Increased Expression of Intestinal P-Selectin and Pulmonary E-Selectin During Intravenous Total Parenteral Nutrition

Kazuhiko Fukatsu, MD; 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: Intravenous total parenteral nutrition (TPN) induces intestinal polymorphonuclear neutrophil recruitment with increased intestinal intercellular adhesion molecule-1 expression. While intercellular adhesion molecule-1 causes firm adhesion of leukocytes to the endothelial cells, P- and E-selectin mediate leukocyte recruitment via rolling. Therefore, manipulation of nutrition may also affect P- and E-selectin expression in organs.

Design: Prospective randomized experimental trials.

Setting: Laboratory.

Materials: Male mice.

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

Main Outcome Measures: After 5 days of diet, mice were administered iodine 125-labeled anti–P-selectin antibody (or iodine 125-labeled anti–E-selectin antibody) and iodine 131-labeled nonbinding antibody to quantify P-selectin (or E-selectin) expression in organs (lung, liver, kidney, small intestine, colon, stomach, pancreas, mesentery, heart, and skeletal muscle).

Results: P-selectin in small intestine, colon, stomach, and pancreas in the intravenous TPN group increased significantly as compared with the chow and the intragastric TPN groups. E-selectin expression was upregulated after intravenous TPN in the lung but not in other sites.

Conclusions: In a time frame (5 days) when intercellular adhesion molecule-1 expression and neutrophil recruitment are increased, intestinal expression of P-selectin remains up-regulated. Early lung inflammatory changes are reflected by increases in E-selectin. This change may reflect early pulmonary dysfunction with intravenous TPN, but its significance requires further study.


Enteral feeding of critically ill or injured patients increases resistance to infection, particularly pneumonia.1-3 Our laboratory has focused on IgA-mediated mucosal immunity as a mechanism for this clinical issue, showing that lack of enteral stimulation causes changes in gut-associated lymphoid tissue and loss of respiratory tract immunity.4-6 Recently, we showed that lack of enteral feeding increased both intestinal intercellular adhesion molecule-1 (ICAM-1) expression and gut polymorphonuclear neutrophil (PMN) accumulation.7 The ICAM-1 expressed on endothelial cells is the ligand counterpart of CD11/18 on PMNs. Expression leads to firm adhesion of PMNs to the surface of the endothelium.8 Since the vascular bed of gastrointestinal tract primes PMNs and exacerbates pulmonary injury to a subsequent insult,9 increased ICAM-1 expression and PMN accumulation in gut during intravenous (IV) total parenteral nutrition (TPN) feeding may unfavorably influence the response to injury or infection.

In addition to ICAM-1, the selectin family of adhesion molecules influences PMN–endothelial cell interaction.8,10-12 The selectins designated L-, P-, and E-selectin are a family of adhesive receptors sharing a common structure of an amniterminal C-type lectin domain, a single epidermal growth factor–like domain, several short consensus repeats, a transmembrane domain, and a short C-terminal cytoplasmic domain.10 P- and E-selectin expressed on the surface of endothelial cells regulate leukocyte–endothelial cell interaction via the rolling of leukocytes.
MATERIALS AND METHODS

ANIMALS

All experimental protocols were approved by the Animal Care and Use Committee of the University of Tennessee, Memphis. Male Institute of Cancer Research mice were purchased (Harlan Laboratories, Indianapolis, Ind) and housed in a conventional facility accredited by the American Association for Accreditation of Laboratory Animal Care. The environment was controlled with regard to temperature and humidity with a 12-hour light-dark cycle. Mice were fed ad libitum chow (RMH3200; Agway Inc, Syracuse, NY) and water for 2 weeks before entry into this study protocol. During feeding protocols, mice were housed in metal metabolism cages with wire grid floors to eliminate coprophagia.

FEEDING PROTOCOL

Fifty-three mice (6-8 weeks old) were randomized to receive chow (n=16), IV TPN (n=21), or intragastric (IG) TPN (n=16). Mice randomized to the chow and IV TPN groups received internal jugular catheters under anesthesia with ketamine hydrochloride (100 mg/kg of body weight) and acepromazine maleate (10 mg/kg of body weight). Through a right jugular approach, a silicone rubber catheter (0.3-mm inner diameter and 0.6-mm outer diameter; Baxter Healthcare Corp, Chicago, Ill) was inserted into the vena cava. The proximal end of the catheter was tunneled subcutaneously over the spine and exited the tail at its midpoint. The mice were placed into metal metabolism cages and partially immobilized by tail restraint to protect the catheter during infusion. The technique is an acceptable method of nutritional support that does not induce physical or biochemical stress.18 Mice randomized to IG TPN received gastrostomy tubes. Through a vertical midline incision, the stomach was mobilized and wrapped around the insertion of the gastrostomy tube with the use of 7-0 silk suture. The proximal end of the catheter was tunneled subcutaneously over the spine and exited the tail at its midpoint.

Catheterized mice were immediately connected to infusion pumps (Instech Laboratories, Plymouth Meeting, Pa) and received 0.9% saline 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% saline IV along with ad libitum chow and water throughout the study. The IV TPN— and IG TPN—fed animals initially received 4 mL of TPN per day 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-nitrogen ratio of 743 kJ/g of nitrogen. This feeding met the calculated nutritional requirement of mice used in the present study. The TPN-fed mice received 1951 kJ of nonprotein energy intake per kilogram per day and 16.4 g of protein per kilogram per day.

After receiving their respective diets for 5 days, 29 animals were used for quantification of P-selectin expression in the lung, liver, kidney, small intestine, colon, pancreas, mesentery, and heart, and 24 were used for E-selectin quantification in the organs. P-selectin expression in the stomach and gastrocnemius muscle was also quantified.

QUANTIFICATION OF P- AND E-SELECTIN EXPRESSION

Monoclonal Antibodies

The mAbs used for the assessment of P-selectin expression were RB40.34, a rat IgG1 directed against mouse P-selectin, and R3-34, a nonbinding rat IgG1 control antibody. To evaluate E-selectin expression, 10E9.6, a rat IgG2a directed against mouse E-selectin, and R3-95, a nonbinding rat IgG2a control antibody (PharMingen Inc, San Diego, Calif) were used.

Radio-iodination of the mAbs

The binding mAbs and the nonbinding mAbs were radiolabeled with iodine 125 (125I) and iodine 131 (131I), respectively.

RESULTS

The preexperiment weights of all groups were similar (Table 1). The mice in the IV TPN and IG TPN groups lost more body weight than chow-fed mice (P<.04), but there were no differences between the IV TPN and IG TPN groups.

P-selectin expression in small intestine, colon, stomach, and pancreas was significantly higher in the IV TPN group than in the chow or the IG TPN groups, with no significant differences between the chow and the IG TPN groups (Figure 1 and Table 2). No significant differ-
by means of the iodo gen method. Briefly, 250 µg of protein was incubated with 9.25 MBq of sodium iodide (125I) (or sodium iodide [131I]) and 125 µg of iodogen. A mixture of 10 µg of 125I-labeled anti–P-selectin mAb or anti–E-selectin mAb was given with an appropriate amount of 131I-labeled nonbinding antibody (400000-600000 cpm) through the jugular vein catheter (total volume, 200 µL). Binding mAb labeled with 125I that is directed against P- (or E-) selectin was injected to evaluate P- (or E-) selectin expression on the endothelial cells, while 131I-labeled nonbinding mAb was given to eliminate the influence of nonspecific binding of anti–P- (or E-) selectin antibody to endothelial cells. A blood sample was obtained through the carotid artery catheter 5 minutes after injection of the mAb mixture. The animals were then treated with 40 U of heparin sodium and rapidly exsanguinated by perfusion of bicarbonate-buffered saline through the carotid artery catheter. This was followed by perfusion of 15 mL of bicarbonate-buffered saline through the carotid artery 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 of the inferior vena cava at the thoracic level. Thus, after removal of all mAbs that did not bind to the endothelial cells, the lungs, liver, kidney, and small intestine were harvested and weighed.

Calculation of Adhesion Molecule Expression

An automated 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. Intercellular adhesion molecule-1 expression 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 in the following equation:

\[
\frac{(125I \text{ cpm/g})}{(131I \text{ cpm Injected})} \times \frac{(125I \text{ cpm/g})}{(125I \text{ cpm Injected})}
\]

WET-TO-DRY LUNG WEIGHT RATIOS

A section of lung tissue was taken from animals and dried at 60°C for 48 hours to evaluate wet-to-dry lung weight ratios.

STATISTICS

Results are presented as means±SEs. Statistical analysis was performed by means of analysis of variance, followed by Fisher protected least significant difference post hoc test or t test. Differences were considered statistically significant at P<.05.

Table 1. Mice Body Weight and Weight Gain

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight Before Feeding, g</th>
<th>Body Weight Change, g</th>
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<tbody>
<tr>
<td>Chow (n = 16)</td>
<td>26.83 ± 0.61</td>
<td>-0.29 ± 0.51</td>
</tr>
<tr>
<td>IV TPN (n = 21)</td>
<td>27.54 ± 0.44</td>
<td>-1.47 ± 0.25†</td>
</tr>
<tr>
<td>IG TPN (n = 16)</td>
<td>27.49 ± 0.60</td>
<td>-2.24 ± 0.50†</td>
</tr>
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</table>

*Values are means±SE. IV indicates intravenous; TPN, total parenteral nutrition; and IG, intragastric.
†P < .05 vs chow group.

COMMENT

Adhesion molecules mediate leukocyte–endothelial cell adhesion and participate in PMN accumulation in tissues. E- and P-selectin expressed on the endothelial cells cause leukocyte rolling, which should precede ICAM-1–mediated firm adhesion. Regulated and coordi
nated expression of adhesion molecules is essential for appropriate tissue repair and host defense against infection.

The PMNs are the major effector of the nonspecific immune response in host resistance to infection. Under some conditions, PMNs could be mediators of tissue-destructive events in a variety of systemic disorders, be-
cause they have little intrinsic ability to differentiate between foreign and host antigens. The PMNs have been implicated as a major mediator for organ injury, particularly lung injury such as adult respiratory distress syndrome, in critically ill or severely injured patients. Therefore, the changes in adhesion molecule expression may be associated with PMN-mediated organ injury or, at least, modulate subsequent response after an insult.

To clarify a mechanism for the increased septic complication in patients fed parenterally, we have focused on IgA-mediated mucosal immunity. Intravenous TPN fails to maintain respiratory antibacterial or antiviral immunity with decreases in IgA levels in both the respiratory tract and the intestine. Changes in gut-associated lymphoid tissue presumably lead to decreased respiratory immunity through depression of common mucosal immunity, establishing a link between the intestine and lung injury after a traumatic insult, as others have reported.

The intestinal vascular bed has been shown to serve as a priming bed for PMNs after traumatic events. Our work strengthens this linkage, since lack of enteral feeding increases ICAM-1 expression and PMN accumulation in intestine.

In this work, we postulated that the type and route of nutrition may affect intestinal expression of other adhesion molecules within the selectin family, since intestinal PMN accumulation with parenteral feeding may have resulted from up-regulated expression of P- or E-selectin as well as increased ICAM-1 expression. Second, we were interested in the changes that occur in nonsplanchnic organs. Since immunohistochemical staining cannot provide accurate quantification and the measurement of messenger RNA expression generates only indirect evidence, we used the dual radiolabeled antibody technique to quantify E- and P-selectin expression in vivo. Otherwise, quantification of the expression would be difficult in uninjured mice.

P-selectin expression was increased only in the gastrointestinal tract, ie, small intestine, stomach, colon, and pancreas. Although constitutive expression was observed in the other organs and tissues, there were no significant

![Figure 1](http://archsurg.jamanetwork.com/pdfaccess.ashx?url=/data/journals/surg/9435/ on 06/16/2017)
differences in expression within those organs among the 3 diet groups. Increased P-selectin is likely to cooperate with ICAM-1 in recruiting PMNs to the intestinal vascular bed. Although we studied uninjured animals manipulated with diet alone, dietary effects may have implications on responsiveness to subsequent injury. P-selectin has also been implicated in the increase in complement deposition after intestinal ischemia reperfusion.27 PB1.3, a monoclonal antibody directed against P-selectin, reportedly limits deposition of the C5b-9 membrane attack complex and reduces the complement-dependent intestinal injury in a rat intestinal ischemia-reperfusion model.27 Consequently, increased intestinal P-selectin in the animals fed parenterally might lead not only to PMN-mediated organ injury, but also to non–PMN-mediated intestinal injury after insult.

Generally, the expression of P-selectin is increased temporarily after stimulation by translocation from Weibel-Palade bodies.8 Rapid internalization and degradation allow the molecule to be expressed on the surface of the endothelium for a short time.8,10 Although P-selectin can also be regulated transcriptionally on cell activation by TNF-α, the elevation of expression may last only 24 hours after a single dose of TNF-α.10 Since gut expression of P-selectin remained up-regulated in the IV TPN group in a longer time frame (5 days), it is possible that failure of rapid degradation occurred or that the cytokine milieu in the intestine changed during lack of enteral feeding. We have no evidence that IV TPN increases the levels of the intestinal proinflammatory cytokines interleukin 1 and TNF-α in our mouse feeding model, but we have noted changes in cytokines related to IgA production within the gut-associated lymphoid tissue. Nevertheless, Ogle et al28 reported that IV TPN up-regulates messenger RNA levels of these cytokines.

The pattern of E-selectin expression was quite different from that of P-selectin. E-selectin was detectable only in lung, liver, and kidney, and only a few animals had any detectable and very low levels in the small intestine, colon, and stomach. Enhanced pulmonary E-selectin expression in mice in the IV TPN group may reflect pulmonary inflammation or the activation of pulmonary endothelium. While IV TPN does impair respiratory IgA-mediated immunity,4,6 the animals did not show any symptom of lung inflammation. It remains unknown whether this increased expression of E-selectin is beneficial or detrimental to the host during parenteral feeding, but this observation warrants further exploration.

In summary, even though P- and E-selectin are believed to be expressed on the endothelium temporarily

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**Table 3. E-Selectin Expression in Organs**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mesentery</th>
<th>Pancreas</th>
<th>Colon</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow (n = 6)</td>
<td>0.00 ± 0.00</td>
<td>0.08 ± 0.08</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>IV TPN (n = 10)</td>
<td>0.06 ± 0.06</td>
<td>0.29 ± 0.26</td>
<td>0.32 ± 0.21</td>
<td>0.90 ± 0.90</td>
</tr>
<tr>
<td>IG TPN (n = 8)</td>
<td>0.00 ± 0.00</td>
<td>0.32 ± 0.32</td>
<td>0.20 ± 0.14</td>
<td>0.32 ± 0.32</td>
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*Values are mean ± SE nanograms of monoclonal antibody per gram of tissue. IV indicates intravenous; TPN, total parenteral nutrition; and IG, intragastric.

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**Figure 2. E-selectin expression in the lung, liver, kidney, and small intestine. All groups of animals received their respective diets for 5 days. IV indicates intravenous; TPN, total parenteral nutrition; IG, intragastric; and mAb, monoclonal antibody. Asterisk indicates P<.01 vs chow group and P<.05 vs IG TPN group.**
Critically ill or severely injured patients fed parenterally are susceptible to infections. Whereas we have focused on IgA-mediated immunity to demonstrate the mechanisms, other investigators have pointed out that derangement of adhesion molecule expression may impair host immunity and cause excessive inflammation. Recently, we showed that lack of enteral feeding increased ICAM-1 expression and neutrophil accumulation in the intestine. Since neutrophils are primed in the intestinal vascular bed and exacerbate distal organ injury to a subsequent insult, these data suggested that route of nutrient delivery also affects response to insults via changes in adhesion molecule expression. The present work further pursues the influence of nutrition on adhesion molecule expression, demonstrating increased gut P-selectin expression and pulmonary E-selectin expression. Enteral feeding seems to maintain appropriate immune responses to insults partly by maintaining normal P- and E-selectin expression in addition to ICAM-1 expression.

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Reprints: Kenneth A. Kudsk, MD, 956 Court Ave, Suite E228, Memphis, TN 38163.

REFERENCES