ANNALS OF SURGERY
Vol. 223, No. 1, 84–93

Route of Nutritional Supply Influences Local, Systemic, and Remote Organ Responses to Intraperitoneal Bacterial Challenge

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Objective

The authors' aim was to investigate whether antecedent nutritional routes influence immune responses after surgical insult.

Summary Background Data

Total parenteral nutrition (TPN) may influence host responses to infection. To the best of the authors' knowledge, however, no study has focused on the mechanisms underlying the influence of nutritional route on local, systemic, and remote organ (lung) responses after surgical insult.

Methods

Sixty-eight rats were divided into TPN and total enteral nutrition (TEN) groups. The two groups received identical nutrients for 7 days and were then challenged intraperitoneally with 3×10^8 *Escherichia coli*. In the first experiment, the rats were observed for survival. In the second experiment, the rats were killed before (0 hours) challenge or 2 or 6 hours after challenge. Peritoneal exudative cells (PEC) and bronchoalveolar cells (BALC) were harvested and cultured *in vitro*. Colony-forming units of bacteria in the peritoneal lavage fluid (PLF) were determined. Tumor necrosis factor (TNF), interleukin-1α (IL-1α), interferon-γ (IFN-γ) levels in serum, PLF, bronchoalveolar lavage fluid (BALF), and cell culture supernatants were measured.

Results

The 48-hour survival rate was higher in TEN than in TPN rats. Local immunity was depressed in the TPN group. Bacterial colony counts in PLF were significantly higher in the TPN group than in the TEN group after challenge. The number of PECs was significantly lower, and at 2 hours, local cytokine (TNF and IL-1α) responses were diminished in the TPN group compared with the TEN group at 2 hours. The number of PECs showed a significant positive correlation with levels of local cytokines in the TEN group but not in the TPN group. Elevation of local IFN-γ was significant from 0 to 6 hours in the TEN group but not in the TPN group. *In vitro* production of TNF by PEC was impaired in the TPN rats before challenge. Remote organ (lung) responses were suppressed in the TPN group. The number of BALCs and the TNF levels in BALF declined significantly between 0 and 2 hours in the TEN group but not in the TPN group. Interferon-gamma levels in BALF were higher in the TEN group than in the TPN group at 2 hours. Systemic cytokine responses were disturbed in the TPN group. Production of systemic TNF was greater, but the IFN-γ response was diminished in the TPN group compared with the TEN group after intraperitoneal bacterial challenge.

Conclusion

Local, systemic, and remote organ (lung) immune responses to intraperitoneal bacterial challenge are suppressed in TPN-treated animals, leading to poor survival after challenge. Enteral nutrition before surgical insult may enhance host immune responses after the insult as compared to parenteral nutrition.

Previous investigations have suggested that enteral nutrition is more advantageous than parenteral nutrition after surgical insult. The advantages include maintenance of intestinal barriers,[1,2] improvement of systemic and local protein metabolism,[1,3] and better stress hormone responses.[4] The precise mechanisms underlying enteral nutrition's superiority to parenteral nutrition after surgical insult, however, are unknown.[5,6] Injury and infection stimulate the production of a variety of endogenous mediators. These mediators, in turn, initiate immunologic, hematologic, and metabolic alterations that are important for host responses to injury. Tumor necrosis factor (TNF), interleukin (IL)-1α, and interferon (IFN)-γ are several cytokines that play important roles in these host responses.[7-10] Previous studies have focused on the serum kinetics of cytokines after intravenous administration of lipopolysaccharide (LPS), rather than live bacteria, to compare the influences of nutritional routes.[4,11] However, pneumonia and intraperitoneal abscess are the most common infectious complications observed in patients receiving total parenteral nutrition (TPN) after surgery,[12,13] thereby suggesting that live bacteria are more useful than LPS in clinical situations. In addition, earlier studies were designed to investigate the response of a single organ or tissue, even when live bacterial challenge was used.[14] To the best of our knowledge, the simultaneous influences of nutritional routes on local, systemic, and remote organ responses have not been examined. Therefore, we designed this study to investigate how antecedent nutritional route influences local, systemic, and remote organ immunologic responses after an intraperitoneal *Escherichia coli* challenge.

MATERIALS AND METHODS

Animals

Sixty-eight male Wistar rats (Japan SLC Company, Tokyo, Japan), age 10 to 12 weeks (250–350 g), were used. In accordance with our institutional guidelines, the rats suffered no unnecessary discomfort, pain, or injury and received proper care and maintenance. All rats were housed for at least 1 week before initiation of the experiments. They were exposed to constant temperature (24 C.) and humidity (60%) and were fed standard rat chow. After this stabilization period, they were randomly divided into TPN and total enteral nutrition (TEN) groups.

Surgical Procedure

Using general anesthesia by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight), we performed all procedures aseptically. Rats in the TEN group underwent upper midline laparotomy, and gastrostomy was made with a silicon rubber tube (Dow Corning Inc., 0.062-inch inner diameter/0.095-inch outer diameter, Midland, MI). A cut-down wound made in the right jugular area served as a sham operation. The TPN rats underwent implantation of silicon rubber catheters (Dow Corning Inc., 0.025-inch inner diameter/0.047-inch outer diameter) through the right jugular vein, and upper midline laparotomy was done as a sham operation. The catheter was tunneled to exit between the scapulae and was attached to a swivel-spring apparatus, which allowed unrestricted movement of the animals. After surgery, the rats were placed in individual metabolic cages.

Nutritional Fluid and Support

The nutritional contents are listed in Table 1. All formulations were administered under sterile conditions and included glucose, electrolytes (Paremental-B, Morishita-Seiyaku Co., Osaka, Japan), amino acids (Moripron-F, Morishita-Seiyaku Co., Osaka, Japan), and multivitamins (M.V.I.-SS, SS-Seiyaku Co., Tokyo, Japan). The nutritional fluid was delivered by continuous pump-controlled infusion to the TPN rats immediately after jugular vein cannulation. Nutritional fluid, diluted to one-third strength, was given on the first experimental day, two-thirds strength on the second day, and full strength (198 kcal/kg/day, 0.912 g nitrogen/kg/day) from the third day until the end of the experiment. The same nutritional fluid as that used in the TPN group at the same incremental strengths was given to the TEN group through a gastrostomy tube immediately after the rats had recovered from anesthesia. All oral intake, including water, was restricted.

Preparation of Bacteria

Approximately 40 hours before bacterial challenge, a culture of *E. coli* ATCC25922 (courtesy of Clinical Laboratory Department, University of Tokyo) was incubated in goat brain heart infusion broth in a 37 C. oscillating water bath for 18 hours. The culture infusion was centrifuged at 2000 rpm for 5 minutes, and the resulting pellets were washed with 0.9% sodium chloride. Twenty-four hours before bacterial challenge, the infusions were serially diluted and plated. Blood agar plates (Nissui-Sei-

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Presented in part at American Society of Parenteral and Enteral Nutrition's 19th Clinical Congress, Miami Beach, Florida, January 18, 1995; and at the 15th Annual Meeting of Surgical Infection Society; April 20, 1995; Louisville, KY.

Accepted for publication April 10, 1995.

Table 1. COMPOSITION OF NUTRITIONAL FORMULATION

Ingredients	Amount (1000 kcal/1100 mL)
Free amino acid (Moripron-F)	
Isoleucine	1.680 g
Leucine	3.750 g
Lysine	3.720 g
Methionine	1.050 g
Phenylalanine	2.805 g
Threonine	1.950 g
Tryptophan	0.390 g
Valine	1.350 g
Alanine	1.860 g
Arginine	2.370 g
Aspartic acid	0.760 g
Cysteine	0.300 g
Glutamic acid	1.950 g
Histidine	1.800 g
Proline	0.990 g
Serine	0.660 g
Tyrosine	0.105 g
Glycine	3.210 g
Carbohydrates (Paremental-B)	
Glucose	250.4 g
Minerals	
Sodium	50 mEq
Potassium	30 mEq
Magnesium	6 mEq
Chloride	14 mEq
Sulphate	6 mEq
Acetate	50 mEq
Phosphate	16 mM
Vitamins (M.V.I.-SS)	
A	10,000 IU
D_2	1,000 IU
E	5 mg
B_1	50 mg
B_2	10 mg
B_6	15 mg
Nicotinamide	100 mg
Biotine	25 mg
C	500 mg

yaku Co., Tokyo, Japan) consisting of blood agar base supplemented with 4% defibrinated horse blood were used to count viable bacteria. The plates for aerobic culture were incubated at 37 C. for 24 hours, and viable bacterial colony counts were determined. The bacterial infusion was then kept at 4 C. overnight. Adjustments were made to achieve a final concentration of 3×10^8 bacteria/mL.

Experimental Design

After 7 days of receiving either TPN or TEN, the rats were challenged intraperitoneally with 3×10^8 *E. coli.* Nineteen rats (TEN group, n = 10; TPN group, n = 9) were observed for survival. The remaining rats were killed just before challenge (0 hours) or 2 or 6 hours after bacterial challenge. At harvest, the abdomen was reopened aseptically. The peritoneal cavity was lavaged with 10 mL of normal saline. Peritoneal exudative cells (PECs) were harvested and cultured *in vitro.* The bronchoalveolar tree was lavaged three times with 10 mL of normal saline. Bronchoalveolar lavage cells (BALCs) were harvested and cultured *in vitro.* Colony-forming units of bacteria in peritoneal lavage fluid (PLF) were determined. TNF, Interleukin-1 α (IL-1α), interferon-γ (IFN) levels in serum, PLF, bronchoalveolar lavage fluid (BALF), and cell culture supernatant were measured as described below.

Culture of Peritoneal Exudative Cells and Bronchoalveolar Lavage Cells *In Vitro*

We centrifuged PLF and BALF at 1500 rpm for 10 minutes. The supernatant was stored for measurement of TNF, IL-1α, and IFN-γ. The cell sediments were washed with Hanks' balanced solution twice, and the erythrocytes were lysed with distilled water. The remaining PECs and BALCs were resuspended in RPMI (Nikken Biomedical Lab, Tokyo, Japan), supplemented with 5% fetal calf serum (Gibco, Grand Island, NY) and 1% antibiotic-antimycotic (Gibco, 10,000 units/mL Penicillin G, 10,000 μg/mL streptomycin sulfate, 25 μg/mL amphotericin B). The suspension containing 10^5 cells/mL RPMI was introduced to 24-well plates and then cultured with and without LPS (10 μg/well, from *E. coli* serotype O111:B4, Sigma, St. Louis, MO) in a sterile incubator at 37 C. with 5% carbon dioxide in air for 24 hours. After incubation, the supernatants were collected and stored at −80 C. for later measurement of TNF, IL-1α, and IFN-γ.

Assay of Tumor Necrosis Factor, Interleukin-1α, and Interferon-Gamma

A highly sensitive mouse fibrosarcoma cell line, L929, was used to measure TNF in serum, PLF, BALF, and the cell culture supernatant, as originally described by Espevik and Nissen-Meyer.[15] Threefold serial dilutions of the samples were tested, and the amounts of TNF were extrapolated from a standard curve using recombinant human TNF-α (Endogen Inc., Boston, MA). Interleukin-1α and IFN-γ levels were measured using commercially available enzyme-linked immunosorbent assay methods (IL-1α from Genzyme Immunobiologicals, Cambridge, MA; INF-γ from BioSource International, Camarillo, CA). For each assay, a standard curve using recombinant cytokine was constructed.

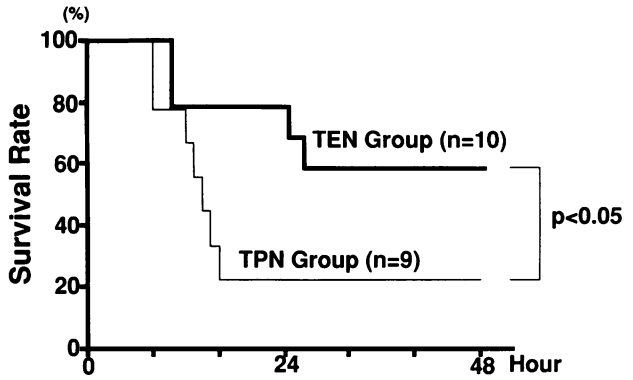

Figure 1. Survival rate after *E. coli* peritoneal challenge. Six of ten rats (60%) in the TEN group survived, whereas only two of nine rats (22%) in the TPN group survived for 48 hours after bacterial challenge. The survival rate was significantly higher among TEN rats than among TPN rats ($p < 0.05$).

Data Analysis

Data are expressed as mean plus or minus standard error of the mean. Analysis of variance followed by Duncan's new multiple range tests was used to compare means. Survival rates were analyzed using the generalized Wilcoxon test. Linear regression analysis was also conducted.

RESULTS

Survival Rate

There were six survivors among the ten rats (60%) in the TEN group, whereas only two of nine rats (22%) in the TPN group were still alive 48 hours after bacterial challenge. The survival rate was thus significantly higher for the TEN rats than for the TPN rats ($p < 0.05$) (Fig. 1).

Peritoneal Bacterial Colony Counts and Peritoneal Exudative Cells

No viable bacteria were detected in the peritoneal cavity in either the TPN or the TEN rats before challenge. Bacterial colony counts in PLF were significantly higher in the TPN group than in the TEN group at 2 and 6 hours after bacterial challenge. In addition, PLF bacterial colony counts in the TPN group but not in the TEN group rose significantly, from 2 to 6 hours (Table 2). The number of PECs increased significantly, from 0 to 2 hours in the TEN group. However, this increase of PECs was delayed for 6 hours in the TPN group. The number of PECs was significantly higher in the TEN group than in the TPN group at 2 hours (Table 2, Fig. 2A). The number of PECs showed a significant inverse correlation with the number of bacteria in PLF at 2 hours ($p < 0.05$, $r = -0.645$) (Fig. 3).

Local Cytokine Responses

Before bacterial challenge, TNF levels in PLF were significantly higher in the TPN group than in the TEN group. Tumor necrosis factor in PLF increased after bacterial challenge in both groups. The levels were higher in the TEN group than in the TPN group at 2 hours (Table 2). The TNF levels in PLF showed a significant positive correlation with the number of PECs in the TEN group ($r = 0.748$, $p < 0.01$), but no such correlation was seen in the TPN group at 2 hours (Fig. 4). Similar trends were observed in the local IL-1α response. The IL-1α level in PLF rose after bacterial challenge in both groups. The levels tended to be higher in the TEN group than in the TPN group 2 hours after challenge (Table 2). Interleukin-1α in PLF showed a significant positive correlation with the number of PECs in the TEN group ($p < 0.01$, $r = 0.572$), but no correlation was apparent in the TPN group at 2 hours. Interleukin-1α in PLF continued to increase in the TPN group, but decreased in the TEN group from 2 to 6 hours. The levels were significantly higher in the TPN group than in the TEN group at 6 hours (Table 2). Local (peritoneal) IFN-γ levels were nearly zero before challenge but rose after inoculation in both groups. Interferon-gamma levels in PLF were higher in the TEN group than in the TPN group after challenge. Interferon-gamma in PLF rose significantly from 0 to 6 hours in the TEN group (Table 2), but the increase was not significant in the TPN group.

In Vitro Production of Cytokines by Peritoneal Exudative Cells

In vitro production of TNF by PEC stimulated with LPS was significantly lower in the TPN group than in the TEN group before bacterial challenge (Table 2). This tendency persisted after bacterial challenge. In both groups, production of TNF by PEC stimulated with LPS decreased after bacterial challenge (Table 2). The *in vitro* production of IFN-γ by PEC showed a similar trend (Table 2).

Systemic Cytokine Responses

Serum TNF levels were extremely low before challenge but increased after bacterial inoculation in both groups. Serum TNF levels were higher in the TPN group than in the TEN group after challenge (Table 3) (Fig. 5A). Serum TNF showed inverse correlations with TNF levels in PLF ($p < 0.01$, $r = -0.43$) (Fig. 6) and with the number of PECs ($p < 0.05$, $r = -0.47$) at 2 hours. Serum

Table 2. LOCAL IMMUNE RESPONSES

	Total Enteral Nutrition			Total Parenteral Nutrition		
	0 Hrs	2 Hrs	6 Hrs	0 Hrs	2 Hrs	6 Hrs
PLF CFU-b ($\times 10^6$)	0.0 ± 0.0	9.5 ± 3.1	30.7 ± 13.9	0.0 ± 0.0	63.1 ± 18.0*	137.8 ± 58.9†‡
No. of PECs ($\times 10^6$)	2.5 ± 0.6	13.0 ± 3.3§	7.6 ± 2.1§	1.8 ± 0.6	2.6 ± 1.1*	8.9 ± 2.9†‖
PLF TNF ($\times 10^2$u/mL)	5.1 ± 1.3	53.0 ± 22.9	12.5 ± 5.9	14.8 ± 4.1§	25.0 ± 14.9	291 ± 269
PLF IL-1 (pg/mL)	13.0 ± 9.8	25.6 ± 10.7	19.9 ± 11.2	11.0 ± 10.1	12.6 ± 4.0	55.9 ± 18.8‡
PLF IFN (pg/mL)	0.0 ± 0.0	20.3 ± 10.1	140.8 ± 59.0*§	0.0 ± 0.0	5.2 ± 3.6	53.3 ± 30.7
In vitro						
PEC-TNF ($\times 10^2$ u/mL)	4.7 ± 1.8	1.4 ± 0.8§	7.5 ± 5.1	6.7 ± 1.4	1.2 ± 1.0‖	0.4 ± 0.3
PEC + TNF ($\times 10^2$u/mL)	70.8 ± 27.1	11.4 ± 6.0§	0.1 ± 0.06*	19.9 ± 5.2§	4.5 ± 2.1‖	0.3 ± 0.3†
PEC + IL-1 (pg/mL)	15.6 ± 7.7	7.7 ± 4.1	29.3 ± 10.7	24.4 ± 11.0	1.4 ± 1.0	22.0 ± 13.0
PEC + IFN (pg/mL)	166 ± 160	0.0 ± 0.0	7.1 ± 6.4	11.1 ± 9.8	0.0 ± 0.0	1.3 ± 0.9

Data are means ± SEM.
PLF = peritoneal lavage fluid; CFU = colony-forming unit; PECs = peritoneal exudative cells; TNF = tumor necrosis factor; IL-1 = interleukin-1; IFN = interferon.
PEC-TNF: TNF production by PEC without LPS; PEC + TNF: TNF production by PEC with LPS; PEC + IL-1, PEC + IFN: IL-1, IFN production by PEC with LPS.
* $p < 0.05$ *vs.* 2-hr TEN.
† $p < 0.05$ *vs.* 2-hr TPN.
‡ $p < 0.01$ *vs.* 6-hr TEN.
§ $p < 0.05$ *vs.* 0-hr TEN.
‖ $p < 0.05$ *vs.* 0-hr TPN.

IFN-γ levels were low before challenge. Bacterial challenge resulted in elevated production of systemic IFN-γ in both groups. Serum IFN-γ rose significantly from 0 to 6 hours in the TEN group, but the increase was not significant in the TPN group. The levels were significantly higher in the TEN group than in the TPN group at 6 hours (Table 3) (Fig. 5B). Serum IL-1α levels were very low both groups before and after bacterial challenge (Table 3).

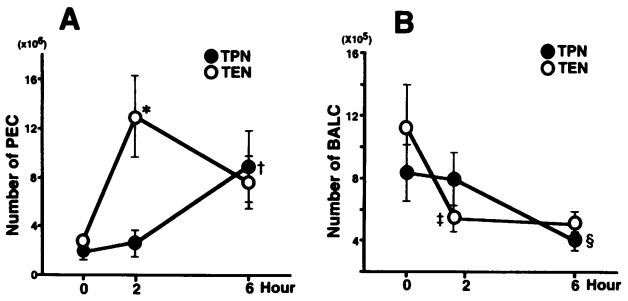

Figure 2. (A) Number of PECs. The number of PECs increased significantly from 0 to 2 hours in the TEN group. However, this increase was delayed for 6 hours in the TPN group. The number of PECs was significantly higher in the TEN group than in the TPN group at 2 hours (*$p < 0.05$ *vs.* 2-hour TPN and 0-hour TEN) (†$p < 0.05$ vs. 0-hour TPN). (B) Number of BALCs. In contrast to the number of PECs, the number of BALCs decreased significantly from 0 to 2 hours in the TEN group but not in the TPN group. The BALC decrease in the TPN group was delayed until 6 hours after challenge (‡$p < 0.05$ *vs.* 0-hour TEN, §$p < 0.05$ *vs.* 0-hour TPN). These results suggest that the mobilization of PECs and BALCs may be more effective in TEN-treated animals than in TPN-treated animals.

Remote Organ (Lung) Responses

The number of BALCs, TNF in BALF, and TNF production by BALCs without LPS declined significantly from 0 to 2 hours in the TEN group but not in the TPN group (Table 4) (Fig. 2B). The BALC decline in the TPN group was delayed for 6 hours after challenge. The number of BALC and TNF levels in BALF correlated significantly in the TEN group ($p < 0.05$, $r = 0.596$) but showed no correlation in the TPN group 2 hours after bacterial peritoneal challenge. Moreover, the number of PECs

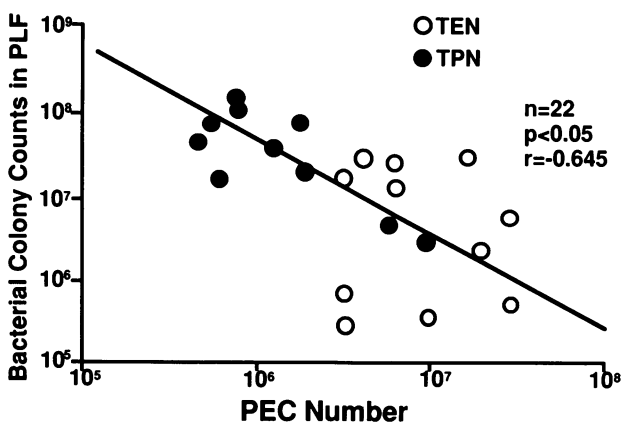

Figure 3. The number of PECs *versus* bacterial colony counts in PLF at 2 hours. The number of PEC showed a significant inverse correlation with the number of bacteria in PLF at 2 hours ($p < 0.05$, $r = -0.645$), suggesting that the number of PECs plays a key role in bacterial clearance in the early septic phase (2 hours).

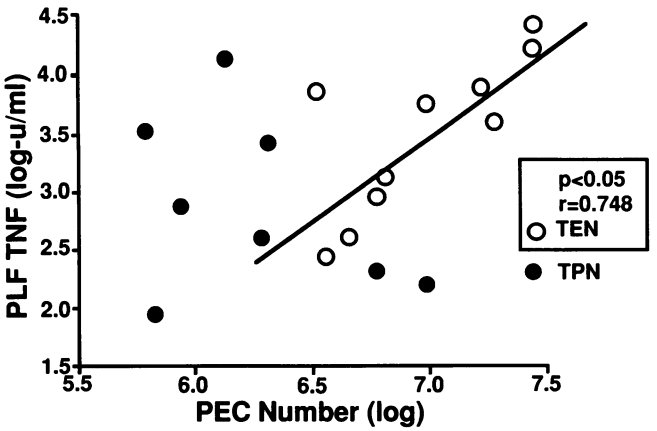

Figure 4. The number of PECs *versus* TNF levels in peritoneal lavage fluid (PLF). Tumor necrosis factor levels in PLF correlated significantly with the number of PECs in the TEN group, but no correlation was observed in the TPN group. This result suggests that the regulatory mechanism of cytokine production in PEC was lost in the TPN group.

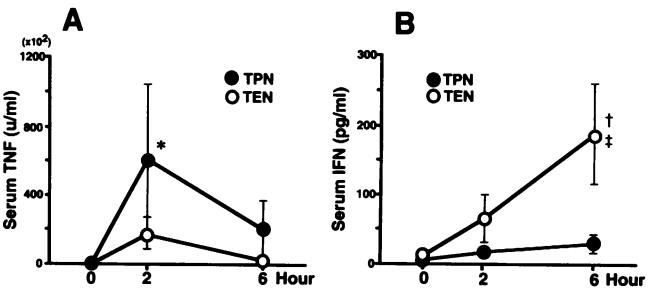

Figure 5. The antecedent nutritional route influences the systemic responses of different cytokines after insult. (A) Serum TNF levels were very low before challenge but rose after infection in both groups. Serum TNF levels were higher in the TPN group than in the TEN group after challenge (*$p < 0.08$ *vs.* 2-hour TEN). (B) Serum IFN-γ levels were extremely low before challenge. Bacterial challenge resulted in the increased production of systemic IFN-γ in both groups. Serum IFN-γ rose significantly from 0 to 6 hours in the TEN group, but the increase was not significant in the TPN group. The levels were significantly higher in the TEN group than in the TPN group at 6 hours (†$p < 0.05$ *vs.* 0.2-hour TEN, ‡$p < 0.05$ *vs.* 6-hour TPN). These results demonstrate distinct differences in systemic cytokine responses after bacterial intraperitoneal challenge between the TEN and TPN groups.

showed significant inverse correlations with the number of BALCs ($p < 0.05$, $r = -0.412$) as well as TNF levels in BALF ($p < 0.05$, $r = -0.511$) in the TEN group. No correlations were observed in the TPN group. In contrast, IFN-γ in BALF rose significantly from 0 to 2 hours in the TEN group. However, IFN-γ levels in BALF declined from 0 to 2 hours in the TPN group. The levels at 2 hours were significantly higher in the TEN group than in the TPN group. (Table 4).

DISCUSSION

In our model, TPN rats had a lower survival rate than TEN rats after intraperitoneal bacterial challenge. We showed that there were fewer PECs; defective cytokine production by PECs; impaired bacterial clearance; suppressed mobilization of immune cells from a remote organ, the lung; and disturbed systemic cytokine production in TPN rats.

Kudsk et al. reported an increased mortality rate after *E. coli* was injected intraperitoneally in parenterally fed rats compared with enterally fed rats.[14,16] The Veterans Administration Cooperative Study showed a significant increase in postoperative infectious complications in patients who received preoperative TPN compared with patients who received enteral nutrition.[13] Therefore, the antecedent nutritional route used may play an important role in host defense after surgical insult. The mechanisms underlying the influence of the antecedent nutritional route on host responses after insult, however, remain unknown.

Malnutrition and nutritional contents, such as specific amino acids (glutamine and arginine), lipids, and phos-

Table 3. SYSTEMIC CYTOKINE RESPONSES

	Total Enteral Nutrition			Total Parenteral Nutrition		
	0 Hrs.	2 Hrs.	6 Hrs.	0 Hrs.	2 Hrs.	6 Hrs.
Serum TNF ($\times 10^2$ u/mL)	0.5 ± 0.3	175.4 ± 93.2	21.8 ± 10.2	1.2 ± 1.0	603.1 ± 434.1*	204.6 ± 165.9
Serum IL-1 (pg/mL)	5.8 ± 2.1	3.4 ± 2.0	0.9 ± 0.4	3.4 ± 0.3	5.0 ± 1.9	2.3 ± 0.9
Serum IFN (pg/mL)	11.9 ± 8.3	67.3 ± 34.2	188.6 ± 72.5†‡	6.1 ± 5.1	18.4 ± 6.0	29.6 ± 13.4§

TNF = tumor necrosis factor; IL-1 = interleukin-1; IFN = interferon.
Data are means ± SEM.
* $p < 0.08$ *vs.* 2-hr TEN.
† $p < 0.05$ *vs.* 0-hr TEN.
‡ $p < 0.05$ *vs.* 2-hr TEN.
§ $p < 0.01$ *vs.* 6-hr TEN.

phate, may influence the host immune response.[17–20] However, we used exactly the same nutrients for both groups. Therefore, we believe that the route by which nutrition is supplied is in itself extremely important for the host responses that follow surgical insult. Total parenteral nutrition can cause hyperglycemia, thereby possibly impairing immune function.[21] However, hyperglycemia was not observed in a previous study in which rats received TPN for 1 week.[22]

There may be several reasons why the parenterally fed animals were susceptible to bacterial challenge. Our results showed that the number of PECs obtained 2 hours after *E. coli* challenge in the TEN group was significantly higher than in the TPN group and that the number of viable bacteria in the peritoneal cavity were significantly lower in the TEN group than in the TPN group. Moreover, there was a significant inverse correlation between number of PECs and viable bacterial counts in the peritoneal cavity. The increase in the number of PECs in the TPN group was delayed for 6 hours after challenge. These data indicate that the fewer PECs found in the early septic phase in TPN rats play a key role in delayed clearance of bacteria.

Peritoneal exudative cells include macrophage/monocytes, neutrophils, lymphocytes, and other inflammatory cells.[23] Although we did not differentiate the cellular components of PEC in our model, it has been shown that the majority of resident PECs are macrophages and that the majority of recruited cells in peritonitis are polymorphonuclear cells and macrophages from the spleen and bone marrow.[23–25] The gut is another possible source for these recruited immune cells, because gut-associated lymphoid tissues (GALT) constitute 50% of bodily lymphoid tissues.[26] In addition, our results suggest that immune cells in bronchus-associated lymphoid tissue may be another source of PEC.

An inhibitory effect of parenteral nutrition on recovery of neutrophil locomotory function in patients with blunt trauma has been reported.[27] Our study showed that the number of BALCs declined significantly from 0 to 2 hours in the TEN group only. This decrease in the number of BALCs in the TPN group was delayed for 6 hours. In addition, the number of PECs and BALCs showed a significant inverse correlation in the TEN group only. Bronchoalveolar lavage cells come from bronchus-associated lymphoid tissue. These immune cells can migrate and differentiate as they pass through the circulatory system or the lymphatic supply in mucosa-associated lymphoid tissues, including GALT and bronchus-associated lymphoid tissue.[28–31] To the best of our knowledge, there have been no reports concerning the influence of nutritional route on cell migration in mucosa-associated lymphoid tissues. However, we speculate that the migration of immune cells from bronchus-associated lymphoid tissue to sites of local inflammation is more effective in the TEN group than in the TPN group in the early septic phase (2 hours). Possibly, the use of the gut route beneficially affects the migration of immune cells from remote organs, not only from bone marrow and spleen but also from bronchus-associated lymphoid tissue (lung) and GALT (intestine), to the local inflammatory site.

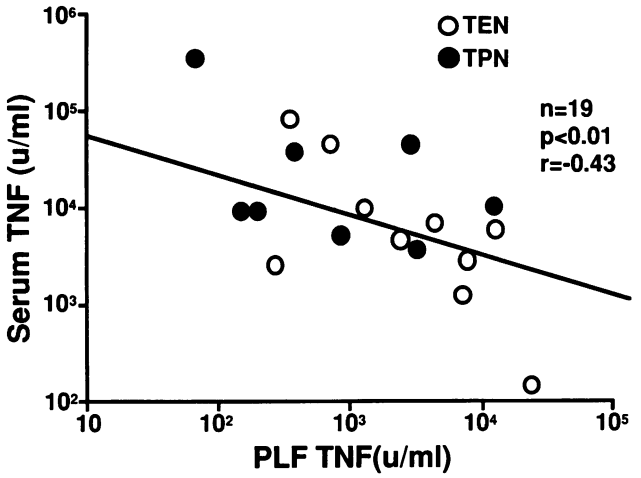

Figure 6. Tumor necrosis factor levels in PLF *versus* serum TNF levels 2 hours after bacterial challenge. Tumor necrosis factor levels in PLF and serum TNF levels showed a significant inverse correlation at 2 hours, suggesting that the local inflammatory site was not the major source of systemic TNF in the early septic phase. There are other potentially significant sources of systemic cytokines, such as liver Kupffer cells, lung macrophages, and immune cells in other organs.

Various cytokines, such as TNF, IL-1α, and IFN-γ, are important humoral factors in the mobilization of leukocytes.[32–34] We found that the level of cytokines (TNF and IL-1α) in PLF were higher in the TEN group than in the TPN group 2 hours after bacterial challenge. These results suggest that increased amounts of peritoneal cytokines exerted chemotactic effects on leukocytes in the TEN group. Moreover, IFN-γ levels in BALF were significantly higher in the TEN group than in the TPN group at 2 hours. The increased IFN-γ levels in TEN-group BALF may have facilitated the mobilization of immune cells from the lung to the peritoneal cavity.

We found it interesting that TNF levels in PLF in the TPN group before bacterial inoculation were significantly higher than those in the TEN group. Possible sources of peritoneal cytokines are immune cells in the peritoneal cavity and/or the gut wall. It has been suggested that TPN promotes not only bacterial translocation, but also translocation of bacterial products from the gut to the peritoneal cavity.[35–37] The bacteria or bacterial products translocated from the gut to the peritoneal cavity were able to stimulate PEC even before bacterial challenge. However, no living bacteria were detected in the peritoneal lavage fluid before bacterial

Table 4. REMOTE ORGAN (LUNG) RESPONSES

	Total Enteral Nutrition			Total Parenteral Nutrition		
	0 Hrs.	2 Hrs.	6 Hrs.	0 Hrs.	2 Hrs.	6 Hrs.
No. of BALCs ($\times 10^5$)	11.3 ± 2.7	5.4 ± 0.9*	5.0 ± 0.7*	8.4 ± 1.8	8.0 ± 1.7	4.0 ± 0.6†‡
BALF TNF ($\times 10^2$ u/ml)	4.4 ± 1.6	0.28 ± 0.13*	10.8 ± 8.6	1.56 ± 0.77	1.53 ± 0.80	8.0 ± 4.4
BALF IL-1 (pg/ml)	2.3 ± 0.8	5.1 ± 3.9	5.0 ± 3.3	6.1 ± 2.9	11.0 ± 8.8	0.5 ± 0.5
BALF IFN (pg/ml)	2.7 ± 0.9	27.3 ± 18.0*§	0.2 ± 0.2	11.4 ± 9.6	0.0 ± 0.0‖	4.0 ± 1.8
In vitro						
BALC − TNF ($\times 10^2$ u/ml)	21.7 ± 4.9	0.62 ± 0.33*	4.6 ± 3.2	20.7 ± 11.1	2.7 ± 1.7	24.5 ± 21.3
BALC + TNF ($\times 10^2$ u/ml)	222 ± 42.5	505 ± 266	1065 ± 246*	176 ± 46	394 ± 210	549 ± 179

BALCs = bronchoalveolar cells; BALF = bronchoalveolar lavage fluid; TNF = tumor necrosis factor; IL-1 = interleukin-1; IFN = interferon.
Data are means ± SEM.
BALC-TNF: TNF production by BALC without LPS; BALC + TNF: TNF production by BALC with LPS.
*$p < 0.05$ *vs.* 0-hr TEN.
† $p < 0.05$ *vs.* 0-hr TPN.
‡ $p < 0.05$ *vs.* 2-hr TPN.
§ $p < 0.05$ *vs.* 6-hr TEN.
‖ $p < 0.05$ *vs.* 2-hr TEN.

challenge. Therefore, translocated bacterial products such as endotoxin may stimulate resident peritoneal cells rather than the bacteria themselves. Another possible source of the increased amount of peritoneal TNF in the TPN group before bacterial inoculation is immune cells in the GALT. These cells include macrophages, lymphocytes, and mast cells as well as Paneth and M cells. The cells in GALT are able to produce a variety of cytokines.[38,39] Possibly, a portion of the peritoneal TNF present before bacterial inoculation in TPN rats comes from these GALT cells.

Our data demonstrate that LPS-stimulated production of TNF by PEC was significantly diminished in the TPN group before bacterial challenge. Additionally, a significant positive correlation between the number of PECs and the amount of peritoneal cytokines (TNF and IL-1α) existed only in the TEN group at 2 hours. Furthermore, local IFN-γ levels rose significantly from 0 to 6 hours in the TEN group but not in the TPN group. These data indicate that local production of cytokines was well regulated in the TEN group but not in the TPN group. Previous investigation showed that the TNF-binding capacity of neutrophil was lost when incubated with agents stimulating migratory and secretory responses.[40] Loss of TNF receptors from PEC due to stimulation by prechallenged peritoneal TNF may have decreased their responsiveness to postchallenged peritoneal TNF in our TPN rats.

Our results also indicate that there are differences in systemic cytokine responses between the two groups after bacterial challenge. Serum TNF levels after bacterial challenge were higher in the TPN group than in the TEN group. The depressed local bacterial clearance in the TPN group may lead to systemic spread of bacteria, which may ultimately initiate greater systemic production of TNF. Another reason for higher serum TNF was altered gut–liver interaction in the TPN group. Parenteral nutrition may prime splanchnic reticuloendothelial cells due to the translocated bacteria and/or endotoxin, leading to exaggerated production of systemic TNF and stress hormones after subsequent LPS administration.[4]

In contrast, serum IFN-γ levels were lower in the TPN group than in the TEN group after bacterial intraperitoneal challenge. Interferon-gamma is a lymphokine produced by T cells in response to bacteria, viruses, and other antigenic stimuli. The spleen was reported to be the major organ expressing mRNA of IFN-γ after *E. coli* challenge had been administered intravenously in rats.[41] The defect in IFN-γ production existed not only in the systemic circulation, but also at the local inflammatory site (peritoneum) and in the remote organ (lung) examined in our TPN rats. To the best of our knowledge, no previous investigations have been made regarding the influence of nutritional route on production of IFN-γ. We believe that T-cell dysfunction occurs in TPN-treated animals. Nutrition through the enteral route may play a role in T-cell differentiation and GALT function.[42] Further study must be done to elucidate the mechanisms of T-cell dysfunction in parenteral nutrition. Whatever the underlying mechanisms, exaggerated production of systemic TNF may induce multiple organ failure and thus be fatal to TPN rats.[7] In contrast, increased systemic IFN-γ levels may protect TEN rats from infection and thereby effect better survival.[43,44]

Taken together, these observations suggest mechanisms that may be responsible for the better host re-

sponses to intraperitoneal bacterial challenge seen with enteral nutrition. Local factors include more PECs, increased PEC cytokine production, and more favorable regulation of PEC TNF production. These will result in more effective bacterial killing and reduced systemic spread of bacteria. In addition, immune cells in remote organs can be mobilized to the peritoneal cavity more effectively. Systemically, TNF production is diminished but IFN-γ production is enhanced after bacterial challenge. These advantageous biologic responses may lead to fewer deaths in association with enteral nutrition.

The results that we obtained with our model may also account for the advantages of immediate enteral feeding after surgical stress. Immediate enteral nutrition decreases infectious complications, especially pneumonia and intra-abdominal abscess, in trauma patients.[13,45,46] Therefore, enteral nutrition before or after surgical insult may enhance immunity. On the basis of our findings, we recommend the use of enteral nutrition over parenteral nutrition, when feasible, for critically ill patients.

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