorable effects on systemic phagocytic activity and the production of T cell –derived cytokine in spenocytes. Also, no survival benefit was observed in Gln supplemented rats with gut-derived sepsis. It is possible that Gln predominantly augments the immune response in the location of injurious stimulus, the effect of Gln on systemic immune response may not potent enough to evoke a survival benefit under the present experimental condition. Since survival was only noted for 3 days, whether Gln supplementation may improve survival over a longer period required further investigation.

ACKNOWLEDGEMENTS

This study was supported by research grant NSC 91-2320-B-038-017 from the National Science Council, ROC.

References

1. Smith RJ, Willmore DW: Glutamine nutrition and requirements. J Parenteral Enter Nutr 14:94S-99S,1990

2. Souba WW: Glutamine: A key substrate for the splanchnic bed. Annu Rev Nutr 11:285-308, 1991

3. Wilmore DW, Shabert JK: Role of glutamine in immunologic responses. Nutrition 14:618-626, 1998

4. Gismondo MR, Drago L, Fassina MC, Vaghi I, Abbiati R, Grossi E. Immunostimulating effect of oral glutamine. Dig Dis Sci 1998; 43:1752-1754

5. Heberer M, Babst R, Juretic A, et al. Role of glutamine in the immune response in critical illness. Nutrition 1996;12(suppl):S71-S72

6. Calder PC: Glutamine and the immune system. Clin Nutr 13:2-8, 1994

7. Lacey JM, Wilmore DW: Is glutamine a conditionally essential amino acid? Nutr Rev 1990;48:297

8. Zellweger R, Ayala A, DeMaso CM, et al: Trauma-hemorrhage causes prolonged depression in cellular immunity. Shock 4:149-153, 1995

9. Askanazi J, Carpentier YA, Michelson CB, et al. Muscle and plasma amino acids following injury. Influence of intercurrent infection. Ann Surg 1980;192:78

10. Hammerquist F, Wernerman J, Al R, Vonder D, Vinners E. Addition of glutamine to total parenteral nutrition after elective abdominal surgery spares glutamine in muscle, counteracts the fall in muscle protein synthesis and improves nitrogen balance. Ann Surg 1989;209:455

11. Roth E, Fundvies J, Muhlbacher F, et al. Metabolic disorders in severe abdominal sepsis: glutamine deficiency in skeletal muscle. Clin Nutr 1982;1:25

12. Parry-Billings M, Evans J, Calder PC, et al: Does glutamine contribute to immunosuppression after major burns? Lancet 336:523-525, 1990

13. Ogle CK, Ogle JD, Mao JX, et al: Effect of glutamine on phagocytosis and bacterial killing in normal and pediatric burn patient neutrophils. J Parenter Enter Nutr 18:128-133, 1994

14. Furukawa S, Saito H, Inoue T, et al: Supplemental glutamine augments phagocytosis and reactive oxygen intermediate production by neutrophils and monocytes from postoperative patients in vitro. Nutrition 16:323-329, 2000

15. Wallace C, Keast D: Glutamine and macrophage function. Metabolism 41:1016-1020, 1992

16. Shou J, Daly JM. Enteral glutamine augments host immune function. Surg Forum 1994;45:33

17. Ringer TV, Zimmermann JJ: Inflammatory host responses in sepsis. Crit Care Clin 8:163-189, 1992

18. Sawyer DW, Donowitz DR, Mandell GL: Polymorphonuclear neutrophils: an effective antimicrobial force. Rev Infect Dis 11:1532-1544, 1989

19. Wichterman KA, Baue AE, Chaudry IH: Sepsis and septic shock-a review of laboratory models and a proposal. J Surg Res 29:189-201, 1980

20. Smith RJ, Panico K: Automated analysis of o-phthalaldehyde derivatives of amino acids in physiological fluids of reverse phase high performance liquid chromatography. J Liq Chromatogr 8:1783-1795, 1985

21. Böhmer RH, Trinkle LS, Staneck JL: Dose effects of LPS on neutrophils in a whole blood flow cytometric assay of phagocytosis and oxidative burst.

Cytometry 13: 525-531, 1992

22. Schiffrin EJ, Rochat F, Aeschlimann JM, et al: Immunomodulation of human blood cells following ingestion of lactic acid bacteria. J Dairy Sci 78:491-497, 1995

23. Kew S, Wells SM, Yaqoob P, et al: Dietary glutamine enhances murine T-lymphocyte responsiveness. J Nutr 129:1524-1531, 1999

24. Wells SM, Kew S, Yaqoob P, et al: Dietary glutamine enhances cytokine production by murine macrophages. Nutrition 15:881-884, 1999

25. Navaratnam RL, Morris SE, Traber DL, Flynn J. Woodson L. Linares H. et al. Endotoxin (LPS) increases mesenteric vascular resistance (MVR) and bacterial translocation (BT). J Trauma 1990; 30: 1104-1113

26. Gardiner K, Barbul A. Intestinal amino acid absorption during sepsis. J Parenter Enter Nutr 1993; 17: 277-283

27. Gardiner KR, Gardiner RE, Barbul A. Reduced intestinal absorption of arginine during sepsis. Crit Care Med 1995; 23: 1227-1232

28. Alverdy JA, Aoys E, Weiss-Carrington P, Burke DA. The effect of glutamine-enriched TPN on gut immune cellularity. J Surg Res 1992;52:34-38

29. DiPiro JT. Cytokine networks with infection: mycobacterial infections, leishmaniasis, human immunodeficiency virus infection, and sepsis. Pharmacotherapy 1997; 17: 205-223

30. Parronchi P, DeCarli M, Manetti R, Simonelli C. Sampognaro S. Piccinni MP. et al. IL-4 and IFN (and) exert opposite regulatory effects on the development of cytolytic potential of Th1 or Th2 human T cell clones. J Immunol 1992; 149:

2977-2983

31. Rohde T, Maclean DA, Klarlund Pedersen B. Glutamine, lymphocyte proliferation and cytokine production. Scand J Immunol 1996;44:648-650

FIGURE LEGENDS

Fig. 1. The numbers of macrophage in peritoneal lavage fluid (PLF) in the five groups 3 days after CLP ($p > 0.05$).(group 1, n = 12; group 2, n = 13; group 3, n =12; group 4, n = 11).

Fig. 2. Phagocytic activity of peritoneal macrophages. After subtraction the background of the control wells, the phagocytic activities of groups 3 (+/-) and 4 (+/+) were significant higher than those of groups 1 (-/-) and 2 (-/+) ($p < 0.05$). (group 1, n = 8; group 2, n = 11; group 3, n =9; group 4, n = 9).

Fig. 3. Phagocytic activity of peripheral blood neutrophils by flow cytometry. No significant differences were observed among the 4 groups ($p > 0.05$). (group 1, n = 8; group 2, n = 10; group 3, n =10; group 4, n = 9).

Fig. 4. The concentrations of cytokines released by PHA-stimulated splenocyte for 24 hours (group 1, n = 8; group 2, n = 11; group 3, n =9; group 4, n = 9)..

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

Ingredients	Gln-supplemented	Without Gln
Casein	165	220
Gln	45	--
Total nitrogen	34.4	34.4
Corn starch	667	657
Soybean oil	44	44
Vitamin[*]	10	10
Salt mixture[†]	35	35
Methyl-cellulose	30	30
Choline chloride	1	1
DL-methionine	3	3

[a] The salt mixture contains the following (mg/g): calcium phosphate diabasic 500, sodium chloride 74, potassium sulphate 52, potassium citrate monohydrate 220, magnesium oxide 24, manganese carbonate 3.5, ferric citrate 6, zinc carbonate 1.6, cupric carbonate 0.3, potassium iodate 0.01, sodium selenite 0.01, and chromium potassium sulphate 0.55.

[b] The vitamin mix contains the following (mg/g): thiamin hydrochloride 0.6, riboflavin 0.6, pyridoxine hydrochloride 0.7, nicotinic acid 3, calcium pantothenate 1.6, D-biotin 0.02, cyanocobalamin 0.001, retinyl palmitate 1.6, DL-α-tocopherol acetate 20, cholecalciferol 0.25, and menaquinone 0.005.

Table 2. Composition of the TPN solution (mL/L)

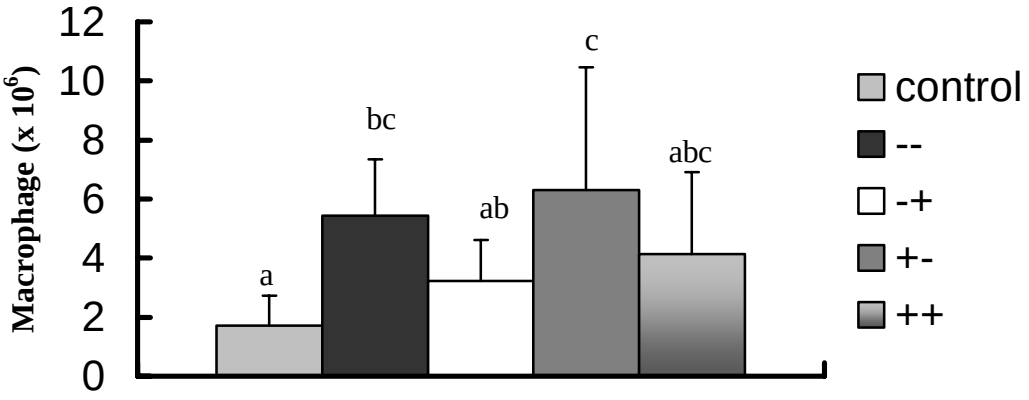
	Gln-supplemented	Without Gln
Glucose 50%	418	412
Fat emulsion 20%	50	50
Moriamin-SN 10%*	417	556
Gln	11g	--
Infuvita†	8	8
NaCL 3%	35	35
KCL 7%	10	10
K_3PO_4 8.7%	10	10
Ca-gluconate	10	10
$MgSO_4$	4	4
Zn SO_4	2	2
Choline chloride (g)	1	1

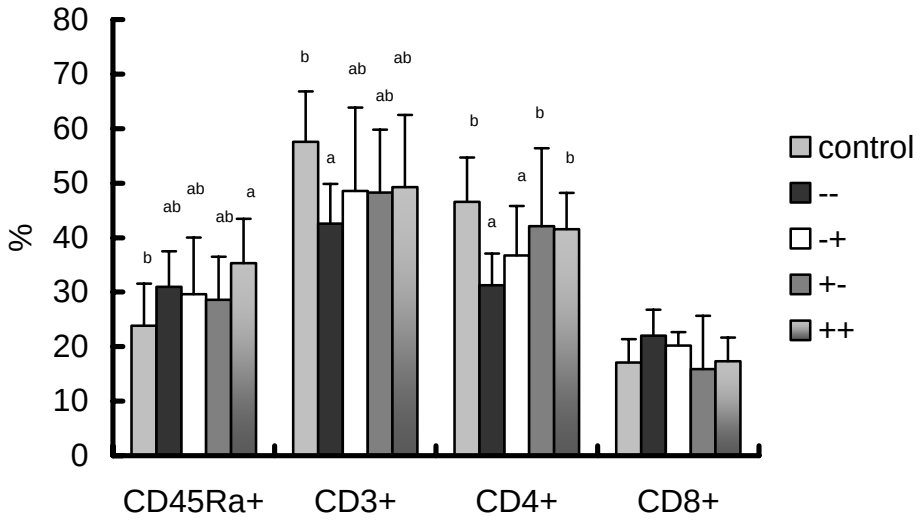
* From Chinese Pharmaceuticals, Taipei, Taiwan

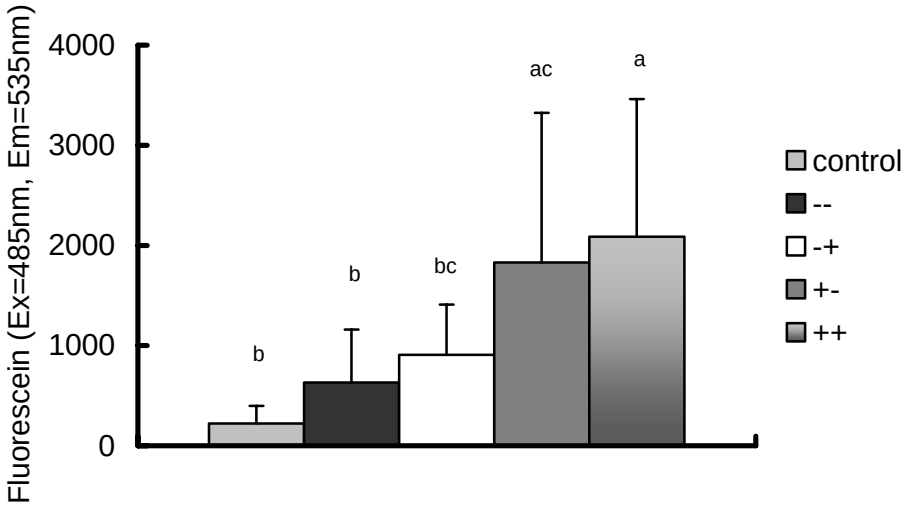
Per deciliter contains: Leu 1250 mg, Ile 560 mg, Lys acetate 1240 mg, Met 350 mg, Phe 935 mg, Thr 650 mg, Trp 130 mg, Val 450 mg, Ala 620 mg, Arg 790 mg, Asp 380 mg, Cys 100 mg, Glu 650 mg, His 600 mg, Pro 330 mg, Ser 220 mg, Tyr 35 mg, Aminoacetic acid (Gly) 1570 mg.

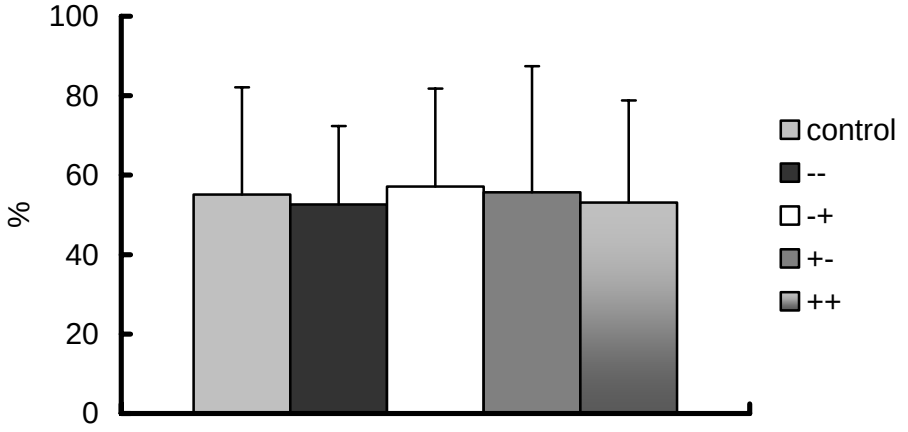
**From Yu-Liang Pharmaceuticals, Taoyuan, Taiwan

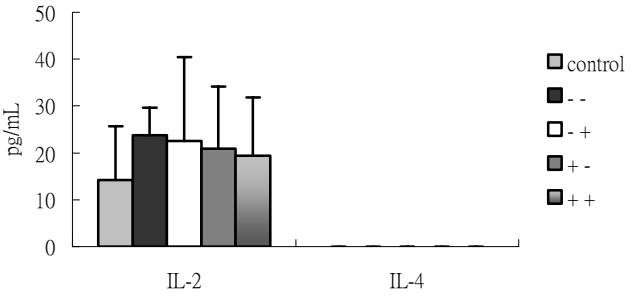
Per milliliter contains: ascorbic acid 20 mg, vitamin A 660 IU, ergocalciferol 40 IU, thiamine HCl 0.6 mg, riboflavin 0.72 mg, niacinamide 8 mg, pyridoxine HCl 0.8 mg, D-panthenol 3 mg, dl-α-tocopheryl acetate 2 mg, biotin 12 ug, folic acid 80 ug, cyanocobalamin 1 ug.

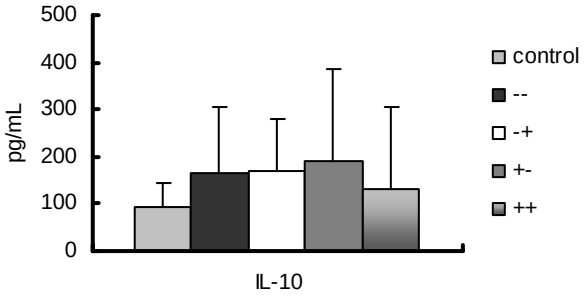

Macrophage (x 10^6)
12
10
8
6
4
2
0
a
bc
ab
c
abc
control
--
-+
+-
++

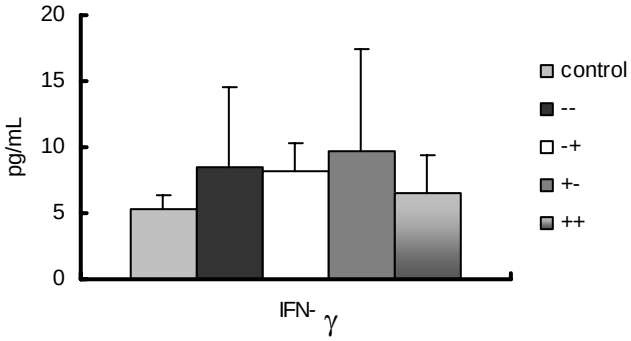

80
70
60
50
40
30
20
10
0
%
b
ab
ab
ab
a
b
a
ab
ab
ab
b
a
a
b
b
CD45Ra+
CD3+
CD4+
CD8+
control
--
-+
+-
++


Fluorescein (Ex=485nm, Em=535nm)
4000
3000
2000
1000
0
b
b
bc
ac
a
control
--
-+
+-
++


%
100
80
60
40
20
0
control
--
-+
+-
++


50
40
30
20
10
0
pg/mL
control
- -
- +
+ -
+ +
IL-2
IL-4


500
400
300
200
100
0
pg/mL
control
--
-+
+-
++
IL-10


20
15
10
5
0
pg/mL
control
--
-+
+-
++
IFN- γ