Decline in intestinal mucosal IL-10 expression and decreased intestinal barrier function in a mouse model of total parenteral nutrition

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Sun X, Yang H, Nose K, Nose S, Haxhija EQ, Koga H, Feng Y, Teitelbaum DH. Decline in intestinal mucosal IL-10 expression and decreased intestinal barrier function in a mouse model of total parenteral nutrition. Am J Physiol Gastrointest Liver Physiol 294: G139–G147, 2008. First published November 8, 2007; doi:10.1152/ajpgi.00386.2007.—Loss of intestinal epithelial barrier function (EBF) is a major problem associated with total parenteral nutrition (TPN) administration. We have previously identified intestinal intraepithelial lymphocyte (IEL)-derived interferon-γ (IFN-γ) as a contributing factor to this barrier loss. The objective was to determine whether other IEL-derived cytokines may also contribute to intestinal epithelial barrier breakdown. C57BL6J male mice received TPN or enteral nutrition (control) for 7 days. IEL-derived interleukin-10 (IL-10) was then measured. A significant decline in IEL-derived IL-10 expression was seen with TPN administration, a cytokine that has been shown in vitro to maintain tight junction integrity. We hypothesized that this change in IEL-derived IL-10 expression could contribute to TPN-associated barrier loss. An additional group of mice was given exogenous recombinant IL-10. Ussing chamber experiments showed that EBF markedly declined in the TPN group. TPN resulted in a significant decrease of IEL-derived IL-10 expression. The expression of several tight junction molecules also decreased with TPN administration. Exogenous IL-10 administration in TPN mice significantly attenuated the TPN-associated decline in zonula occludens (ZO)-1, E-cadherin, and occludin expression, as well as a loss of intestinal barrier function. TPN administration led to a marked decline in IEL-derived IL-10 expression. This decline was coincident with a loss of intestinal EBF. As the decline was partially attenuated with the administration of exogenous IL-10, our findings suggest that loss of IL-10 may be a contributing mechanism to TPN-associated epithelial barrier loss. tight junction; epithelial barrier function; adherens junctional molecule 1 (JAM-1); claudins

EPI THELIAL BARRIER FUNCTION (EBF) is essential for the intestine to maintain an effective defense against intraluminal toxins, foreign antigens, and bacteria, as well as to allow the epithelium to effectively absorb nutrients. Such a defense mechanism requires an intact epithelial layer. One of the major physiological consequences of total parenteral nutrition (TPN) administration is a loss of intestinal EBF (34, 35, 74, 76, 77, 81, 82). This loss of EBF is significant because it could lead to the transmigration of luminal endotoxins or potentially bacteria into the host. These cross-barrier actions may contribute to the observation that patients dependent on TPN have a much higher rate of septicemia compared with patients who are enterally fed (20, 55, 56).

The precise mechanism(s) responsible for this TPN-associated loss of EBF are unknown. A number of changes in the intestinal mucosal immune system have been observed, including changes in cytokine expressions in mice after TPN administration (76, 83, 85, 86), and these changes of IEL-derived cytokine expression may play an important role in the loss of EBF (3). One of the first such cytokines shown to modulate EBF was interferon-γ (IFN-γ). Madara and colleagues (10) demonstrated a significant loss of EBF when epithelial monolayers were exposed to IFN-γ in vitro. A study from our group has found that, with TPN administration, there is an increase in intraepithelial lymphocyte (IEL)-derived IFN-γ expression and that, similar to these in vitro studies, IFN-γ plays an important role in the loss of EBF with the administration of TPN (81, 83). However, in a subsequent study using IFN-γ knockout mice, we found that the loss of EBF was only partially corrected by the elimination of IFN-γ, which implies that other cytokines (factors) may also contribute to this TPN-associated loss of EBF (76, 81, 83).

IL-10 has also been shown to play an important role in the regulation of intestinal epithelial integrity (5, 47, 51, 58, 69). Endogenous IL-10 appears to be a central regulator of the mucosal immune system in vivo (5); a loss of IL-10 expression by using knockout mice can result not only in a colitis model, but may also lead to an increase in epithelial permeability (4, 48). Previous studies from our own group and Fukatsu et al. (20) have observed that IEL-derived IL-10 expression declined in a mouse TPN model. However, it is not clear what effects this decline in IL-10 has on EBF (20, 75, 76). We hypothesized that this decrease of IEL-derived IL-10 expression after TPN administration may play an important role in the loss of EBF.

MATERIALS AND METHODS

Parenteral Nutrition Model

Animals. C57BL6J male specific-pathogen-free mice (8 wk old) were obtained from Jackson Laboratory (Bar Harbor, ME) and maintained under temperature-, humidity-, and light-controlled conditions. Mice were initially fed ad libitum with standard mouse chow and water and allowed to acclimate. During the administration of intravenous solutions, mice were housed in metabolic cages to prevent coprophagia. The studies conform to the Guidelines for the Care and Use of Laboratory Animals established by the University Committee

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on Use and Care of Animals at the University of Michigan, and protocols were approved by that committee (no. 7703).

**TPN Model**

Administration of TPN was performed as previously described (36). Mice were infused with a crystalloid solution at 4 ml/day. After 24 h, mice were randomized into two groups (n = 6 per group). The control group received the same intravenous saline solution at 7 ml/24 h, in addition to standard laboratory mouse chow and water ad libitum. The TPN groups received an intravenous TPN solution at 7 ml/24 h. The TPN solution has been described in detail previously (36) and contained a balanced mixture of amino acids, lipids, and dextrose in addition to electrolytes, trace elements, and vitamins. Caloric delivery was based on estimates of caloric intake by the control group and from previous investigators (42) so that caloric delivery was essentially the same in both groups. All animals were euthanized at 7 days by using CO₂.

**Exogenous IL-10 administration.** To further assess the role of IL-10 during TPN administration exogenous, recombinant IL-10 (R&D Systems, Minneapolis, MN) was given at a dose of 10 μg·kg⁻¹·day⁻¹ via the intravenous catheter starting on the first day of TPN administration and continuing daily through the first week of TPN administration. This amount was based on established therapeutic dosings (8, 40, 53).

**Electrophysiological Experiments**

Using chambers were used to assess intestinal barrier function, and methods are identical to our previous studies (82). The permeability of the small intestine was assessed with [³H]mannitol. After a 20-min equilibration period, [³H]mannitol (Sigma Chemical, St. Louis, MO) was added to the mucosal compartment. One-millilitre samples were taken every 15 min from the serosal compartment for subsequent analysis of [³H]mannitol and replaced by fresh Krebs buffer for a 90-min incubation period. A scintillation counter (Beckman LS-1801; Beckman Instruments, Fullerton, CA) determined the amount of radioactivity. The apparent permeability coefficient (Papp) was calculated according to a previously published equation (24).

**Bacteriological Cultures**

Bacteriological cultures were used for detecting bacteria translocation (35, 72). After euthanasia, samples of tissue from mesenteric lymph nodes, liver, and spleen were excised by using sterile technique. Each tissue sample was incubated in thioglycolate broth for 24 h at 37°C and then subcultured onto MacConkey media or colistin-resistant blood agar plates. Positive cultures indicated bacterial translocation. Bacteriological cultures were also studied by transepithelial resistance. The transmembrane resistance was determined with the use of Ohm’s law.

**Mucosal Cell Isolation**

Small bowel IEL and epithelial cells (ECs) were isolated as previously described (36). Briefly, the small bowel was placed in tissue culture medium (RPMI 1640 with 10% FCS; Life Technologies, Rockville, MD). Mesenteric fat and Peyer’s patches were removed. The intestine was then opened longitudinally and agitated to remove mucus and fecal material. The intestine was then cut into 5-mm pieces, washed three times in an IEL extraction buffer (1 mM EDTA, 1 mM DTT in PBS), and incubated in the same buffer with continuous brisk stirring at 37°C for 20 min. The supernatant was then filtered rapidly through a glass wool column. This resulted in a mixture of IEL and ECs. To enrich each population, a series of magnetic beads conjugated with antibody to CD45 (lymphocyte specific) were used (BioMag SelectaPure Anti-Mouse CD45 Antibody Particles; Polyscience, Warrington, PA), thus resulting in purified IEL and EC populations, where cells bound to beads were considered purified IEL; the ECs remained in the supernatant. Flow cytometry confirmed purity of sorted IEL, which was greater than 99%, based on a control sample stained with anti-CD45 antibody, or anti-G8.8 antibody (specific for ECs) (16).

**Real-Time PCR**

Methods of RNA isolation, purification, and PCR are identical to those previously described (81). Oligomers were designed by using an optimization program (Lasergene 6; DNASTar, Madison, WI). Sequences of specific primers are (presented as GeneBank accession number: forward, reverse primers): ZO-1 (D14340): 5'-AACC CGAC TGA TGC TGT GGAG TA-3', 5'-AAA TGG TCG GGC CAG ACG ACT TGT GTA-3'; occludin (NM_008756): 5'-ATG TCC GCC CGC TGA TCT TCA AGT ACG TGG TAT CTA-3', 5'-TCG GCT GGT ATG GAG GTA-3'; E-cadherin (X06115): 5'-GCA CAT ATG TAG CTC TCA TC-3', 5'-CCT TCA CAG TCA CAC ACA TG-3'; ZO-2 (NM_011597): 5'-CCG CCG CCG ACT ATC TGA-3', 5'-CTG CGG CCA GTG TAT CCT-3'; IL-10 (NM_010548): 5'-AAT AAG AGC AGC GCA GTG GA-3', 5'-GGG ATG ACA GTA GGG GAA CC-3'; claudin-2 (NM016675): 5'-GGC TGT TAG GCA CAT CCA T-3', 5'-TGG CAC CAA CAT AGG AAC TC-3'; claudin-3 (NM016887): 5'-AGG GTC TGC TGT CCT CTT-3', 5'-GT ACG CAT CTG TTT CA-3'; claudin-15 (NM021719): CAG CTT GGA TAA ATG GTA-3', 5'-CAG TGG GAC AAC AAA TGG TG-3'; ZO-3 (M12481): 5'-AATCGTGCGTGACATC-3', 5'-AAG GAA GCC TGG AAA AGC AG-3'. Real-time PCR (RT-PCR) was performed using a Smart Cycler (Cepheid, Sunnyvale, CA) with intercalation of SYBR green I used to determine the amount of DNA by using previously published techniques (80). Specificity of the RT-PCR samples was documented with gel electrophoresis and resulted in a single product with the desired length. Additionally, cDNA was extracted from gels and sequenced to insure it matched targeted GenBank mRNA sequences. Expression of results were normalized to β-actin expression by using the Ct method, by which the change (Δ) in Ct values between each study group were compared with the Ct value of β-actin (ΔCt), and the ΔΔCt is the difference between the ΔCts from the TPN groups compared with the control (enteral) group, taken as 2^(-ΔΔCt) (44).

**Intracellular Cytokine Staining and Flow Cytometry**

Intracellular staining of IL-10 was performed to better define the subpopulation of IEL, which was the predominant source of IL-10. The surface and intracellular staining protocol has previously been described (83). Cell surface staining consisted of the following: anti-CD4, anti-CD8α, and anti-CD8β (BD Pharmingen), each conjugated with either FITC or Texas red. Intracellular staining was performed with anti-IL-10 antibody conjugated with phycoerythrin (Pharmingen, 2 μg/1 × 10⁶ cells). IEL were identified by forward and side-scatter characteristics. Expression of IL-10 positive cells was based on control cells, which were stained with a phycoerythrin-conjugated nonspecific, isotype control antibody, and is the percentage of each subpopulation of gated IEL that expressed IL-10.

**Western Immunoblotting**

**Antibodies.** The following antibodies were used for Western blot analysis: rabbit anti-ZO-1 Ab (Zymed, South San Francisco, CA), mouse anti-E-cadherin Ab (BD Transduction Laboratories; cytoplasmic domain specific), goat anti-JAM-1 Ab (Santa Cruz Bio-technology, Santa Cruz, CA), goat anti-occludin Ab (Santa Cruz; extracellular/cytoplasmic domain), and rabbit anti-ZO-2 Ab (Zymed Laboratories, Invitrogen). Mouse anti-claudin-2 was from Zymed Laboratories, Invitrogen; mouse anti-β-actin Ab was from Sigma.
Secondary antibodies including anti-rabbit horseradish peroxidase (HRP)-conjugated Ab (Zymed Laboratories, Invitrogen), goat antimouse-HRP (Santa Cruz), and rabbit anti-goat HRP (Sigma) were used for Western blot analysis.

**Western blot analysis.** Briefly, isolated ECs were homogenized on ice in lysis buffer (79). Protein determination was performed by using a Micro BCA Protein Assay Kit (PIERCE, Rockford, IL). Immunoblotting was identical to that previously described (83). Blots were then stripped and reprobed with monoclonal mouse anti-β-actin antibody (1:8,000 in blocking solution, Sigma) to confirm equal loading of protein. Quantification of results was performed using Kodak 1D image quantification software (Kodak). Results of immunoblots are expressed as the relative expression of proteins to β-actin expression.

**Data Analysis**

Data are expressed as means ± SD. Statistical analysis employed the t-test for comparison of two means and a one-way ANOVA for comparison of multiple groups (with a Bonferroni post hoc analysis to assess statistical differences between groups). The chi-square test was used for categorical data (Prism software; GraphPad Software, San Diego, CA). Statistical significance was defined as P < 0.05.

**RESULTS**

IEL-Derived IL-10 Is Downregulated with TPN Administration

After 7 days of TPN administration, a significantly decreased expression of IEL-derived IL-10 was noted (Fig. 1). There was a nearly twofold decrease of IL-10 mRNA expression when compared with control (P < 0.01). A similar significant (P < 0.05) decrease in protein expression of IL-10 was also observed.

On the basis of this finding and the fact that IL-10 is an important factor in supporting barrier function, we further hypothesized that the decline in IL-10 with TPN administration contributes to the loss of EBF and that exogenous IL-10 administration would attenuate the loss of intestinal barrier function. To address these hypotheses, exogenous recombinant IL-10 was used for Western blot analysis.

**Exogenous IL-10 Administration and TPN-Associated EBF**

To help determine the physiological consequences of the observed loss of IL-10 in TPN mice, two measures of EBF were done: a series of electrophysiological measures of EBF with Ussing chambers, and determination of bacterial translocation rates.

**Small intestine permeability of [3H]mannitol.** To address the physiological significance of the decline in IL-10 expression, we used [3H]mannitol to examine the alterations in epithelial permeability with TPN administration. Mannitol was selected because it is reported to diffuse across the epithelium via intracellular and paracellular pathways; also mannitol passes through tight junctions at both the level of the villus and crypt (6, 65, 70).

There was a linear permeation increment during the 90-min Ussing chamber incubation in all groups (Fig. 2A; i.e., concentration of trace progressively increased in the serosal side over time). The cumulative permeation of [3H]mannitol was 0.21 ± 0.1% in the controls; however, TPN significantly (P < 0.01) increased the permeability value (0.56 ± 0.16%). Giving IL-10 to mice receiving TPN resulted in a significantly lowered [3H]mannitol permeability (0.36 ± 0.15%, P < 0.01, Fig. 2). Expressed as the permeability coefficient (Papp; Fig. 2B), [3H]mannitol permeability in jejenum was also seen to be significantly reduced to levels similar to control values.

**Transepithelial resistance.** Transepithelial resistance (TER) is a measure of intestinal epithelial integrity and tissue viability (67, 68). Baseline TER (Ω·cm²) 20 min after mounting the small bowel segments was 68.5 ± 10.6 Ω·cm² for controls. TPN administration led to a significant decrease in TER (46.0 ± 8.6 Ω·cm², P < 0.01). The observed decline in TER in TPN mice was significantly ameliorated by giving the mice exogenous IL-10, and TER returned toward control levels (63.4 ± 9.9 Ω·cm², P > 0.05, Fig. 3).

**Exogenous IL-10 administration in TPN mice markedly reduced bacterial translocation.** Similar to our previous studies (36), bacterial culture results showed that TPN administration led to a significant increase in bacterial translocation. The
Changes of Tight Junctional Protein Expression with TPN Administration

A major mechanism by which cytokines mediate altered EBF is via changes in epithelial junctional proteins. To address this as a potential mechanism for the loss of EBF, a number of junctional proteins were studied. Tight junctional molecules studied included zonula occludens (ZO)-1, ZO-2, claudin members (2, 7, and 15), occludin, and adherence junctional molecule 1 (JAM-1). Furthermore, because IEL lie adjacent to ECs and comprise part of the epithelial barrier, we also examined the expression of E-cadherin, a specific ligand for these mucosal lymphocytes (29).

RT-PCR results for mRNA expression of junctional factors are shown in Fig. 4. The expressions of many junctional factors in mice receiving TPN were decreased significantly ($P < 0.01$) compared with controls (control vs. TPN groups): ZO-1, $20.24 \pm 5.75$ vs. $57.95 \pm 19.82$; occludin, $8.28 \pm 3.23$ vs. $38.55 \pm 11.55$; E-cadherin, $1.22 \pm 0.57$ vs. $5.09 \pm 2.0$; and ZO-2, $6.52 \pm 1.65$ vs. $15.98 \pm 5.46$ (Fig. 4A). The only exception to this was JAM-1, where despite a 31% decline in expression, the difference was not significant ($5.87 \pm 2.41$ vs. $8.49 \pm 2.67$, $P > 0.05$). Results of the selected Claudins are shown in Fig. 4B. We noted a significant decline in the expression of claudin-2 ($7.49 \pm 2.27$ vs. $2.23 \pm 1.38$) with (37, 39, 63). To define which subpopulation of the IEL demonstrated the greatest change in IL-10 expression with TPN administration, IEL underwent intracellular staining to detect IL-10, and subfractions of the population were isolated based on surface phenotype (Table 2). Interestingly, a substantial portion of both CD4$^+$ IEL and CD8$^+$ IEL expressed IL-10. However, a significant decline in IL-10 was only noted in CD8$^+$ IEL. This decline occurred in the CD8alpha, as well as the CD8alpha beta subpopulations. This was interesting, as we have previously noted that the number of CD8 IEL are preserved with TPN administration, yet the number of CD4 IEL significantly decline (36).

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E-cadherin, 0.62

The downregulation of junctional factor expression was significantly ameliorated by the exogenous administration of IL-10 to TPN mice. This IL-10 administration led to an increase in the expression of each of these factors toward that of control levels, and these levels were not significantly different ($P > 0.05$) from control values (TPN + IL-10 vs. controls): ZO-1, 36.45 ± 9.34 vs. 57.95 ± 18.23; claudin-2, 8.49 ± 2.65 vs. 7.49 ± 2.27; occludin, 31.39 ± 10.52 vs. 38.55 ± 11.55; and ZO-2, 10.17 ± 4.89 vs. 15.98 ± 5.46 (Fig. 4, A and B). Relatively little change was noted with IL-10 for claudin-15 (7.05 ± 1.55). Interestingly, although claudin-7 did not appreciably change with TPN administration, the addition of IL-10 led to a marked increase (though not significant) in claudin-15 expression (3.5 ± 1.87). On the basis of these findings and the fact that claudin-2 may have particularly strong control of intestinal EBF (62), we confined protein analysis of the claudin family to claudin-2.

The expressions of E-cadherin, JAM-1, occludin, claudin-2, and ZO-2 proteins were also examined by Western blot analysis (Fig. 5). Similar to RT-PCR analysis, the expression of the junctional proteins claudin-2, E-cadherin, occludin, and ZO-2 were significantly ($P < 0.01$) decreased with TPN administration compared with controls: claudin-2, 0.25 ± 0.11 vs. 0.32 ± 0.20; E-cadherin, 0.40 ± 0.12 vs. 0.74 ± 0.14; occludin, 0.54 ± 0.11 vs. 0.81 ± 0.1; and ZO-2, 0.58 ± 0.08 vs. 0.79 ± 0.1. Although claudin-2 and JAM-1 declined in the TPN group, the difference was not significant. Similar to the PCR results, treatment of TPN mice with IL-10 partially prevented the TPN-associated decline in these junctional proteins. For claudin-2, E-cadherin and occludin levels rose to values that were not significantly ($P > 0.05$) different from control levels: E-cadherin, 0.62 ± 0.14 vs. 0.74 ± 0.14 and occludin, 0.7 ± 0.08 vs. 0.81 ± 0.1 (Fig. 5).

DISCUSSION

This study examined a potential mechanism of intestinal EBF loss associated with TPN administration. Major alterations in the IEL phenotype and function of the mucosal immune system have been found with TPN, as well as a loss of enteral nutrition intake (41, 49, 60, 76). IEL, located within the intestinal mucosa, are an important component of the gastrointestinal immune system (26, 88). This cell population is a rich source of cytokines, which have been demonstrated to have an important role in the regulation of epithelium integrity (27, 78, 83). Our group has previously shown that expression of IFN-γ in the intestinal epithelium was increased with TPN administration (76, 81, 83). A subsequent study suggested that the upregulation of IEL-derived IFN-γ expression is one mechanism responsible for the loss of EBF associated with TPN (83). However, using IFN-γ knockout mice, we also identified that this loss of intestinal barrier function was not fully explained by IFN-γ. As a number of other cytokines can modulate EBF, we hypothesized that altered expression of other cytokines may also contribute to this loss of intestinal EBF (71).

IL-10 has been reported to play an important role in the modulation of EBF (47, 48). Furthermore, Fukatsu et al. (20) showed that IL-10 is significantly decreased in a mouse model of TPN. IL-10 can suppress the release of many proinflammatory cytokines and chemokines, including tumor necrosis factor-α (TNF-α), IL-1, IL-6, and IL-8 (19, 38). The IEL is a rich source of IL-10 (1, 20, 22), and IL-10 has been well demonstrated to prevent loss of barrier function in colonic and small intestine, as well as hepatocyte epithelium (47, 50, 61). In this study, we found that IEL-derived IL-10 in mice receiving TPN was significantly decreased. Simultaneously, a disruption of EBF was detected by a striking increase in bacterial translocation, as well as a significant decrease in transepithelial resistance and increased permeability to tracer molecules. Interestingly, we also showed that exogenous IL-10 administration prevented the loss of EBF associated with TPN administration. IL-10 also substantially reduced bacterial translocation in TPN mice, and this decline in translocation was significant for gram-positive cultures.

Our study also indicated that IL-10 was detected in both CD4+ as well as CD8+ IEL. Further, the TPN-associated decline in the percent of IEL-expressing IL-10 was found to be confined to CD8+ IEL, including both CD8αα and CD8αβ subgroups. Our group previously observed a significant decline in CD4+ IEL with TPN (35); however, despite the decline in number of CD4+ IEL, the percentage of CD4+ IEL that expressed IL-10 did not change. The implications of this differential decline in IL-10 by the CD8+ IEL population are uncertain. However, it has been shown that CD8+ IEL plays a key role in the generation of IL-10 and the prevention of intestinal inflammatory conditions (22). It may well be that, although the number of CD8+ IEL cells does not change with TPN, their functional role is markedly altered, contributing to the loss of IL-10 and EBF.

Table 1. Bacterial translocation: number of organs and positive rate with bacteria translocation in different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Positive</th>
<th>Negative</th>
<th>% of Positive</th>
<th>Positive</th>
<th>Negative</th>
<th>% of Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPN (n = 18)</td>
<td>23</td>
<td>31</td>
<td>42.56†</td>
<td>29</td>
<td>25</td>
<td>53.70*</td>
</tr>
<tr>
<td>TPN + IL-10 (n = 8)</td>
<td>8</td>
<td>16</td>
<td>33.3</td>
<td>3</td>
<td>21</td>
<td>12.5</td>
</tr>
<tr>
<td>Control (n = 16)</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>2</td>
<td>16</td>
<td>6.3</td>
</tr>
</tbody>
</table>

χ² test: Compared with TPN + IL-10 group, *P < 0.01; †P > 0.05. TPN, total parenteral nutrition.

TPN. A significant decline in claudin-15 was also noted with TPN (27.15 ± 5.55 vs. 3.54 ± 0.78); however, claudin-7 did not show any change with TPN administration (1.82 ± 0.70 vs. 1.75 ± 0.92).

Table 2. Expression of IL-10 using intracellular staining and flow cytometry

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-10+ (CD4+)</th>
<th>IL-10- (CD8+αα)</th>
<th>IL-10- (CD8+αβ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.1±5.3</td>
<td>24.2±8.2</td>
<td>26.5±7.5</td>
</tr>
<tr>
<td>TPN</td>
<td>20.2±8.9</td>
<td>11.9±4.2*</td>
<td>11.6±0.6</td>
</tr>
</tbody>
</table>

Values are the percent of IL-10 positive cells from each intraepithelial lymphocyte (IEL) subpopulation. Thus, for control mice, 20.1% of the CD4+ IEL are IL-10 positive. Note the alteration in the expression of IL-10 in the various IEL subpopulations. *P < 0.05 vs. Control group using t-test.
The intestinal epithelial barrier is comprised of multiple components. Intestinal epithelial cells form a continuous relatively impermeable and highly selective barrier to prevent paracellular crossing of luminal contents. This barrier is maintained by a well-organized system of epithelial junctional proteins (17, 25, 43). These proteins include the tight junction, adherence junction, and desmosomes (14, 17, 71, 73). It has been demonstrated that increased permeability in several intestinal pathophysiological conditions is associated with diminished junctional integrity and disruption of junctional proteins (45, 58) and internalization of some of these proteins, such as occludin (66). Cytokine regulation of epithelial junction makeup may represent an important mechanism for dysregulation of EBF in some clinically relevant intestinal conditions (9, 25, 46, 62, 66).

To better demonstrate the mechanism of EBF loss after TPN administration, we examined the changes in the expression of several mucosally derived junctional molecules at both the mRNA and protein level. Among these, ZO (1 and 2), Claudins, and occludin are the most important and critical components in the structural and functional organization of tight junctions (15, 54, 62, 66). Claudins were selected based on the relevance to maintaining barrier function, and specific Claudins were selected based on the fact that these were the most prevalent in the small intestine (28, 62). Claudins appear fairly tightly localized to the expression of ZO-1 in the small intestine (28). Claudin-2 may have a special role in maintenance of barrier function. In a recent study examining expression of the claudin family after ischemia and reperfusion, several Claudins recovered in a time-dependent fashion; however, claudin-2 and -4 expression was altered and appeared to cover a more comprehensive portion of the crypt-villus axis (30). All epithelial cells forming tight junctions express both ZO-1 and ZO-2. These molecules bind to occludin and actin and act as a bridge between the plasma membrane and cytoskeleton proteins (21, 54). Occludin has been shown to have functional importance both for EBF and in cell-to-cell adhesion (21). In addition, E-cadherin is another critical cell-to-cell adhesion molecule involved in the maintenance of integrity in the gut epithelium. It is thought to contribute to intercellular integrity on the lateral cell membrane of enterocytes and also facilitates IEL localization and adhesion to ECs (51). It was interesting that another study that examined changes in junctional proteins in an animal model of TPN failed to observe a decline in junctional protein expression (32). In this study, pigs were given TPN for 1 wk. These authors failed to detect a decline in claudin-2, occludin, or ZO-1 and actually noted an increase in claudin-1 expression. Clearly, the change in the relative amount of these proteins may not necessarily correlate with EBF function, and the differences between our study and that of Kansagra et al. may be due to species differences.

Our present study showed that the TPN-associated decline in the expression of IL-10 was accompanied by a decline in the expression of all examined junctional proteins. Interestingly, exogenous IL-10 administration in mice receiving TPN attenuated the decline of these tight junction protein expressions.

Fig. 4. A: mRNA expression of tight junction genes from intestinal mucosal specimens measured by RT-PCR. Results are expressed as 2^(-ΔΔCt) in relation to β-actin gene expression, n = 7 mice in each group. mRNA expression in TPN mice were significantly decreased compared with controls except for JAM-1. Administration of IL-10 to TPN mice returned the expression of these factors toward control values, and expression was not significantly different from control values for zonula occludens (ZO)-1, occludin, and ZO-2 (* P < 0.05; # P < 0.05). B: mRNA expression of selected Claudins are shown separately. Note the significant decrease in the expression of claudin-2 and claudin-15 with TPN; for claudin-2 a reduction of this decline in expression was noted with administration of IL-10 in the TPN group (as represented in the graphs by the IL-10 group (* P < 0.05; # P > 0.05).
These results demonstrate that IL-10, which may affect tight junctional protein expression, plays a critical role in the regulation of EBF in mice receiving TPN. The role of IL-10 observed in our study was similar to that observed in an inflammatory bowel disease model using IL-10 knockout mice (50). In this study, IL-10 knockout mice showed a disruption in barrier function and abnormal claudin-1 and ZO-1 expression and distribution. Oshima et al. (58) reported that, in vitro, IFN-γ also reduced epithelial barrier (monolayer electrical resistance and increased albumin permeability) and reduced tight junction (occludin) expression and staining. These effects were reversed by pretreatment of monolayers with IL-10 (58). Our laboratory has previously demonstrated the contributory role that IFN-γ has on EBF in TPN mice (83). This present study suggests that modulation in the expression of IL-10 also has a contributory role in parenteral nutrition-associated loss of EBF. It is important to appreciate that neither the elimination of IFN-γ, using knockout mice (83), nor the exogenous administration of IL-10 (in the present study) were able to completely return barrier function to normal. This suggests that both cytokines have important contributory roles. Future studies that look at the combination of IFN-γ knockout mice and exogenous IL-10 administration may allow us to understand whether the combined derangement in these two cytokines is the sole contributory factor to the loss of barrier function with TPN.

It is possible that other cytokines or factors may also have a role in the modulation of junctional proteins. Prasad et al. (62) has shown that IFN-γ and TNF-α can downregulate the expression of several Claudin family members, whereas IL-13 can upregulate Claudin-2 expression in a model of inflammatory bowel disease. IL-17 can also upregulate the expression of Claudin-2 in T84 cells (33). Additionally, other cytokines such as IL-4 and IL-6 may contribute to EBF (11, 13, 87); however, previous work by our group has failed to demonstrate significant changes in the expression of these two cytokines with TPN administration (X. Sun and D. H. Teitelbaum, unpublished results). In addition to cytokines, other factors such as the presence of oxidants, trefoil factor, or changes in mucins may modulate the expression of tight junctions and/or barrier function (7, 12, 18, 57, 59, 89). A lack of enteral nutrition itself may result in a loss of the antioxidant glutathione and a decline in IGF-1 expression, both of which can contribute to a loss of barrier function (23, 31).

The alterations of tight junctional protein expression may lead to a perturbation of electrochemical gradients established by ion transport and lead to an increase in paracellular permeability. Objectively, the function of tight junctions can be measured by TER. The reduction in TER is thought to be a useful measure of the disruption of tight junction complex structure and/or function (64). In animal experiments, the correlation between a decrease in occludin levels and the perturbation of the tight junction permeability barrier has been confirmed (2, 52). Importantly, the change of TER and increase in bacterial translocation after TPN administration was closely linked to a decline in tight junctional protein expression, and this was partially prevented with exogenous IL-10 administration.

In conclusion, TPN administration led to a marked decline in IEL-derived IL-10 expression. This decline was accompanied by a decreased expression of several tight junctional proteins and was associated with an increase in intestinal epithelial permeability and bacteria translocation. This decline in EBF after TPN administration was partially attenuated with exogenous IL-10 administration. The down-regulation of IL-10 after TPN administration may be a contributing factor to TPN-associated epithelial barrier loss through downregulation of tight junction-related protein expression.


