Route of Nutrition Influences Intercellular Adhesion Molecule-1 Expression and Neutrophil Accumulation in Intestine

Kazuhiro Fukatsu, MD, PhD; Andrew H. Lundberg, MD; M. Keith Hanna, MD; Yong Wu, MD; Henry G. Wilcox, PhD; D. Neil Granger, PhD; A. Osama Gaber, MD; Kenneth A. Kudsk, MD

Hypothesis: The levels of intestinal interleukin 10 and interleukin 4, inhibitors of intercellular adhesion molecule-1 (ICAM-1) expression, decline with total parenteral nutrition (TPN). These cytokine changes induced by lack of enteral nutrition may increase ICAM-1 expression, resulting in polymorphonuclear neutrophil accumulation in intestine.

Design: Prospective randomized experimental trials.

Setting: Laboratory.

Materials: Male mice.

Interventions: Sixty-three mice were randomized to chow, intravenous TPN, or intragastric TPN.

Main Outcome Measures: Experiment 1: After diet manipulation, iodine 125–labeled anti–ICAM-1 antibody and iodine 131–labeled nonbinding antibody were injected to quantify ICAM-1 expression on endothelial cells in the lung, liver, kidney, and small intestine. Measurement of myeloperoxidase was used to quantify polymorphonuclear neutrophil accumulation in the organs. Experiment 2: Intestine was harvested for both ICAM-1 and myeloperoxidase levels after chow refeeding of mice in the intravenous TPN group.

Results: In experiment 1, uninjured mice fed intravenous TPN showed significantly increased intestinal ICAM-1 expression and polymorphonuclear neutrophil accumulation with no significant changes in the lung, liver, or kidney. No changes occurred in mice fed chow or intragastric TPN. In experiment 2, reinstitution of enteral feeding returned intestinal ICAM-1 and myeloperoxidase levels to normal.

Conclusion: Gut changes associated with lack of enteral feeding induce endothelial changes and an immunologic response, which may influence subsequent responses to injury.


Experimentally, lack of enteral feeding produces mucosal atrophy, reduces gut immunity, alters gut cytokine levels, and increases intestinal permeability. Clinically, septic morbidity is higher in posttrauma patients receiving total parenteral nutrition (TPN) than in those fed enteral nutrition. To date, local inflammatory responses induced by lack of enteral feeding have not been scrutinized experimentally.

Polymorphonuclear neutrophils (PMNs) are the major effector of the non-specific immune response in host resistance to infection. Regulated adhesion, stimulation, and migration of PMNs through endothelial cells are essential steps in acute inflammation, with the PMN itself contributing to tissue injury by releasing enzymes or toxic oxygen products. Intercellular adhesion molecule-1 (ICAM-1) expressed by endothelium, the ligand counterpart of CD11/18 integrins on PMN, is believed to cause firm adhesion between the endothelial cell and the PMN. Through PMN-endothelium interaction via ICAM-1, PMNs are activated, transmigrate into interstitium, and cause tissue injury. Since derangement of PMN-endothelium interaction and PMN accumulation causes susceptibility to bacterial infection or PMN-mediated tissue injury, the investigation of ICAM-1 expression and myeloperoxidase activity in organs may provide information that is clinically useful for managing critically ill patients.

Our recent work noted that intravenous TPN (IV TPN) alters the balance of gut cytokine levels. While interferon γ, a potent stimulant of ICAM-1 expression, remained unchanged with IV TPN, interleukin (IL) 10 and IL-4 levels declined rapidly. Both IL-10 and IL-4 are important inhibitors of ICAM-1 expression.
MATERIALS AND METHODS

ANIMALS

All experimental protocols were approved by the Animal Care and Use Committee of The University of Tennessee, Memphis. Male ICR (Institute of Cancer Research) mice (Harlan, Indianapolis, Ind) were housed in an American Association for Accreditation of Laboratory Animal Care–accredited conventional facility. The environment had controlled temperature and humidity with a 12-hour light:dark cycle. Mice were fed ad libitum chow (RMLH3200 Agway, Syracuse, NY) and water for 2 weeks prior to study protocol. During feeding protocols, mice were housed in metal metabolism cages with wire grid floors to eliminate coprophagia.

FEEDING PROTOCOL

Experiment 1: Effects of Nutrition on ICAM-1 Expression and Myeloperoxidase Levels in Organs

Forty-eight mice (6-8 weeks old) were randomized to receive chow (n = 16), IV TPN (n = 15), or intragastric (IG) TPN (n = 17). Mice randomized to the chow and IV TPN groups received chow (n = 16), IV TPN (n = 15), or intragastric (IG) TPN (n = 17). Mice randomized to the chow and IV TPN groups received internal jugular catheters with ketamine hydrochloride (100 mg/kg of body weight) and acepromazine maleate (10 mg/kg of body weight) anesthesia as previously described.2,4 Catheterized mice received 9.0% isotonic sodium chloride solution at 4 mL/d for 48 hours with ad libitum access to chow and water. On postoperative day 2, mice received their respective feeds. Chow-fed animals received 4 mL of 0.9% isotonic sodium chloride solution along with ad libitum chow and water. The IV TPN– and IG TPN–fed animals initially received 4 mL/d of TPN and were advanced to a goal rate of 10 mL/d by the third day of feeding. The TPN solution contained 4.1% amino acids, 34.3% glucose (4878 kJ/L), electrolytes, and multivitamins with a nonprotein energy-to-nitrogen ratio of 743 kJ/g nitrogen, meeting the calculated daily nutritional requirements of mice (1951 kJ/kg of nonprotein energy and 16.4 g of protein/kg).

After receiving diets for 5 days, 24 animals were used for quantification of ICAM-1 expression in the lungs, liver, kidney, and small intestine, while 24 were used for measurement of myeloperoxidase to assess PMN accumulation in the organs.

Experiment 2: Effects of Reinstitution of Enteral Feeding on Intestinal ICAM-1 Expression and Myeloperoxidase Levels

Fifteen mice were divided into chow (n = 6) and IV TPN (n = 9) groups. After receiving diets for 5 days, animals were refed chow for 4 days with continuous infusion of 4 mL/d of 0.9% isotonic sodium chloride solution. Then, ICAM-1 expression and myeloperoxidase levels were measured in the small intestine.

QUANTIFICATION OF ICAM-1 EXPRESSION

Monoclonal Antibody

The monoclonal antibodies (mAbs) used for the assessment of ICAM-1 expression were as follows: 3E2, a hamster IgG1 directed against mouse ICAM-1; and A19-3, a nonbinding hamster IgG1 control Ab (PharMingen Inc, San Diego, Calif).

Radioiodination of the mAbs

The binding mAb 3E2 and the nonbinding mAb A19-3 were radioiodlated with iodine 125 and iodine 131, respectively, using the iodogen method. Briefly, 250 µg of protein was incubated with 9.25 MBq of sodium iodine 125 (or sodium iodine 131) and 125 µg of iodine (1,3,4,6-tetrachloro-3a,6a-Di-phenylglycoursil; Sigma-Aldrich Corp, St Louis, Mo) at 4°C for 12 minutes. Phosphate-buffered saline was then added to bring the total volume to 2.5 mL. The radioiodlated mAb was then separated from free125I (or 131I) by gel filtration on a Sephadex PD-10 column (Pharmacia LKB, Upppsala, Sweden). Phosphate-buffered saline with 1% bovine serum albumin was used to equilibrate the column and to elute the radioiodlated Ab. Two 2.5-mL fractions were collected, with the second fraction containing the labeled mAb. Radiolabeled mAbs were stored at 4°C.

Animal Ab Infusion Procedures

The mice were anesthetized with ketamine and acepromazine maleate. The left jugular vein and the right carotid artery were cannulated with a silicone rubber catheter and a polyethylene tubing (PE10; Becton Dickinson, Sparks, Md). A mixture of 10 µg of 125I-labeled anti–ICAM-1 mAb (3E2) and 40 µg of unlabeled anti–ICAM-1 mAb was given with an appropriate amount of 131I-labeled A19-3 (400 000–600 000 cpm) through the jugular vein catheter (total volume, 200 µL). 125I-labeled binding mAb that is directed against ICAM-1 was injected to evaluate ICAM-1 expression on the endothelial cells, while 131I-labeled nonbinding mAb was given to eliminate the influence of nonspecific binding of anti–ICAM-1 Ab to endothelial cells. Unlabeled anti–ICAM-1 mAb was administered to saturate all receptors on the endothelial cells. Pilot data showed that the combination of 10 µg of 125I 3E2 and 40 µg of cold 3E2 provided optimum activity to accurately assess ICAM-1 expression and to ensure receptor saturation. A blood sample was obtained through the carotid artery catheter 5 minutes after injection of the mAb mixture. Then, the animals were heparinized (40 U of sodium heparin) and rapidly exsanguinated by perfusion of bicarbonate-buffered saline through the jugular vein catheter with simultaneous blood withdrawal through the carotid artery catheter. This was followed by perfusion of 15 mL of bicarbonate-buffered saline through the carotid artery catheter after severing the inferior vena cava at the thoracic level. Thus, after removing all mAbs that did not bind to the endothelial cells, the lungs, liver, kidney, and small intestine were harvested and weighed.

Continued on next page
We speculated that the route of nutrition may influence ICAM-1 expression and PMN accumulation in organs and sought to evaluate the influences of nutrition on ICAM-1 expression and on PMN sequestration in organs.

RESULTS

EXPERIMENT 1

Body Weight Change

The preexperiment weights of all groups were similar (Table 1). The mice fed IV TPN and IG TPN lost body weight more than chow-fed mice (P<.05), but there were no differences between IV TPN and IG TPN groups.

Influence of Nutrition on ICAM-1 Expression in Organs

Intestinal ICAM-1 expression was significantly higher in the IV TPN group than in the chow and IG TPN groups, with no differences between chow and IG TPN groups (Figure 1). Lung and kidney ICAM-1 expression also tended to be higher in the IV TPN group, but did not reach statistical significance. No differences were observed in the liver among the 3 groups.

Influence of Nutrition on Tissue Myeloperoxidase Activity

Mice fed IV TPN showed significantly higher intestinal myeloperoxidase activity levels than those fed chow or IG TPN (Table 2), with no significant changes in other organs.

Wet-to-Dry Lung Weight Ratios

There were no differences in the wet-to-dry lung ratios between any groups (chow, 5.426 ± 0.113; IV TPN, 5.447 ± 0.108; IG TPN, 5.623 ± 0.207).

EXPERIMENT 2

After refeeding with chow for 4 days, there were no significant differences in ICAM-1 expression or myeloperoxidase levels of small intestine between chow and IV TPN groups (Figure 2).

CALCULATION OF ICAM-1 EXPRESSION

A counting system (Cobra Automated Gamma Counting System; Packard Instrument, Meriden, Conn) was used to count 125I (binding mAb) and 131I (nonbinding mAb) activities in each organ and in a 50-µL plasma sample. A 2-µL aliquot of the preinjection mixture of radiolabeled mAbs was measured to determine total injected activity of each labeled mAb. The amount of radioactivity remaining in the tube used to mix the mAbs and the syringe used to inject the mAb mixture was subtracted from the total calculated injected activity. Expression of ICAM-1 was determined by subtracting the accumulated activity of the nonbinding mAb from that of binding mAb, and expressed as micrograms of mAb per gram of tissue (µg of mAb/g tissue) in the following equation:

\[
\text{ICAM-1 Expression (µg mAb/g Tissue)} = \left( \frac{125\text{I cpm/g}}{125\text{I cpm Injected}} - \frac{131\text{I cpm/g}}{131\text{I cpm Injected}} \right) \times \left( \frac{125\text{I cpm/50 µL of Serum}}{125\text{I cpm Injected}} \right) \times \left( \frac{131\text{I cpm/50 µL of Serum}}{131\text{I cpm Injected}} \right) \times \text{Total Injected Binding mAb (µg)}.
\]

MEASUREMENT OF MYELOPEROXIDASE ACTIVITY

Tissue myeloperoxidase activity was determined in the lungs, liver, kidney, and small intestine. The organs were harvested, rinsed with ice-cold phosphate-buffered saline, blotted dry, weighed, and frozen at −80°C. The samples were thawed and homogenized in 10 volumes of 0.45% isotonic sodium chloride solution. The homogenate of the lungs and intestine was centrifuged at 20 000g for 15 minutes at 4°C. The supernatant was then discarded and the pellet was homogenized with 10 volumes of phosphate-buffered solution (pH 6.0) containing 0.3% hexadecyl trimethyl ammonium bromide (Sigma-Aldrich, St Louis, Mo). Samples were freeze-thawed (for 20 minutes at −80°C) and sonicated, followed by centrifugation at 20 000g for 15 minutes at 4°C. The supernatant myeloperoxidase activity was assayed by measuring the hydrogen peroxide–dependent oxidation of 3,3′,5,5′-tetramethylbenzidine. The absorbance was measured at 655 nm (model LS50B; Perkin-Elmer, Oak Brook, Ill). Tissue myeloperoxidase activity was expressed as activity units per gram of wet weight. Liver and kidney myeloperoxidase activities were measured in the same manner except that these tissues were incubated at 60°C for 2 hours after homogenization in 0.45% sodium chloride solution to eliminate factors that can interfere with the assay.18,19

In experiment 2, part of the small intestine was obtained from the middle of the proximal intestine and the middle of the distal intestine after perfusion. Then, myeloperoxidase activity was determined as described above.
Enteral feeding reduces septic morbidity of critically ill patients. Lack of enteral nutrition increases intestinal permeability; alters metabolic, hormonal, and pro-inflammatory cytokine responses to subsequent injury; and alters intestinal mucosal immunity. Lack of enteral feeding also influences the inflammatory responses within the intestinal tract.

Our preliminary data demonstrated an increase in myeloperoxidase level within the gastrointestinal tract of IV-fed rats, suggesting localization of PMNs in a setting of increased permeability and altered gut cytokine profile. Our prior work in mice demonstrated that lack of enteral feeding reduced gut-associated lymphoid tissue mass as well as intestinal and respiratory tract IgA levels and reduced the Th2-IgA–stimulating cytokines IL-4 and IL-10 within the intestine. Since IL-4 and IL-10 inhibit ICAM-1 expression on endothelial cells, the present research accomplished 2 objectives. First, it confirmed the increase in myeloperoxidase accumulation with lack of enteral feeding in mice, and second, it confirmed expression of the adhesion molecule, ICAM-1, in conjunction with this PMN accumulation.

Until recently, investigators struggled with accurate quantification of adhesion molecule expression on intact endothelial cells. Immunohistochemical staining can evaluate adhesion molecule expression in vivo, but quantification is difficult. Measurement of adhesion molecule messenger RNA generates only indirect evidence for changes in adhesion molecule expression. A dual radiolabeled monoclonal antibody technique, based on the use of radiolabeled binding and nonbinding mAbs, enabled quantification of endothelial cell surface adhesion molecule expression accurately in vivo. This technique has yielded novel information about the kinetics and magnitude of ICAM-1 expression on endothelial cells.

In this study, mice were randomized to chow, IV TPN, or IG TPN feeding with no additional insults.
Enteral delivery of nutrients significantly reduces septic morbidity, particularly pneumonia, in critically ill and critically injured patients. Previous investigators have noted that neutrophils are primed by the gastrointestinal tract and exacerbate pulmonary injury to a subsequent insult. The present work strengthens the link between the gastrointestinal tract and pulmonary dysfunction in critical illness by demonstrating that lack of enteral nutrition up-regulates adhesion molecule expression in the intestinal tract, attracting neutrophils to the intestinal vascular bed. Enteral nutrition appears to prevent inflammation within the intestinal tract and maintain normal intestinal and extraintestinal barrier integrity.

ing with IG TPN served as an isoenergetic, isonitrogenous enteral control diet. Expression of intestinal ICAM-1 increased along with myeloperoxidase in the IV TPN–fed group. Expression of ICAM-1 is regulated by various proinflammatory and antiinflammatory cytokines, 25–28 several of which are affected by route and type of nutrition. Interferon γ increases whereas IL-4 and IL-10 inhibit ICAM-1 expression. 26–28 Feeding with IV TPN decreases intestinal IL-4 and IL-10 levels with no changes in interferon γ compared with chow, while IG TPN produces moderate decreases in IL-4 but no change in IL-10 or interferon γ. Whether these cytokines alone were the primary stimulus for ICAM-1 expression on endothelial cells in vivo or whether there were additional influential factors, such as increases in gut permeability, is unclear, but endothelial up-regulation of ICAM-1 was isolated to the intestine alone with no other significant changes in the lung, liver, or kidney. These intestinal alterations are readily reversible with the reinstitution of enteral feeding.

These observations may have important implications. Moore et al 3 showed that postischemic gut serves as a priming bed for circulating PMNs. Polymorphonuclear neutrophils accumulate and are primed by various inflammatory mediators released from the postischemic tissue. Since lack of enteral nutrition increases gut permeability and the proinflammatory cytokines, tumor necrosis factor α, IL-1, and IL-6, within the rat intestine, 29 it is possible that the accumulated PMNs are stimulated and primed in situ, however, we have no evidence that IV TPN increases levels of IL-1, IL-6, or tumor necrosis factor α or primes the neutrophil in our model. Although it is unknown whether gut increases in ICAM-1 expression and PMNs are beneficial or detrimental to the host administered IV feeding, subsequent responses to injury may be altered through these changes.

In summary, uninjured mice fed IV TPN significantly increased intestinal ICAM-1 expression and PMN accumulation without changes in the lung, liver, or kidney. Changes were rapidly reversible with reinstitution of chow feeding. These results imply that lack of enteral feeding induces endothelial changes and an immunologic response that may influence a subsequent response to injury or inflammation. Changes occur in association with a cytokine profile characterized by a reduction in cytokines inhibitory to ICAM-1 expression but no change in the cytokine interferon γ, which is known to up-regulate ICAM-1 expression. One must be suspicious of changes in permeability and alterations in intestinal cytokines as important modifiers of adhesion molecule expression.

Supported by grant 5 R01 GM53439 from the National Institutes of Health, Bethesda, Md.


We thank Doris Parson for help in preparing and editing the manuscript.

Reprints: Kenneth A. Kudsk, MD, 956 Court Ave, Suite E228, Memphis, TN (e-mail: dparsons@utmem.edu).

REFERENCES


DISCUSSION

Cora K. Ogle, MD, Cincinnati, Ohio: This is a well-written manuscript again by this group who has been investigating the effects of TPN on the immune system. In the introduction in the manuscript, the authors indicate that interferon gamma was not changed, which was a little disappointing to me, because we have a poster, poster 21 of Dr Valente’s work, that interferon gamma stabilizes the message for IL-8, but nevertheless, we have shown that IL-8 can be increased in Caco cells with IL-6 stimulation.

Your second experiment, where you have shown that you can ameliorate the effects of TPN, was very interesting and very clinically applicable. I have 2 questions for you. Do you think you would obtain the same results if you had used a compromised animal, a sepsis, or burn, or hemorrhage, or a trauma model? And the second question, what role do you think the intraepithelial lymphocytes are playing? Do you think it is the gut cells that are making the cytokines or that the intraepithelial lymphocytes are being modulated?

Dr Fukatsu: In this study, we used uninjured mice fed with IV TPN or chow or IG TPN. We are now studying injured mice using LPS [lipopolysaccharide]. LPS does not increase ICAM-1 expression, and we cannot detect any difference between the groups. We are now using an ischemia reperfusion injury model, and hopefully I can report these data in the near future.

With regard to the second question, intraepithelial lymphocyte cells may produce proinflammatory cytokines, and many papers report that IL-1 or TNF-alpha cause an increased expression of ICAM-1 and other adhesion molecules. We have focused on the Ig production and did not measure intestinal IL-1 or TNF levels since they do not stimulate IgA production. Since now we have increased expression of ICAM-1 in the IV TPN-fed mice, we are now trying to evaluate those cytokine levels.

As for IL-6, we have already measured those cytokine levels, but note no difference among these groups. In this model, chow and IG TPN served as control groups to identify complications associated with catheterization. IG TPN studies the influence of nutritional route on ICAM-1 expression.

Edwin A. Deitch, Newark, NJ: Since Dr Kudsk was kind enough to discuss my paper, I thought I would give him the favor of returning the favor.

First, I agree with what you see. About 10 years ago we published a paper that was presented at the SUS showing that treating animals with the same models you used caused a nutritionally associated impairment of the immune response. In this study, we looked at all the lymphoid compartments in terms of circulating lymphocytes, spleen, mesenteric lymph node, and GALT [gut-associated lymphoid tissue] lymphocytes as well as neutrophil function. We found that after refeeding the animals, they got better.

Considering we think the gut is important and feeding through the gut, we then did another series of experiments where we changed the gut flora, and just causing bacterial overgrowth will in fact modulate the immune response. In fact, what Dr Magnott showed earlier was that just by increasing the gut flora, you in fact change the entire ability of the animal to respond.

So the question I am going to ask you relates to the mechanism. When you give animals oral or IV TPN, you know as well as I do that the gut flora changes and the level of enterics increases by 2 to 3 to 4 logs. You are now in a situation where bacterial translocation occurs in most of the animals, or at least the bacteria crossed the mucosal barrier. You now have stimulus within the mucosa. That stimulus in fact can set up a number of factors.

So my question is, if you reduce the gut flora or if you use an animal that the gut flora is not allowed to increase, will you see the same changes in the immune system locally and in induction of the various adhesion molecules, and if you don’t, clearly that could implicating the gut crossing as a trigger even though it causes no infection?

Dr Fukatsu: So your question is the effect of intestinal flora, am I right?

Dr Deitch: You are right. Do you think the intestinal flora changes could be causing this? Have you done any experiments to lower the intestinal flora or to control for that variable between the groups?

Dr Fukatsu: It is possible that the changes of intestinal bacteria flora may affect ICAM-1 or other adhesion molecule expression. And so far we don’t have any data about this issue, but I think it is a very nice, good idea.

Cathy L. White-Owen, MD, Cleveland, Ohio: This may be a little bit of a simplistic question, but if I read your abstract correctly, you have very small amounts of ICAM in the small intestine—actually, significantly lower levels in the small intestine than any other tissue you looked at, but dramatically increased levels of myeloperoxidase. Is it possible that what you have done is maybe look at changes that are occurring in the circulating neutrophils as opposed to changes that are occurring in the endothelium?

Did you look at CD11b expression in your neutrophils following changes, differences, in your feeding patterns? And did you look at any other adhesion molecules that may be present in higher concentrations in this small intestine that are not as significantly different in other tissues?

Dr Fukatsu: This is a very, very important question. We have not yet studied the changes in neutrophil function. The neutrophils in the peripheral blood and the neutrophils in the vascular bed or small intestine, these may differ from each other.

Our next step is to evaluate these neutrophil changes using flow cytometry or immunohistochemical staining technique.

Avery B. Nathens, MD, Seattle, Wash: My question relates to your control group. You mentioned that they are ener- terally fed. Did they also have central venous access like the TPN group? And if not, how do you know that your effects aren’t due to the excessive handling of the TPN-fed animals?

Dr Fukatsu: In our model, chow animals were also included with jugular vein catheters, and these animals received 4 mL of normal saline per day.