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DIETARY ARGININE ENHANCES ADHESION MOLECULE AND T HELPER 2 CYTOKINE EXPRESSION IN MICE WITH GUT-DERIVED SEPSIS

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ABSTRACT—This study investigated the effects of arginine (Arg) on cellular adhesion molecules and intracellular Th1/Th2 cytokine expressions in mice with polymicrobial sepsis. Myeloperoxidase activity in organs was also analyzed to identify the extent of tissue injury resulting from neutrophil infiltration. Mice were randomly assigned to a normal group (NC), a control group, or an Arg group. The NC group was fed a standard chow diet. The control group was fed a common semipurified diet, and in the Arg group, part of the casein was replaced by Arg, which provided 2% of the total calories. After 3 weeks, sepsis was induced by cecal ligation and puncture (CLP) in the control and Arg groups. Mice in the experimental groups were sacrificed at 0, 6, 12, and 24 h after CLP, whereas mice in the NC group were sacrificed when the CLP was performed. Blood and organ samples were immediately collected for further analysis. Results showed that compared with the control group, plasma intracellular adhesion molecule-1 levels were significantly higher in the Arg group 12 and 24 h after CLP. Lymphocyte interferon-γ expression in the Arg groups was significantly lower, whereas interleukin (IL)-4 expression was higher than the control group at various time points after CLP. The expression of lymphocyte CD11a/CD18 was significantly higher in the Arg group 6, 12, and 24 h after CLP than those of the corresponding control group and the NC group. PMN expressions of CD11b/CD18 in the Arg groups were higher than those in the control group at 12 and 24 h after CLP. The Arg group had higher IL-6 levels at 6 and 12 h in the kidney and intestine and 12 h in the lung after CLP. Higher myeloperoxidase activities were observed in the Arg groups at 24 h after CLP than those in the control group in various organs. These findings suggest that pretreatment with an Arg-supplemented diet enhances adhesion molecule and inflammatory cytokine expression during sepsis, which may aggravate the inflammatory reaction and increase neutrophil infiltration into tissues. In addition, Arg supplementation reduced intracellular interferon-γ and enhanced IL-4 expression. This change may promote the Th2-type response and suppress the cellular immune response in gut-derived sepsis.

KEYWORDS—Arginine, cecal ligation and puncture, CD11a/CD18, CD11b/CD18, interleukin-4, interferon-γ

INTRODUCTION

Sepsis is defined as a systemic response to infection, and it remains the leading cause of death in surgical intensive care units. A previous study showed that overexpression of inflammatory cytokine and adhesion molecules on endothelium cells and leukocytes may contribute to tissue injury and multiple organ dysfunction syndrome (MODS) in sepsis (1–3). Adhesion molecules play a key role in cell-cell interactions and cell-extracellular matrix interactions. Members of the immunoglobulin family of adhesion molecules such as intercellular adhesion molecule (ICAM)-1 and its ligands (CD11a/CD18 and CD11b/CD18) are important mediators of host defense localized in the earliest lesions of inflammation (4). Studies have shown that the concentration of the soluble form ICAM-1 increases in volunteers given endotoxin or patients with septic multiple organ failure (5, 6). Clinical trials have also shown that increased plasma ICAM-1 levels are correlated with the development of MODS and death in neonatal and adult sepsis (4, 7).

The cytokine profiles are associated with and related to the severity of various types of infection. The cytokine profiles are determined by two functional subsets of T lymphocytes, Th1 and Th2. Th1 cytokines enhance cell-mediated immunity, whereas Th2 cytokines enhance humoral immunity (8). Previous studies showed that a shift from a Th1- to a Th2-type response occurs during sepsis, and marked depression of Th1-type immunity results in increased mortality in sepsis (9–11).

Arginine (Arg) is a nonessential amino acid for healthy adults. It is the specific precursor for nitric oxide (NO), and it has been shown to possess numerous useful physiological properties. Previous reports have revealed that supplemental dietary Arg enhances the immune function in injured rats (12–14). Also, Arg increases the mitotic response of peripheral blood lymphocytes to concanavalin A and phytohemagglutinin in healthy humans and in postoperative patients (15, 16). Arg is often used in immunonutrition regimens. Some studies showed that immune-enhancing diets containing Arg were found to be beneficial for injured or surgical patients (17, 18). Braga et al. (19) also demonstrated that preoperative oral Arg and n-3 fatty acid supplementation improves the immunometabolic response and decreases the infection rate in patients with colorectal resection. However, meta-analysis of several studies focusing on immunonutrition indicated that Arg supplementation has no effect on infection complications and may increase mortality in critically ill patients (20). Because immune-enhancing diets contain other pharmacologically active components, whether Arg is responsible for the modulatory

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effects remains controversial. Animal studies exclusively with Arg supplementation for nonseptic burned or injured rodents indicate that it appears to be beneficial (12–14, 21), but the results of studies investigating the effect of Arg on sepsis are inconsistent (22, 23). In addition, the effects of Arg supplementation on inflammation-related adhesion molecule expression and cytokine profiles in sepsis have not been addressed. Therefore, the aim of this study was to investigate the effects of Arg on cellular adhesion molecules and intracellular Th1/Th2 cytokine expressions in polymicrobial sepsis. Because oxyradicals released from neutrophils that accumulate in organs may damage organ cells and induce MODS (24), we analyzed the myeloperoxidase (MPO) activities in organs as an indicator for identifying the extent of tissue injury resulting from neutrophil infiltration (25, 26).

MATERIALS AND METHODS

Animals

Male ICR mice weighing 20 to 25 g were used in this study. All mice were housed in stainless steel cages maintained in a temperature- and humidity-controlled room with 12-h light-dark cycle. All mice were allowed free access to a standard chow diet and water for 1 week before the study. The care of the laboratory animals was established by Taipei Medical University, and protocols were approved by the Animal Care Committee.

Study protocol

Mice were randomly assigned to a normal group (NC), a control group, or an Arg group. The normal group was fed a chow diet. The control group was fed a common semipurified diet, and the Arg group was supplied an identical diet except that part of the casein was replaced by Arg, which provided 2% of the total energy intake (Table 1). This amount of Arg was found to have regulatory effects on the immune response (23). After feeding the respective diets for 3 weeks, polymicrobial sepsis was induced in mice in the control and Arg groups by cecal ligation and puncture (CLP). CLP was performed as previously described by Ayala et al. (10). Mice were lightly anesthetized with ether. A midline incision (1.5–2.0 cm) was made below the diaphragm, exposing the internal organs. 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 in two places with a 22-G needle and was replaced into the abdomen. The abdominal wound was closed in two layers. Mice in the control and Arg groups were sacrificed 0, 6, 12, and 24 h after CLP, respectively. Mice in the normal group were sacrificed at the time CLP was performed in the experimental groups. All mice were anesthetized and sacrificed by cardiac puncture. Blood samples were collected in tubes containing heparin for analysis. Tissues including lung, kidney, liver, and intestine were rapidly harvested and stored at –70°C for further analysis.

Measurements of sICAM-1 concentrations in the circulation during sepsis

Concentrations of sICAM-1 in the circulation were measured by a commercially available enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN). Antibodies specific for mice ICAM-1 were coated onto the wells of the microtiter strips provided. Procedures followed the manufacturer's instructions.

Analysis of intracellular IL-4 and IFN-γ expression

Populations of lymphocyte IL-4 and IFN-γ expression in fresh blood were analyzed by flow cytometry (Coulter, Miami, FL). After sacrificing the mice, 50 μL of fresh blood was immediately incubated with 100 μL of Leucoperm (Serotec, Oxford, UK) reagent A for 15 min at room temperature to fix the leukocytes, and then 5 mL of phosphate-buffered saline (PBS) was added and centrifuged for 5 min at 300*g*. After discarding the supernatants, 100 μL of Leucoperm reagent B was added to the cell pellets to penetrate the leukocytes, then 10 μL of fluorescein-conjugated rat monoclonal anti-mouse IFN-γ (Serotec) and 5 μL of phycoerythrin (PE)-conjugated rat monoclonal anti-mouse IL-4 (Serotec) were incubated together for 30 min at room temperature. Leukocytes were washed with PBS. After removing the supernatant and resuspending cells in the sheath fluid, lymphocytes were gated on the basis of their forward scatter and side scatter profile. Lymphocytes capable of IL-4 and IFN-γ expression were assessed using dual intracellular cytokine staining and flow cytometry (Coulter). The results are presented as a percentage of cytokine-producing cells in 1×10^5 lymphocytes.

TABLE 1. **Composition of the experimental diets**

Component (g/kg)	Control	Arg
Soybean oil	100	100
Casein	200	158
Argz	0	20.9
Salt mixture†	35	35
Vitamin mixture‡	10	10
Methyl cellulose	31	31
Choline chloride	1	1
Methionine	3	3
Corn starch	620	641.1

†Salt mixture contained the following (mg/g): calcium phosphate diabasic, 500; sodium chloride, 74; potassium sulfate, 52; potassium cutrate monohydrate, 20; magnesium oxide, 24; manganese carbonate, 3.5; ferric citrate, 6; zinc carbonate, 1.6; curpric carbonate, 0.3; potassium iodate, 0.01; sodium selenite, 0.01; and chromium potassium sulfate, 0.55.

‡Vitamin mixture contained the following (mg/g): thiamin hydrochloride, 0.6; ricoflavin, 0.6; pyridoxine hydrochloride, 0.7; nicotinic acid, 3; calcium pantothenate, 1.6; D-biotin, 0.05; cyanocobalamin, 0.001; retinyl palmitate, 1.6; DL-α-tocopherol acetate, 20; cholecalciferol, 0.25; and menaquinone, 0.005.

Analysis of CD11a/CD18 distributions in lymphocytes and PMN expression of CD11b/CD18

One hundred microliters of fresh blood was incubated with 10 μL of fluorescein isothiocyanate (FITC)-conjugated rat monoclonal anti-mouse CD11a and PE-conjugated rat anti-mouse CD18 (Serotec) for 15 min at 4°C. Afterward, red blood cells were lysed with lysing buffer (Serotec). The proportions of CD11a/CD18 expression on lymphocytes were analyzed by flow cytometry (Coulter). Fluorescence data were collected, and the results are presented as a percentage of CD11a-presenting cells in 1×10^5 lymphocytes. To determine the CD11b/CD18 expressions on PMNs, FITC-conjugated rat monoclonal anti-mouse CD11b, and PE-conjugated rat anti-mouse CD18 (Serotec) were added to 100 μL of the PMN suspension. Fluorescence data were collected on 1×10^5 viable cells and were also analyzed by flow cytometry (Coulter). The results are presented as a percentage of CD11b-presenting cells in 1×10^5 PMNs. Lymphocytes and PMNs were gated on the basis of forward scatter and side scatter profile, and were analyzed for the expression of CD11a/CD18 and CD11b/CD18, respectively.

Measurements of IL-6 concentrations in organ tissues

Organs including the lung, kidney, liver, and intestine (including the proximal, middle, and distal small intestine) were removed and parts of the samples (0.5 g) were placed in 10 mL of lysing buffer containing protease inhibitors (2 mmol/L phenylmethylsulfonyl fluoride, and 2 μg/mL leupeptin, pepstatin A, and aprotinin; Sigma, St. Louis, MO) at 4°C. Samples were homogenized and ultracentrifuged at 15,000 rpm for 45 min at 4°C. The concentrations of IL-6 in the supernatants were determined with a commercially available enzyme-linked immunosorbent assay kit. Antibodies specific for mice IL-6 were coated onto the wells of the microtiter strips provided (BioSource International, Camarillo, CA).

Measurements of MPO activity in organs

MPO activity was measured using the method modified by Laight et al. (25). Liver, lung, kidney, and intestine samples were homogenized in 50 mM potassium phosphate buffer (PB, pH 6.0), and centrifuged at 20,000*g*, at 4°C for 15 min. The supernatants were discarded and the pellets were suspended in 50 mM PB containing 0.5% hexadecyltrimethylammonium bromide. After freezing and thawing for three cycles, samples were centrifuged at 20,000*g* for 15 min. Aliquots (0.3 mL) were added to 2.3 mL of the reaction mixture containing 50 mM PB, *o*-dianisidine, and 20 mM of an H_2O_2 solution. MPO activity is presented as units per milligram of tissue protein. The concentrations of tissue protein were measured by Lowry's method. The absorbance at 460 nm was immediately measured for 3 min, and the rate of change in the absorbance was used to calculate the activity of MPO.

Statistical analysis

Data are expressed as the mean ± SD. Differences among groups were analyzed by two-way analysis of variance with Fisher's test. $P < 0.05$ was considered statistically significant.

RESULTS

Plasma sICAM-1 levels

Plasma concentrations of sICAM-1 increased with the progression of sepsis and reached a peak at 12 h in both groups, then decreased by 24 h after CLP. Compared with the control group, plasma sICAM-1 levels were significantly higher in the Arg group 12 and 24 h after CLP (Fig. 1).

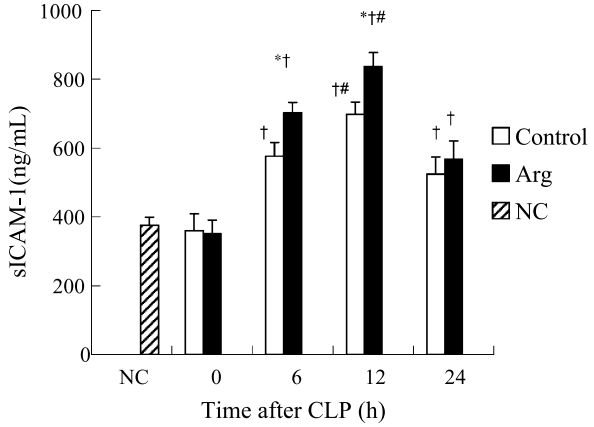

FIG. 1. **Concentration of sICAM-1 in circulation during sepsis.** *Significantly different from the control group at the same time point; †Significantly different from the NC group and time 0 h in the same group; #Significantly different from the same group at different time points.

Intracellular IFN-γ and IL-4 distribution

Lymphocyte expression of IFN-γ decreased, whereas IL-4 expression increased in both groups as sepsis progressed. Lymphocyte IFN-γ expression in the Arg groups was significantly lower, and IL-4 levels were higher than the control group at various time points after CLP (Fig. 2).

CD11a/CD18 expression on lymphocytes and CD11b/CD18 expression on PMNs

The expression of lymphocytes CD11a/CD18 was significantly higher in the Arg group 6, 12, and 24 h after CLP than those of the corresponding control group and the NC group, whereas no significant changes were observed in the control group at various time points after CLP. Compared with the NC group and 0 h, CD11b/CD18 expression on PMNs had significantly increased in both groups by 6 and 12 h after CLP and even at 24 h in the Arg group. PMN expression of CD11b/CD18 in the Arg groups was higher than those in the control group at 12 and 24 h after CLP (Table 2).

IL-6 levels in the liver, lung, kidney, and intestines

IL-6 levels in the lung, kidney, and intestine homogenates in both groups were significantly higher 6, 12, and 24 h after CLP than in the normal group and at 0 h. The IL-6 levels reached a peak at 6 h in the lung and intestine and at 6 and 12 h in the kidney after CLP. The Arg group had higher IL-6 levels at 6 and 12 h in the kidney and intestine and 12 h in the lung after CLP. Contrary to results for the lung, kidney, and intestines, IL-6 levels decreased as sepsis progressed in the liver regardless of whether Arg was supplemented or not (Table 3).

MPO activities in various organs

The activities of MPO in the lung, liver, kidney, and intestine increased in both groups as sepsis progressed and reached a peak at 6 or 12 h after CLP, then gradually decreased at 24 h. The Arg groups had higher MPO activities at 24 h after CLP than those of the control group in various organs (Table 4).

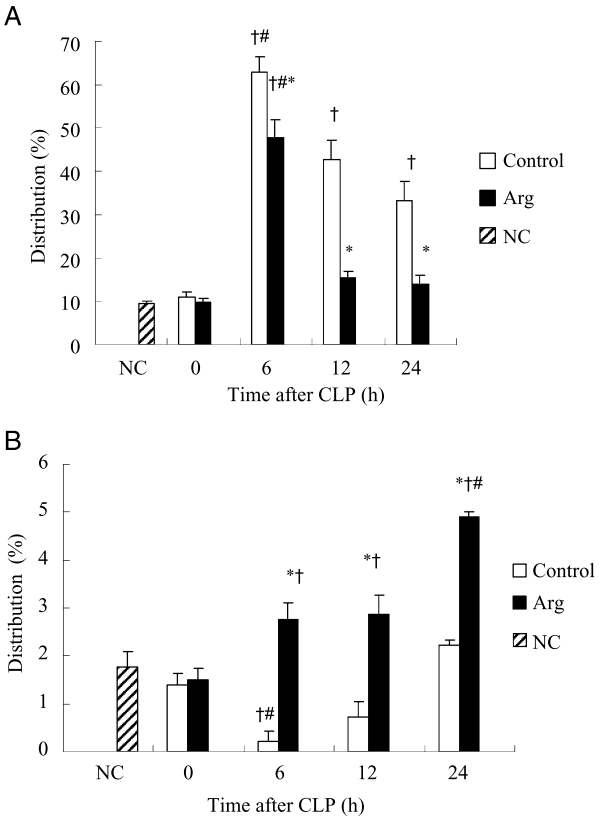

FIG. 2. **Distributions of intralymphocytes interferon-γ (A) and IL-4 (B) at the times indicated after CLP-induced sepsis in mice.** *Significantly different from the control group at the same time point; †Significantly different from the NC group and at time 0 h in the same group; #Significantly different from the same group at different time points.

DISCUSSION

In this study, we directly measured intracellular IFN-γ and IL-4 production to investigate the effect of Arg on the Th1/Th2-type response during sepsis. IFN-γ is produced by Th1 lymphocytes and IL-4 is a Th2 cytokine. The effects of Th1 and Th2 lymphocytes are counter-regulatory (8). The results showed that in accordance with the progression of sepsis, a predominant Th2-type response was observed in both groups. Compared with the control group, Arg administration had more pronounced IFN-γ-suppressive and IL-4-enhancing effects during sepsis. This finding suggests that a more

TABLE 2. **Expression of leukocytes CD11a/CD18 and CD11b/CD18 during sepsis**

	CD11a/CD18	CD11b/CD18 (%)
NC	5.23 ± 1.65	9.56 ± 1.98
0 h		
Control	4.87 ± 1.61	10.78 ± 0.26
Arg	4.08 ± 0.24	11.38 ± 1.74
6 h		
Control	3.6 ± 1.51	19.58 ± 1.25†,‡
Arg	14.65 ± 1.96*,†	17.2 ± 1.94†
12 h		
Control	3.06 ± 1.79	13.68 ± 0.54†
Arg	25.38 ± 4.5*,†	25.22 ± 2.16*,†,‡
24 h		
Control	6.13 ± 2.45	9.83 ± 0.59
Arg	43.03 ± 3.28*,†,‡	26.2 ± 1.39*,†,‡

Data are presented as the mean ± SD. NC, normal control group.
*Significantly different from the corresponding control group at the same time point.
†Significantly different from the NC group and time 0 h in the same group.
‡Significantly different from the same group at various time points.

predominant Th2 response occurs that may lead to a greater extent of cellular immunosuppression. IL-6 is an important mediator in the early phase of infection. IL-6 has been demonstrated to be associated with septic mortality and is an inducer of the systemic inflammatory response syndrome (27). A previous study showed that IL-6 expression in the lungs and kidneys was enhanced after surgery with sepsis induced by LPS (28). In this study, we found that IL-6 levels in the lungs, kidneys, and intestines in the Arg groups were higher than those of the control group at various time points. Because IL-6 is positively correlated with IL-4 production and Th2 differentiation (9), the higher IL-6 levels are comparable with the predominant Th2 response in the Arg groups observed in nonhepatic organs. In this study, we observed that hepatic IL-6 levels were diminished as sepsis progressed in both groups. This finding was consistent with our previous report in a similar sepsis model (29). In our study, the cecum of animals was ligated and punctured twice to induce gut-derived sepsis. This model of peritonitis was found to result in an irreversible hepatic dysfunction (30). A study by Tu et al. (31) also showed that Arg enhanced NO production and was involved in cellular failure in the liver of septic rats. It is possible that the ability of hepatic IL-6 production impaired after CLP under the present experimental condition.

Blood leukocytes are mediators of host defenses and inflammation at the site of injury. Under a condition of inflammation and tissue injury, patches of arterial endothelial cells express selective adhesion molecules on their surfaces that bind to various classes of leukocytes (32). CD11a and CD11b are members of the leukocyte adhesion molecules β_2 integrin. CD11a/CD18 are exclusively expressed on leukocytes and interact with ICAM to promote a variety of homotypic and heterotypic cell adhesion processes required for normal and pathogenic functions of the immune system (33). CD11b/CD18 are abundant in PMNs and contribute to neutrophil migration into sites of inflammation. A previous study showed that expression of CD11b/CD18 was significantly increased in patients with septic shock (34). CD11a/CD18 and CD11b/CD18 are thought to play central roles in mediating the firm adhesion of leukocytes to endothelial cells. Excessive expression of these intergins may induce an inflammatory response and tissue injury (32, 33). In this study, we observed that plasma concentrations of soluble ICAM-1 were significantly higher in the Arg group than in the control group at 6 and 12 h after the CLP, which is consistent with the higher CD11a/CD18 and CD11b/CD18 expression on leukocytes. The finding indicates that CAM-mediated cell interactions may be aggravated when Arg is administered in a septic condition.

MPO is a neutrophil-specific enzyme and is considered to be an index of neutrophil infiltration (26). Excessive MPO released by neutrophils is linked to the pathological process in inflammatory disorders (35). The results of this study showed that compared with the NC group and 0 h, MPO activities in the various organs increased as sepsis progressed, indicating that an inflammatory reaction was induced by sepsis.

TABLE 3. **Concentrations of IL-6 in lung, kidney, intestine, and liver homogenates during sepsis**

	(pg/mg tissue)			
	Lung	Kidney	Intestines	Liver
NC	1.33 ± 0.24	12.1 ± 0.9	1.05 ± 0.7	22.72 ± 2.05
0 h				
Control	3.18 ± 1.16	12.4 ± 0.9	1.1 ± 0.77	26.1 ± 3.17
Arg	2.56 ± 0.8	12.22 ± 1.41	1.25 ± 0.89	27.84 ± 2.34
6 h				
Control	26.67 ± 2.07†,‡	32.6 ± 1.07†,‡	25.58 ± 0.9†,‡	10.37 ± 1.72†
Arg	25.68 ± 2.8†,‡	41.95 ± 1.8*,†,‡	30.24 ± 1.17*,†,‡	10.31 ± 1.5†
12 h				
Control	12.5 ± 1.06†	26.9 ± 1.08†	4.21 ± 1.67†	8.48 ± 0.79†
Arg	17.89 ± 0.87*,†	46.29 ± 2.12*,†,‡	9.14 ± 1.89*,†	7.15 ± 1.5†
24 h				
Control	14.09 ± 1.25†	20.46 ± 0.7†	3.41 ± 0.81†	7.07 ± 2.01†
Arg	14.43 ± 2.5†	20.18 ± 1.7†	4.4 ± 0.76†	7.03 ± 1.7†

Data are presented as the mean ± SD. NC, normal control group.
*Significantly different from the corresponding control group at the same time point.
†Significantly different from the NC group and time 0 h in the same group.
‡Significantly different from the same group at various time points.

TABLE 4. **Activities of MPO in organ homogenates during sepsis**

	(U/mg protein)			
	Lung	Liver	Kidney	Intestines
NC	0.789 ± 0.219	0.503 ± 0.104	0.701 ± 0.203	0.269 ± 0.106
0 h				
Control	0.737 ± 0.181	0.54 ± 0.101	0.704 ± 0.117	0.279 ± 0.106
Arg	0.628 ± 0.201	0.5 ± 0.21	0.649 ± 0.109	0.296 ± 0.065
6 h				
Control	2.061 ± 0.299†	1.094 ± 0.054†	1.361 ± 0.194†	0.506 ± 0.114†,‡
Arg	2.03 ± 0.156†	1.06 ± 0.103†	1.103 ± 0.236†	0.574 ± 0.203†
12 h				
Control	2.855 ± 0.328†,‡	1.091 ± 0.069†	3.156 ± 0.275†,‡	0.443 ± 0.109†,‡
Arg	2.737 ± 0.236†,‡	1.08 ± 0.125†	3.41 ± 0.21†,‡	0.441 ± 0.108†
24 h				
Control	1.262 ± 0.45	0.826 ± 0.088†,‡	1.834 ± 0.29†	0.222 ± 0.031
Arg	2.09 ± 0.26*,†	1.09 ± 0.059*,†	2.7 ± 0.106*,†	0.439 ± 0.056*,†

Data are presented as the mean ± SD. NC, normal control group.
*Significantly different from the control group at the same time point.
†Significantly different from the NC group and time 0 h in the same group.
‡Significantly different from the same group at different time points.

Compared with the control group, the Arg group had higher MPO activities at 24 h after sepsis. This finding may indicate that mice with Arg administration have greater neutrophil infiltration in the late stage of sepsis in these organs.

NO can act as a signal transducer and cellular messenger in homeostasis and host defense. However, NO can be induced by inflammatory mediators such as cytokines and endotoxin. Uncontrolled production of NO is detrimental and can contribute to tissue injury and organ failure (36). Previous studies have shown that Arg administration increases the production of NO in endotoxemic animals (37) and shortened the survival time in rats with hemorrhagic shock (38). In addition to synthesizing NO, superoxide is also formed during sepsis. NO is known to react with superoxide anions to form a highly cytotoxic compound, peroxynitrite (39). Peroxynitrite has been reported to cause cell destruction and tissue injury (39, 40). We speculated that the adverse effects of Arg on the immune response observed in this study could be attributed to the increased substrate for NO synthase, and this may have consequently resulted in peroxynitrite-mediated tissue injury. Many studies showed that Arg supplementation had beneficial effects on immune function in healthy or injured subjects (12–16), and the conditions were distinct from sepsis. Most experimental studies used LPS or endotoxin to induce a septic state (23, 41), both of which have limitations as analogs of human sepsis and may not reflect actual septic condition. In this study, we used CLP as a sepsis model. The presence of live multiplying bacteria in blood and tissue mimics the clinical problems of sepsis more closely than models using LPS. A study by Gianotti et al. (22), who also used CLP as a sepsis model, showed that Arg supplementation improved survival in septic mice. In their study, the cecum of animals was ligated and punctured once with a 25-G needle, whereas in our study, we punctured the cecum twice with a 22-G needle. The model of peritonitis performed here was found to result in more severe and irreversible organ dysfunction and may result in different results. We did not observe a higher mortality in the Arg group 24 h after CLP (8 survivors of 16 mice in the Arg group vs. 9 of 14 in the control group, $P > 0.05$). Because survival was only observed for 24 h in the present study, determining whether Arg supplementation affects survival over a longer period or if overproduction of inducible NO or peroxynitrite is responsible for the adverse immune response requires further investigation.

In summary, this study demonstrates that pretreatment with an Arg-supplemented diet resulted in more pronounced Th2 cytokine production, enhanced leukocyte CD11a/CD18 and CD11b/CD18 expressions, increased circulating ICAM-1 levels, and increased IL-6 as well as MPO activities in various organs in gut-derived sepsis. These findings suggest that Arg administration before sepsis enhances adhesion molecule and inflammatory cytokine expression, which may aggravate the inflammatory reaction and increase neutrophil infiltration into tissues under the present septic conditions. In addition, Arg supplementation reduced lymphocyte IFN-γ and enhanced IL-4 expression. These changes may promote the Th2-type response and suppress the cellular immune response in gut-derived sepsis. These results suggest that the use of Arg in a critical condition should be carefully evaluated.

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