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Original Communications

Arginine Supplementation Enhances Peritoneal Macrophage Phagocytic Activity in Rats With Gut-Derived Sepsis

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ABSTRACT. *Background:* Previous reports have shown that arginine (Arg) enhances phagocytic activity of macrophages and is required for macrophage-mediated toxicity toward tumor cells. Few studies have addressed the importance of Arg supplementation on macrophage and neutrophil function after infection and sepsis. This study examined the effect of Arg-supplemented diets before and Arg-enriched 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:* Male Wistar rats were assigned to 4 groups. Groups 1 and 2 were fed a semipurified diet, while groups 3 and 4 had part of the casein replaced with 2% of total calories as Arg. After the experimental diets were administered for 10 days, sepsis was induced by cecal ligation and puncture (CLP); at the same time, an internal jugular vein was cannulated. All rats were maintained on TPN for 3 days. Groups 1 and 3 were infused with conventional TPN, while groups 2 and 4 were supplemented with Arg, replacing 10% of total amino acids in the

TPN solution. Survival rates were recorded for 3 days after CLP, and all surviving rats were killed 3 days after CLP to examine their immune responses. *Results:* Aerobic and anaerobic bacteria colony counts in peritoneal lavage fluid were significantly reduced, and 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 groups. *Conclusions:* Enteral Arg supplementation before sepsis significantly enhanced peritoneal macrophage phagocytic activity and reduced total bacterial counts in peritoneal lavage fluid. Arg administered before and after CLP seemed to have a synergistic effect on enhancing phagocytic activity and on bacterial clearance. However, IV Arg administration after CLP had no favorable effects on phagocytic activity or survival rates in rats with gut-derived sepsis. (*Journal of Parenteral and Enteral Nutrition* 27:235–240, 2003)

Arginine (Arg) is a semiessential amino acid that has been shown to possess numerous useful physiologic properties.¹ Previous reports have revealed that supplemental dietary Arg reduces net protein catabolism and enhances the immune function in rats.^{2–4} Also, Arg increases the mitotic response of peripheral blood lymphocytes to concanavalin A and phytohemagglutinin in healthy humans⁵ and in postoperative patients.⁶ Although a recent meta-analysis of several studies focusing on immunonutrition indicated that Arg supplementation has no effect on infectious complications and may increase mortality in critically ill patients, immunonutrition with Arg is associated with a reduction in infectious complication rates and a shorter length of hospital stay without any adverse effect on mortality in elective surgical patients.⁷ Arg is considered an essential amino acid for patients with catabolic diseases.^{1,8}

Sepsis is a cause of death in patients who have undergone major surgery. Sepsis is initiated by bacte-

ria and their related toxins. When bacterial toxins insult the body, profound alterations in immune responses and organ functions may occur.⁹ A study by Madden et al¹⁰ demonstrated that Arg given orally before injury or started IV after cecal ligation and puncture (CLP) improved the survival of rats. Also, Gianotti et al¹¹ showed that Arg-supplemented diets improved survival in mice subjected to blood transfusions and peritonitis. The benefit of Arg appears to be mediated by improved bactericidal mechanisms via the Arg-nitric oxide (NO) pathway.¹¹ NO is an intermediate metabolite generated during the biochemical transformation of Arg to citrulline. Stuehr and Marletta¹² noted that mouse peritoneal macrophages produce nitrite and nitrate when cultured with lipopolysaccharide. An *in vitro* study by Hibbs et al¹³ showed that the key role of Arg in macrophage- and lymphocyte-mediated toxicity toward infected cells is largely via the production and release of NO. A report by Tachibana et al¹⁴ showed that an Arg-enriched solution enhanced phagocytic activity of alveolar macrophages in tumor-bearing rats. However, few studies have addressed the importance of Arg supplementation on macrophage and neutrophil function after infection and sepsis.

Phagocytic cells are an essential arm of the host defense against microbes. Macrophages and neutro-

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TABLE I
Composition of the semipurified diet (g/kg)

Ingredients	Arg-supplemented	Without Arg
Casein	180	220
Arg	20	—
Total nitrogen	35.2	35.2
Corn starch	677	657
Soybean oil	44	44
Vitamin†	10	10
Salt mixture*	35	35
Methyl-cellulose	30	30
Choline chloride	1	1
DL-methionine	3	3

† The vitamin mixture 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.

* The salt mixture contains the following (mg/g): calcium phosphate dibasic 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.

phils are involved in the early, nonspecific host-defense responses and play an important role in the pathophysiology or protection against sepsis.^{15,16} To the authors' knowledge, the effects of Arg on phagocytic activity in gut-origin sepsis have not been previously studied. Therefore, the aim was to investigate the effect of Arg supplementation before and after sepsis and on the ability of the host defense to kill translocated enteric bacteria in the peritoneal cavity and organisms that invade the systemic circulation. In this study, CLP was used as a sepsis model because CLP is more clinically relevant and is considered to be a simple and reproducible model of gut-derived sepsis in rats.¹⁷

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 200 to 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 3 days before the experiment. The care of the animals used in this study followed standard experimental animal care procedures.

Study Protocol

All rats were assigned to 4 groups. Groups 1 and 2 were fed a semipurified diet. Rats in groups 3 and 4 were fed an identical diet except that part of the casein was replaced by Arg, which provided 2% of the total energy intake (Table I). After the experimental diets were administered for 10 days, sepsis was induced by CLP according to the method of Wichterman et al.¹⁷ 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 2 layers. Immediately after CLP, the right internal jugular vein was cannulated with a Silastic catheter (Dow Corning, Midland, MI). 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 metabolic cages. Two milliliters of TPN per hour was administered on the first day. Full-strength TPN was given thereafter. 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 rats were maintained with TPN for 3 days. Groups 1 and 3 were infused with conventional TPN. Groups 2 and 4 were supplemented with Arg, replacing 10% of total amino acids, which also provided 2% of the total energy of the TPN solution. TPN provided 280 kcal/kg body weight, and the kilocalorie:nitrogen ratio was 119:1. The TPN solutions were isonitrogenous and identical in nutrient composition except for the difference in amino acid content (Table II). There were 4 groups: group 1, without Arg supplementation before and after CLP (-/-); group 2, Arg-supplemented TPN after CLP (-/+); group 3, Arg-supplemented diet before CLP (+/-); and group 4, Arg supplementation before and after CLP (+/+).

Measurements and Analytical Procedures

Survival rates were noted daily after CLP and were recorded for 3 days. TPN was continued until killing 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 was intraperitoneally injected to elute the peritoneal cells. After the peritoneal lavage fluid (PLF) was harvested, the rats were killed by drawing arterial blood from the aorta of the abdomen. Fresh PLF was used for bacteriological

TABLE II
Composition of the TPN solution (mL/L)

	Arg-supplemented	Without Arg
Glucose 50%	412	400
Fat emulsion 20%	50	50
Moriamin-SN 10%*	450	556 ml
Arg	5 g	—
Infuvita†	8	8
NaCl 3%	35	35
KCl 7%	10	10
K ₃ PO ₄ 8.7%	10	10
Ca-gluconate	10	10
MgSO ₄	4	4
ZnSO ₄	2	2
Choline chloride (g)	1	1

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

† 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 -tocopherol acetate 2 mg, biotin 12 μ g, folic acid 80 μ g, cyanocobalamin 1 μ g.

analysis and macrophage phagocytic activity, and the remaining samples were stored at -80°C until assay for NO and tumor necrosis factor (TNF)- α . Blood samples were collected in tubes containing heparin. Whole blood was used for analyzing bacterial counts, and neutrophil phagocytosis was determined by flow cytometry. Plasma was centrifuged from the remaining blood and was stored at -80°C for analysis of NO and amino acid concentrations.

Technique for Quantitative Bacteriologic Culture

A total aerobic bacterial count was made by spreading 50 μL of whole blood or 10-fold serially diluted PLF on tryptic soy agar with 5% sheep blood (TSA) blood agar plates (BBL media; Becton Dickinson, Sparks, MD) and incubating at 37°C overnight. A total anaerobic bacteria count was made by spreading 50 μL of the above samples on CDC blood agar plates (BBL media) and incubating under anaerobic conditions (GasPak System; Becton Dickinson) at 37°C for 2 days. The total number of colonies formed on each plate was counted. The results were expressed as colony forming units per milliliter of blood or PLF.

Phagocytosis Assay of Blood Polymorphonuclear Neutrophils (PMNs)^{18,19}

A flow cytometric phagocytosis test was used to evaluate the phagocytic activity of blood PMNs. One hundred microliters of heparinized whole blood was aliquotted on the bottom of a $12 \times 75\text{-mm}$ Falcon polystyrene tube (Becton Dickinson) and placed in an ice-water bath. Twenty microliters of precooled opsonized fluorescein isothiocyanate-labeled *Escherichia coli* (Molecular Probes, Eugene, OR) was added to each tube. Control tubes remained on ice, and assay samples were incubated for precisely 10 minutes at 37°C 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) 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 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 of propidium iodide solution (1 $\mu\text{g}/\text{mL}$ in HBSS) was added to stain the nuclear DNA 10 minutes before the flow cytometric analysis. Flow cytometry was performed on a FACS Calibur flow cytometer (Becton Dickinson) equipped with a 488-nm argon laser. 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 .

Phagocytosis Assay of Peritoneal Macrophages

A Vybrant Phagocytosis Assay kit (Molecular Probes) was used to evaluate the phagocytic activity of peritoneal macrophages. After washing the peritoneal

macrophages 3 times with HBSS, the cell concentration was counted, and 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 distribution of 100 μL of diluted solutions into each well on 96-well microplates, they were transferred to a 37°C CO_2 incubator for 1 hour to allow the cells to adhere to the microplate surface. The Roswell Park Memorial Institute solution was removed from all microplate wells by vacuum aspiration, and then 100 μL of the prepared fluorescein isothiocyanate-labeled *Escherichia coli* was added to each well for 2 hours. Labeled bacteria were removed by vacuum aspiration, and 100 μL of trypan blue suspension was added to all wells within 1 minute. 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.

Plasma Amino Acid Analysis

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

Measurement of TNF- α Concentration

Concentrations of TNF- α in PLF were determined with a commercially available enzyme-linked immunosorbent assay in microtiter plates. Antibodies specific for rat TNF- α were coated onto the wells of the microtiter strips provided (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Determination of NO

NO is highly unstable in solution and cannot be readily assayed. However, NO is converted to stable nitrite and nitrate ions in aqueous solution. After conversion of nitrate to nitrite using nitrate reductase, nitrite concentrations were measured using the Griess reagent. The concentrations of $\text{NO}_2^-/\text{NO}_3^-$ in plasma and PLF were determined with a commercial kit (Assay Designs, Ann Arbor, MI). Procedures followed the manufacturer's instructions.

Statistical Analysis

Data are expressed as the mean \pm SD. Differences among groups were analyzed by analysis of variance by using Duncan's test. A value of $p < 0.05$ 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 4 groups (data not shown). The Arg-supplemented groups (group 2, 168.6 ± 59.3 ; group 3, 159.6 ± 24.1 ; group 4, 178.4 ± 77.2 nmol/mL) had significantly higher plasma Arg levels than did group 1 (71.6 ± 31.5

nmol/mL). There were no differences in plasma citrulline concentrations among the 4 groups (data not shown).

The numbers of total aerobic or anaerobic bacteria in PLF of groups 3 (+/-) and 4 (+/+) were significantly less than those of groups 1 (-/-) and 2 (-/+). The total bacteria numbers in the whole blood did not differ among the 4 groups (Fig 1). No significant difference in the cell numbers of peritoneal macrophages was observed among the 4 groups (data not shown). Phagocytic activities were significantly higher in groups 3 (+/-) and 4 (+/+) than in groups 1 (-/-) and 2 (-/+; Fig 2). There were no significant differences in the phagocytic activities of blood PMNs among the 4 groups (Fig 3).

Concentrations of $\text{NO}_2^-/\text{NO}_3^-$ in plasma and PLF did not differ among the 4 groups 3 days after CLP (Fig 4). There were no significant differences in PLF concentrations of TNF- α among the 4 groups (Fig 5). No significant differences in survival rates were observed among the groups (Fig 6).

DISCUSSION

In this study, 2% of total energy was supplied by Arg. This amount of Arg was found to reduce mortality in

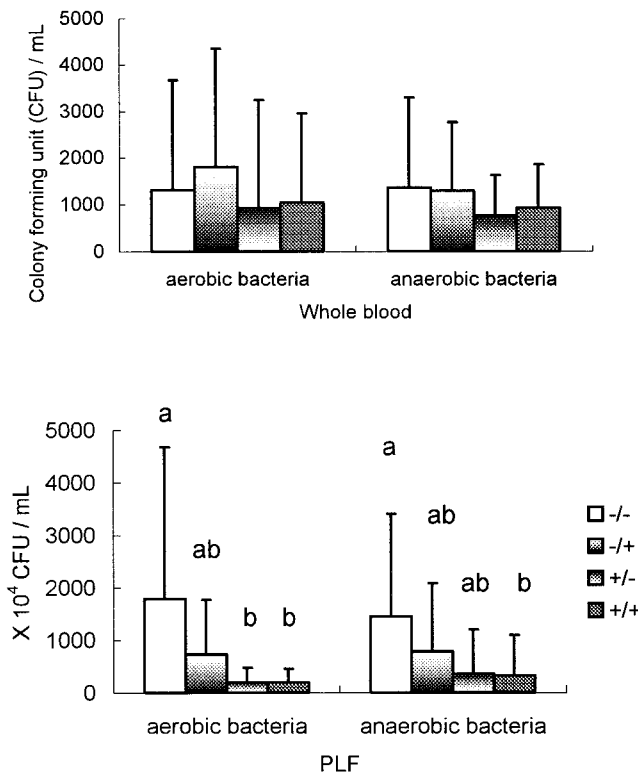


FIG. 1. Total counts of aerobic and anaerobic bacteria in whole blood and PLF in the 4 groups 3 days after CLP. Aerobic bacteria were isolated on TSA blood agar plates and cultured in a 37°C incubator for 24 hours. Anaerobic bacteria were isolated on CDC blood agar plates and cultured in Gas-Pak jar at 37°C for 48 hours. The numbers of total aerobic or anaerobic bacteria in PLF of groups 3 and 4 were significantly less than those of groups 1 and 2 ($p < .05$). Total numbers of bacteria in whole blood did not differ among the 4 groups ($p > .05$; group 1, $n = 12$; group 2, $n = 13$; group 3, $n = 12$; group 4, $n = 11$).

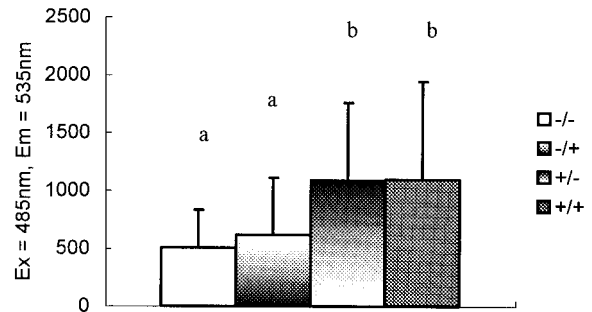


FIG. 2. Phagocytic activity of peritoneal macrophages. After subtraction the background of the control wells, the phagocytic activities of groups 3 (+/-) and 4 (+/+) were significantly higher than those of groups 1 (-/-) and 2 (-/+) ($p < .05$; group 1, $n = 8$; group 2, $n = 11$; group 3, $n = 9$; group 4, $n = 9$).

rodents in gut-derived sepsis.¹¹ We provided oral Arg supplementation for 10 days before sepsis induction. This model mimics the septic complications in patients with abdominal surgery, in whom preventive use of an Arg-supplemented enteral diet may be recommended. TPN was administered after CLP, because sepsis has been shown to adversely affect the barrier and metabolic functions of the small intestine and to reduce mesenteric blood flow and cause histologic damage.^{21,22} A study by Gardiner et al²³ showed that sepsis induced by CLP resulted in impaired intestinal amino acid uptake, and the parenteral rather than the enteral route of Arg therapy may have benefits for survival from septic insult.

The results of this study showed that the phagocytic activity in peritoneal macrophages was much higher in groups with Arg supplementation before CLP (groups 3 and 4), and aerobic and anaerobic bacteria in PLF were significantly reduced in groups 3 or group 4. CLP causes peritoneal contamination with mixed bacteria flora, mostly anaerobic bacteria originating from enteric organisms. These findings indicate that Arg supplementation enhanced the activity of peritoneal macrophages and may consequently modulate bacterial clearance at the site of injury. We did not analyze the enteric organisms either quantitatively or qualitatively in this study. We cannot determine if Arg supplementation before CLP may have caused a change in the intestinal flora and affected the subsequent development of sepsis.

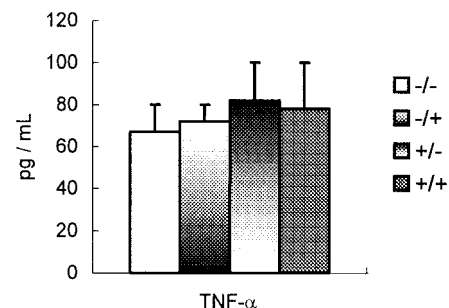


FIG. 3. TNF- α concentrations in PLF of the 4 groups 3 days after CLP. There were no significant differences among the 4 groups ($p > .05$; group 1, $n = 8$; group 2, $n = 11$; group 3, $n = 9$; group 4, $n = 9$).

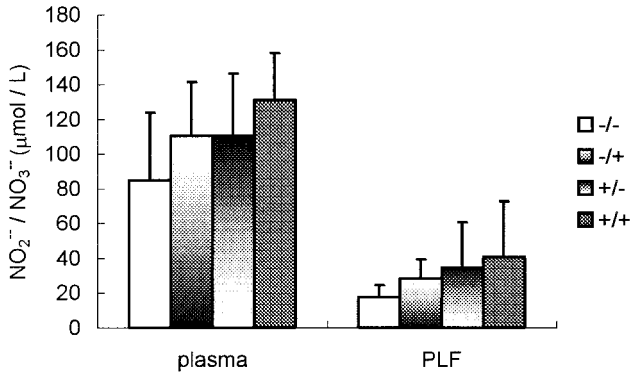


FIG. 4. NO concentrations in plasma and PLF of the 4 groups 3 days after CLP. There were no significant differences among the 4 groups ($p > .05$; group 1, $n = 7$; group 2, $n = 7$; group 3, $n = 9$; group 4, $n = 9$).

Hibbs et al²⁴ demonstrated that L-Arg is required *in vitro* for the cytotoxic effector mechanisms of macrophages, and that the production of NO via the conversion of Arg to citrulline is controlled by the Arg deaminase pathway. In this study, NO₂⁻/NO₃⁻ concentrations in plasma and PLF did not differ among the 4 groups. Plasma citrullin levels not differing among the 4 groups suggests that NO synthetase activities were not stimulated and NO was not induced after Arg supplementation. It is possible that NO synthesis in response to metabolic stress such as with sepsis in this study was already at a peak.²⁵ This result is similar to that of a study by Cui et al²⁶ in which they also found no difference in the plasma NO product between an Arg-supplemented group and a control group after burn injury. Because we did not analyze NO levels within macrophages, whether NO derived from Arg in macrophages is responsible for enhancing phagocytic activity requires further investigation.

TNF- α is a macrophage-derived peptide that has antitumor action and modulates immune and inflammatory reactions.²⁷ Previous reports showed that high plasma TNF- α levels were associated with increased severity of inflammatory diseases.^{27,28} In this study, there were no differences in PLF TNF- α concentrations among the groups. This finding may indicate that the

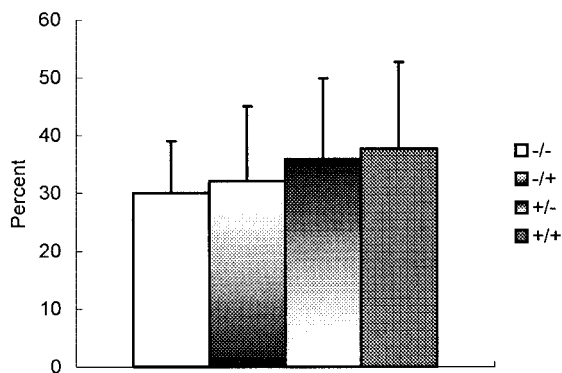


FIG. 5. Phagocytic activity of peripheral blood neutrophils by flow cytometry. No significant differences were observed among the 4 groups ($p > .05$; group 1, $n = 8$; group 2, $n = 10$; group 3, $n = 10$; group 4, $n = 9$).

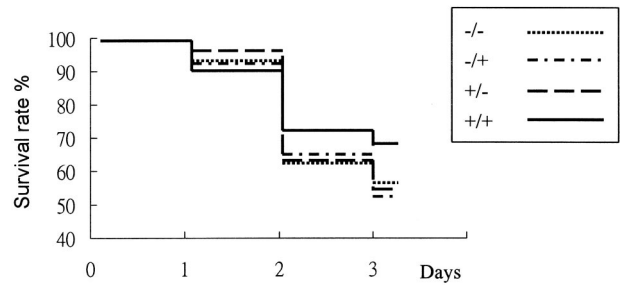


FIG. 6. Survival rates for 3 days of animals with Arg supplementation both before and after CLP. Survival rates did not differ among the 4 groups ($p > .05$; group 1, $n = 10$; group 2, $n = 10$; group 3, $n = 10$; group 4, $n = 10$).

severity of the inflammatory reaction was similar among the groups and that Arg supplementation did not modulate the TNF- α production of peritoneal macrophages. Because interleukin-1 and TNF- α can activate macrophage activity,²⁸ whether interleukin-1 plays a role in enhancing the phagocytic activity of peritoneal macrophages is unclear.

Results of phagocytic activity of peritoneal macrophages and of bacterial counts in PLF showed that there were no differences between groups 3 (+/-) and 4 (+/+), whereas those of group 2 (-/+) were significantly lower than those of group 3 and were no different from those of group 1 (-/-). This finding may indicate that Arg supplementation before sepsis had beneficial effects on enhancing phagocytic activity. Arg administered before and after CLP seemed to have a synergistic effect on phagocytic activity and bacterial killing. No favorable effects were noted when Arg was supplemented after CLP. This result is inconsistent with that of a report by Madden et al,¹⁰ which showed that IV-administered Arg after CLP significantly increased animal survival over those without Arg. The daily dosage used in this study was comparable to that used by Madden et al. However, in that study 100 mg of Arg hydrochloride was infused IV 3 times a day, and the Arg dosage injected at the given times was high. In this study, Arg was provided evenly in 24 hours via TPN, and the plasma Arg concentration was relatively constant. Whether a high-dose injection of Arg in a limited time may activate the immune response and improve the survival after CLP requires further investigation.

PMNs are potent inflammatory cells, and the total number and percentage of circulating PMNs can be induced by acute infection and endotoxin.¹⁵ PMNs efficiently engulf a wide variety of microbes. In this study, the phagocytic activity of blood PMNs cells did not differ among the 4 groups. Also, there were no differences in blood total aerobic and anaerobic bacteria numbers among the groups. Because the peritoneal cavity is the primary site of injury, it is possible that Arg augments phagocytic activity at the location of bacterial invasion. The effect of Arg on phagocytic cells in the systemic circulation was not obvious.

In summary, this study shows that enteral Arg supplementation before sepsis significantly enhances peritoneal macrophage phagocytic activity; the total bacteria number in PLF was also reduced. Arg administered before and after CLP seemed to have a synergistic

effect on phagocytic activity and bacterial clearance at the site of injury. However, IV Arg administration after CLP had no favorable effects on phagocytic activity or on survival in rats with gut-derived sepsis.

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REFERENCES

- Evoy D, Lieberman MD, Fahey III TJ, et al: Immunonutrition: The role of arginine. *Nutrition* 4:611–617, 1998
- Barbul A, Wasserkrug HL, Yoshimura NN, et al: High arginine levels in intravenous hyperalimentation abrogate post-traumatic immune suppression. *J Surg Res* 36:620–624, 1984
- Sietger E, Rettura G, Barbul A, et al: Arginine an essential amino acid for injured rats. *Surgery* 84:224–230, 1978
- Sitren HS, Fisher H: Nitrogen retention in rats fed on diets enriched with arginine and glycine: I. Improved N retention after trauma. *Br J Nutr* 37:195–208, 1977
- Barbul A, Sisto D, Wasserkrug H, et al: Arginine stimulates lymphocyte immune response in healthy human beings. *Surgery* 90:244–251, 1981
- Daly JM, Reynolds J, Thom A, et al: Immune and metabolic effects of arginine in surgical patients. *Ann Surg* 208:512–523, 1988
- Heyland DK, Novak F, Drover JW, et al: Should immunonutrition become routine in critically ill patients? A systematic review of the evidence. *JAMA* 286:944–953, 2001
- De-Souza DA, Greene LJ: Pharmacological nutrition after burn injury. *J Nutr* 128:797–803, 1998
- Zellweger R, Ayala A, DeMaso CM, et al: Trauma-hemorrhage causes prolonged depression in cellular immunity. *Shock* 4:149–153, 1995
- Madden HP, Breslin RJ, Wasserkrug HL, et al: Stimulation of T cell immunity by arginine enhances survival in peritonitis. *J Surg Res* 44:658–663, 1988
- Gianotti L, Alexander JW, Pyles T, et al: Arginine-supplemented diets improve survival in gut-derived sepsis and bacterial clearance. *Ann Surg* 217:644–653, 1993
- Stuehr DJ, Marletta MA: Mammalian nitrate biosynthesis: Mouse macrophages produce nitrite and nitrate in response to *Escherichia coli* lipopolysaccharide. *Proc Natl Acad Sci USA* 82:7738–7743, 1985
- Hibbs JB, Vavrin Z, Taintor RR: L-Arginine is required for expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells. *J Immunol* 138:550–555, 1987
- Tachibana K, Mukai K, Hiraoka I, et al: Evaluation of the effect of arginine-enriched amino acid solution on tumor growth. *JPEN* 9:428–434, 1985
- Ringer TV, Zimmermann JJ: Inflammatory host responses in sepsis. *Crit Care Clin* 8:163–189, 1992
- Sawyer DW, Donowitz DR, Mandell GL: Polymorphonuclear neutrophils: An effective antimicrobial force. *Rev Infect Dis* 11:1532–1544, 1989
- 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
- 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
- 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
- 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
- Navaratnam RLN, Morris SE, Traber DL, et al: Endotoxin (LPS) increases mesenteric vascular resistance (MVR) and bacterial translocation (BT). *J Trauma* 30:1104–1113, 1990
- Gardiner K, Barbul A: Intestinal amino acid absorption during sepsis. *JPEN* 17:277–283, 1993
- Gardiner KR, Gardiner RE, Barbul A: Reduced intestinal absorption of arginine during sepsis. *Crit Care Med* 23:1227–1232, 1995
- Hibbs JB, Taintor RR, Vavrin Z: Macrophage cytotoxicity: Role for L-arginine deaminase and imino nitrogen oxidation to nitrate. *Science* 235:473–476, 1987
- Lindblom L, Cassuto J, Yregard L, et al: Role of nitric oxide in the control of burn perfusion. *Burns* 26:19–23, 2000
- Cui XL, Iwasa M, Iwasa Y, et al: Arginine-supplemented diet decreases expression of inflammatory cytokines and improves survival in burned rats. *JPEN* 24:89–96, 2000
- Beutler B, Cerami A: Tumor necrosis, cachexia, shock and inflammation: A common mediator. *Annu Rev Biochem* 57:505–518, 1988
- DiPiro JT: Cytokine networks with infection: Mycobacterial infections, leishmaniasis, human immunodeficiency virus infection, and sepsis. *Pharmacotherapy* 17:205–223, 1997