Dietary glutamine and oral antibiotics each improve indexes of gut barrier function in rat short bowel syndrome

Junqiang Tian, Li Hao, Prakash Chandra, Dean P. Jones, Ifor R. Williams, Andrew T. Gewirtz, and Thomas R. Ziegler

1Nutrition and Health Sciences Program, 2Emory Center for Clinical and Molecular Nutrition, and Departments of 3Medicine and 4Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia

Submitted 12 March 2008; accepted in final form 13 December 2008


GUT BARRIER DYSFUNCTION is well documented in animal models of short bowel syndrome (SBS) after massive small bowel resection. This is evidenced by translocation of luminal microbes to mesenteric lymph nodes (MLN) and subsequently to blood and peripheral organs such as liver and spleen (3, 9, 26, 31). This phenomenon may correlate with the common incidence of infections due to gut-derived microorganisms in SBS patients requiring parenteral nutrition (28, 36). Our recent studies demonstrate a high incidence of detectable flagellin and lipopolysaccharide (LPS) in the serum of adults with SBS and elevated specific immunoglobulins (Ig) against these gram-negative bacterial antigens (44). Multiple aspects may be involved in enteric bacterial invasion, including small bowel bacterial overgrowth (SBBO) and impairment of gut-associated anatomic and immune barriers. Intestinal permeability in animal models of SBS has been determined by ex vivo electrophysiological methods and in vivo studies of paracellular permeation of luminal nonmetabolizable sugar markers (24, 27, 32, 33, 37, 39, 41). However, the results of these studies have been conflicting, and permeability studies do not always correlate with concomitant bacterial translocation (24, 27, 32, 33, 37, 39, 41).

Another possible cause of impaired gut barrier function in SBS may be net loss of gut-associated lymphoid tissue (GALT) after massive small bowel and/or colonic resection. Available data from animal models indicate that some components of GALT (e.g., T4 and T8 cell number; T4/T8 ratio) and humoral immune function (systemic B cells and immunoglobulins, mucosal plasma cells) are decreased after massive small bowel resection (4, 10, 30). Most of these observations were made within 1–2 wk after operation. However, bacterial translocation from the gut lumen may elicit adaptive immune responses that develop over a longer time frame, such as secretion into the gut lumen of secretory IgA (sIgA) by mucosal plasma cells.

Glutamine (GLN) is a major fuel substrate for both intestinal epithelial and circulating and fixed immune cells (2, 20, 42, 43). This amino acid is an important substrate for synthesis of purines and pyrimidines, ammonia, glucose, and amino acids and has several other major metabolic functions (20, 43). In addition, GLN inhibits apoptosis and stimulates cell proliferation in both intestinal and immune cells (12, 14, 16, 42). Such functions may be crucial for GALT activation in response to bacterial invasion. Dietary or intravenous GLN supplementation inhibits bacterial translocation in a number of catabolic animal models with intact intestine (5–6, 8, 11, 13, 15, 17, 40). GLN may become a conditionally essential nutrient during catabolic stress owing to the increased need for this substrate by gut epithelia, concomitant with insufficient cellular capacity for endogenous GLN production (21, 35, 43).

The present study was designed to contrast the effect of dietary GLN supplementation and oral antibiotic administration on indexes of gut barrier function in a rat model of combined partial small bowel-colonic resection, a previously utilized translational model for human SBS.

METHODS

Animals. Young (6 wk) male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used. The rats were housed in pathophysiological methods and in vivo studies of paracellular permeation of luminal nonmetabolizable sugar markers (24, 27, 32, 33, 37, 39, 41). However, the results of these studies have been conflicting, and permeability studies do not always correlate with concomitant bacterial translocation (24, 27, 32, 33, 37, 39, 41).

Another possible cause of impaired gut barrier function in SBS may be net loss of gut-associated lymphoid tissue (GALT) after massive small bowel and/or colonic resection. Available data from animal models indicate that some components of GALT (e.g., T4 and T8 cell number; T4/T8 ratio) and humoral immune function (systemic B cells and immunoglobulins, mucosal plasma cells) are decreased after massive small bowel resection (4, 10, 30). Most of these observations were made within 1–2 wk after operation. However, bacterial translocation from the gut lumen may elicit adaptive immune responses that develop over a longer time frame, such as secretion into the gut lumen of secretory IgA (sIgA) by mucosal plasma cells.

Glutamine (GLN) is a major fuel substrate for both intestinal epithelial and circulating and fixed immune cells (2, 20, 42, 43). This amino acid is an important substrate for synthesis of purines and pyrimidines, ammonia, glucose, and amino acids and has several other major metabolic functions (20, 43). In addition, GLN inhibits apoptosis and stimulates cell proliferation in both intestinal and immune cells (12, 14, 16, 42). Such functions may be crucial for GALT activation in response to bacterial invasion. Dietary or intravenous GLN supplementation inhibits bacterial translocation in a number of catabolic animal models with intact intestine (5–6, 8, 11, 13, 15, 17, 40). GLN may become a conditionally essential nutrient during catabolic stress owing to the increased need for this substrate by gut epithelia, concomitant with insufficient cellular capacity for endogenous GLN production (21, 35, 43).

The present study was designed to contrast the effect of dietary GLN supplementation and oral antibiotic administration on indexes of gut barrier function in a rat model of combined partial small bowel-colonic resection, a previously utilized translational model for human SBS.

METHODS

Animals. Young (6 wk) male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used. The rats were housed in...
GLUTAMINE, ANTIBIOTICS, AND GUT BARRIER FUNCTION

individual cages in the animal care facility under controlled conditions of temperature and humidity with a 12-h light, 12-h dark cycle. The animals were given free access to water and standard pelleted rat food (Laboratory Rodent Chow 5001, PMI Feeds, St. Louis, MO) during a 7-day acclimation period. The Institutional Animal Care and Use Committee of Emory University, Atlanta, GA approved the protocol.

Chemicals. Trypsin-chymotrypsin inhibitor (T9777), protein L (P3101), and phenylmethylsulfonyl fluoride (PMSF P7626) were purchased from Sigma-Aldrich (St. Louis, MO). MacConkey agar (DF0113-17-5) and blood agar were purchased from Fisher Scientific (Pittsburgh, PA) and Ward’s Natural Science (Rochester, NY), respectively. Protein block buffer (X0909) was from DAKO (Carpinteria, CA). For tight junction protein Western blotting experiments, the primary antibodies [rabbit anti-zonula occludens-1 (ZO-1) (61-7300) and mouse anti-occludin (33-1500)] were from Zymed. Anti-β-actin was from Sigma Chemical (St. Louis, MO). Secondary antibodies Alexa Fluor 680 goat anti-mouse (A31562) and donkey anti-goat (A21084) were from Molecular Probes (Carlsbad, CA), and IRdye 800 anti-rabbit IgG (KBF001) was from Rockland (Gilbertsville, PA). Protease inhibitor Complete Mini solution was from Roche (Indianapolis, IN). To detect total and anti-LPS IgG by ELISA, the secondary antibody was goat anti-rat IgG [horseradish peroxidase (HRP) conjugated, sc-2006] from Santa Cruz Biotechnology (Santa Cruz, CA). To detect fecal total, anti-flagellin, and anti-LPS IgG by ELISA, the secondary antibody was goat anti-rat IgA (Serotec, Raleigh, NC). The same antibody was also used for gut lamina propria IgA cell staining. Purified flagellin and LPS were provided by Dr. Andrew Gewirtz.

Experimental design. All rats were fed standard pellet rat chow prior to operation. After operation, the rats were fed semipurified diets custom prepared by Harlan Teklad (Madison, WI), with or without L-GLN supplementation (Table 1). The GLN content of GLN-supplemented diet was 4% of total diet weight, approximately fivefold higher than the GLN content derived from casein in the control diet [AIN-93G (29)]. The protein (casein) content in the GLN diet was reduced such that the GLN-supplemented and control diets were isocaloric and isonitrogenous (Table 1).

Operative procedures and pair feeding. Rats were randomized by body weight and fasted overnight before operation. The following day (day 1), rats underwent laparotomy between 9:00 AM and 1:00 PM, as previously described (23). Briefly, animals were anesthetized with intraperitoneal ketamine (100 g/l) and xylazine (20 g/l), and a ventral abdominal midline incision was performed. The locations for bowel transection (TX, operative control) and partial small bowel-colonic anastomosis (RX) were identified by using defined landmarks. The resected rats underwent removal of the distal 60% of the small intestine, the entire cecum, and 1 cm of the proximal colon. The transected rats had small bowel transection at 60 cm proximal to the ileal-cecal valve (23). After TX and RX procedures, the intestinal segments were anastomosed with sutures and 10 ml of saline was injected to the abdominal cavity for fluid resuscitation prior to abdominal closure with sutures and staples. Rats were allowed free access to water after operation and the pelleted study rat food begun on day 2. All groups of rats were pair fed to ensure similar food intake between groups. Body weight and food intake were determined daily from operation to euthanasia.

Experimental design. The study included four groups of rats: TX rats fed the control diet (TX/CON; n = 10), RX rats fed the control diet (RX/CON; n = 12), RX rats treated with an oral antibiotic (ABX) cocktail and fed the control diet (RX/ABX; n = 9), and RX rats fed the GLN-supplemented diet (RX/GLN; n = 13). The oral antibiotic cocktail consisted of neomycin (250 mg·kg⁻¹·day⁻¹), polymyxin B (9 mg·kg⁻¹·day⁻¹), and metronidazole (50 mg·kg⁻¹·day⁻¹), a regimen previously shown to decontaminate the gut lumen of rodents (18). The antibiotics were given in the drinking water, starting from 3 days prior to operation until tissue collection on day 21. Fecal samples were collected for sIgA and anti-LPS and anti-flagellin IgG concentrations 1 day prior to surgery (baseline) and on days 6, 13, and 20 postsurgery, and stored at −20°C. The rats were killed and tissue collected at 21 days after operation. MLN were obtained using sterile techniques for bacterial culture (26) and serum was obtained for specific anti-flagellin and anti-LPS IgG.

Tissue collection. The intestines of anesthetized rats were stripped of mesenteric and vascular connections and sequentially removed from the peritoneum. The lumen was flushed with ice-cold saline to clear intestinal contents and suspended from a ring stand with a constant distal weight. The segments used for the end points of this study were collected sequentially at the equivalent site in each rat. The gut segments used for tight junction protein determination were longitudinally cut, and the mucosa was obtained by gentle scraping with a glass slide and then placed in liquid nitrogen for storage. MLN were dissected from the mesentery and placed in sterile PBS on ice. Blood was drawn by cardiac puncture, from which serum was collected and stored at −80°C.

Bacterial culture and identification. MLN were homogenized with sterile glass tissue grinders (Kendall, MA) with 100 mg tissue/ml PBS and then plated on MacConkey agar to identify gram-negative enteric bacterial pathogens. The positive bacterial colonies were counted after incubation for 24 h at 37°C. Bacterial translocation to MLN was considered present when a sample had ≥10 colony-forming units per gram tissue. The positive colonies were subcultured on blood agar for an additional 24 h at 37°C, and Enterobacteriaceae species were identified by using a commercial clinical diagnosis kit as described by the manufacturer (API 20E, BioMerieux, Durham, NC).

Western blot analysis of apical junction proteins. We used Western blotting to examine expression of occludin and ZO-1, two key apical junction proteins known to regulate intestinal barrier function (22). Frozen mucosal samples from defined segments of jejunum and colon (−1 g) were placed in 6 ml of RIPA buffer with freshly added protease inhibitor mixture, homogenized, and stored on ice for 60 min, then centrifuged at 16,000 g for 25 min at 4°C. Samples (30 µg of total protein) was separated on Tris·HCl 4–20% polyacrylamide gels (Bio-Rad) and transferred to Hybond-ECL nitrocellulose membrane (Amersham Pharmacia). The membranes were incubated with anti-ZO-1 or anti-occludin antibodies (1:500) overnight at 4°C. Bound antibodies were detected with anti-rabbit and goat anti-mouse antibodies, specific for ZO-1 and occludin, respectively. The fluorescent bands of ZO-1 and occludin were visualized by use of an Odyssey Scanner (LI-COR, Lincoln, NE) and quantified by using a Molecular Dynamics Computing Densitometer. The membranes were then washed twice in PBS (20 min) and incubated with anti-cytokertinin (1:500, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C and detected as outlined above. Final intensities of ZO-1 and occludin were expressed as percentage of control samples normalized for cytokeratin expression.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Control Diet, g/kg</th>
<th>GLN-Supplemented Diet, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-casein</td>
<td>200</td>
<td>160</td>
</tr>
<tr>
<td>L-GLN</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Corn starch</td>
<td>397</td>
<td>397</td>
</tr>
<tr>
<td>Maltodextrin*</td>
<td>132</td>
<td>132</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Cellulose† (fiber)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mix‡</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mix§</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitrate</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*Dextrinized corn starch; †Solka-Floc; ‡AIN-93G-MX; §AIN-93G-MX.

Please see METHODS for details of feeding regimens.
Fecal and serum immunoglobulin analysis. Fecal and serum Ig concentrations were determined by ELISA, as described (34, 44). In each case, the secondary antibody was detected with tetramethyl-benzidine and read at 650 nm with a VersaMax Tunable Microplate Reader (Basel, Switzerland). Fresh stool samples were dried for 30 min in a vacuum dryer at room temperature, vortexed for 30 min in PBS with trypsin-chymotrypsin inhibitor (0.1 mg/ml), and centrifuged at 13,000 rpm at 4°C for 15 min. PMSF was added to the supernatant containing sIgA to 1.0 mM concentration. For stool total sIgA concentrations by ELISA, the microplate wells were coated with Protein L (2.5 μg/ml) in bicarbonate buffer (pH 9.6) at 4°C overnight and incubated with the samples at 37°C for 1 h. The sIgA was incubated by secondary antibody (goat anti-rat IgA, HRP conjugated, diluted 2,000×). To detect fecal anti-flagellin IgG by ELISA, the microplate (Luminex, Dynex Technology) was coated with purified flagellin (20 μg/ml) in bicarbonate buffer (pH 9.6) at 4°C overnight, then blocked with 1% BSA for 20 min and incubated with the diluted sample (10×) at 37°C for 1 h. The anti-LPS IgG was incubated with secondary goat anti-rat IgA (HRP conjugated, diluted by 15,000×) at 37°C for 1 h. Analysis for serum anti-LPS and anti-flagellin IgG levels was determined as previously described (34, 44). For serum total IgG by ELISA, the microplate wells were coated with Protein L (2.5 μg/ml) in bicarbonate buffer (pH 9.6) at 4°C overnight, then blocked with 1% BSA for 2 h and incubated with the samples (diluted 100,000 to 10,000×) at 37°C for 1 h. The plate was incubated with secondary antibody goat anti-rat IgG (HRP conjugated, diluted by 15,000×). For the serum anti-LPS ELISA, the microplate was coated with LPS (1 μg/ml) in bicarbonate buffer (pH 9.6) at 4°C overnight, then blocked with 1% BSA for 20 min and incubated with the diluted sample (10×) at 37°C for 1 h. The anti-LPS IgG was incubated with secondary goat anti-rat IgA (HRP conjugated, diluted by 5,000×) at 37°C for 1 h.

Analysis for serum anti-LPS and anti-flagellin IgG levels was determined as previously described (34, 44). For serum total IgG by ELISA, the microplate wells were coated with Protein L (2.5 μg/ml) in bicarbonate buffer (pH 9.6) at 4°C overnight, then blocked with 1% BSA for 2 h and incubated with the samples (diluted 100,000 to 10,000×) at 37°C for 1 h. The plate was incubated with secondary antibody goat anti-rat IgG (HRP conjugated, diluted by 15,000×). For the serum anti-LPS ELISA, the microplate was coated with LPS (1 μg/ml) in bicarbonate buffer (pH 9.6) at 4°C overnight, then blocked with 1% BSA for 20 min and incubated with the diluted sample (10×) at 37°C for 1 h. The anti-LPS IgG was incubated with secondary goat anti-rat IgA (HRP conjugated, diluted by 15,000×) at 37°C for 1 h.

Jejunal lamina propria IgA immunohistochemistry. After deparaffinization, sections of defined portions of proximal jejunum were placed in a solution containing proteinase K (20 μg/ml), Tris–Cl (50 mM), and EDTA (5 mM) for antigen retrieval at 37°C for 10 min. Sections were then quenched in 1% H2O2 at room temperature for 10 min and blocked with blocking buffer for 10 min. IgA cells were detected with goat anti-rat IgA (HRP conjugated) and stained with diaminobenzidine. Sections were counterstained with hematoxylin, dehydrated, and mounted. The IgA-positive cells in the lamina propria of at least 10 well-oriented jejunal villi per rat were counted in a blinded manner (J. Tian) and normalized to total villus area. Total villus area was determined with a digital imaging system (Quantification Imaging, Burnaby, BC, Canada) and image processing software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD).

Statistical analysis. Data were analyzed by one-way ANOVA and the Fisher’s protected least-significant difference post hoc test when a significant difference was indicated by one-way ANOVA. The incidence of bacterial translocation to MLN between groups was compared by the Fisher’s exact test. All statistical analysis was performed by use of SPSS software (Chicago, IL). Data are presented as means ± SE. P values < 0.05 were considered statistically significant.

RESULTS

Food intake and body weight. Mean daily food intake during the 21-day study period was similar between study groups (TX/CON 18.9 ± 0.3, RX/CON 18.3 ± 0.6, RX/ABX 17.9 ± 0.7 and RX/GLN 18.6 ± 0.6 g/day, respectively; not significant (NS)). Mean daily body weight fell in the first few days after operation and then increased during the 3-wk postoperative period, without significant differences between the four study groups (Fig. 1). The body weight of RX rats lagged behind TX rats during the 3-wk postoperative period, likely because of removal of intestinal mass and malabsorption-induced diarrhea with RX.

Bacterial translocation to MLN and serum total and anti-LPS IgG. The incidence of gram-negative bacterial translocation to MLN at day 21 was markedly increased in bowel-resected rats compared with transected control rats [TX/CON 1 of 10 (10%) vs. RX/CON 8 of 12 (75%); P < 0.05] (Fig. 2A). Using the gram-negative bacterial colonies isolated on MacConkey agar at 24 h, we identified three gram-negative facultative gut-derived bacteria (Enterobacteriaceae) present in MLN (Table 2). Escherichia coli accounted for most cases of bacterial translocation (60%), followed by Enterobacter cloacae (27%) and Klebsiella pneumoniae (13%) (Table 2).

Bacterial translocation in the RX/CON group was accompanied by a significant increase in serum total IgG (Fig. 2B) and in serum anti-LPS IgG levels (Fig. 2C). Treatment with an oral antibiotic cocktail (metronidazole, neomycin, and polymyxin B) (18) completely blocked bacterial translocation and was associated with control levels of serum total IgG and LPS-specific IgG (Fig. 2). Postoperative GLN supplementation decreased the incidence of bacterial translocation to MLN at 21 days postoperatively from 75 to 46% (Fig. 2A; NS vs. control, P = 0.214). Dietary GLN did not change total serum total IgG (P = 0.576, Fig. 2B) but was associated with a significant decrease in anti-LPS IgG (P < 0.05, Fig. 2C). We did not detect any changes in anti-flagellin IgG between groups (not shown).

Effects of dietary GLN and oral antibiotics on total sIgA and LPS-specific IgA in the gut lumen. Partial small bowel-colonic resection alone (RX/CON group) significantly increased luminal sIgA by day 13 compared with TX/CON rats (Fig. 3). However, this response was diminished by day 20 after operation, as values became statistically indistinguishable from baseline or day 6 values (Fig. 3). These data suggest possible intestinal immune adaptation to massive bowel resection, possibly in response to bacterial translocation. Treatment with the oral antibiotic cocktail completely inhibited the increase in stool IgA induced by partial small bowel-colonic resection.
(Fig. 3), concomitant with the prevention of bacterial translocation. Supplementation of GLN in the diet after RX did not alter stool IgA at days 6 and 13 postoperatively compared with RX rats fed control diet. However, dietary GLN markedly increased stool IgA levels at day 20 after operation, at a time when values in RX rats given nonsupplemented diet were declining ($P < 0.05$ vs. TX/CON, RX/CON, and RX/ABX groups) (Fig. 3). To determine whether this GLN response was due to increased sIgA production by mucosal plasma cells, we performed immunohistochemistry studies. The data show a marked increase in IgA-positive lamina propria plasma cells in residual jejunum with dietary GLN after RX compared with the other three experimental groups (Fig. 4, A and B).

Recent reports indicate that the interaction of LPS with host Toll-like receptor-4 (TLR4) is essential for *E. coli* transcytosis (25). Likewise, gram-negative bacteria-derived flagellin interaction with TLR5 on the basolateral surface of intestinal epithelia appears to be essential for *Salmonella* sp. invasion (34, 38). S IgA in the gut lumen may serve to block LPS and flagellin as key virulence factors regulating bacterial entry (43). Therefore, we investigated the concentrations of specific anti-flagellin and anti-LPS sIgA in the stool of our models on day 20. The results show that stool levels of anti-flagellin IgA were unchanged with RX, with or without dietary GLN, and tended to decrease with antibiotic administration (NS vs. the other groups) (Fig. 5A). In contrast, RX tended to increase the concentration of anti-LPS sIgA in stool (NS vs. TX/CON), and this response was further and significantly increased by both oral antibiotics and dietary GLN supplementation in RX rats (Fig. 5B).

**Tight junction protein expression.** Expression of the major tight junction proteins occludin and ZO-1 by Western blot (corrected for cytokeratin expression) in jejunum and colon at day 21 was not different between the four groups (Table 3).

**DISCUSSION**

Patients with SBS commonly develop systemic infection with gut-derived microorganisms (5–7), and animal models of SBS demonstrate an increased rate of bacterial translocation from the gut (1–4). In this study, we explored gut barrier function indexes and two potential therapeutic approaches, oral antibiotic administration to diminish luminal microflora and dietary GLN supplementation, in a rat model involving partial small bowel and proximal colonic resection with loss of the

---

**Table 2. Identification of bacteria in MLN**

<table>
<thead>
<tr>
<th></th>
<th>TX/CON</th>
<th>RX/CON</th>
<th>RX/ABX</th>
<th>RX/GLN</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>1/10</td>
<td>4/12</td>
<td>0/9</td>
<td>4/13</td>
<td>60%</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>0/10</td>
<td>3/12</td>
<td>0/9</td>
<td>1/13</td>
<td>27%</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>0/10</td>
<td>1/12</td>
<td>0/9</td>
<td>1/13</td>
<td>13%</td>
</tr>
</tbody>
</table>

Bacteria were isolated from mesenteric lymph nodes (MLNs) and identified as described in methods. TX, small bowel transection; CON, control diet; RX, small bowel resection; ABX, oral antibiotics; GLN, glutamine-supplemented diet. Data presented in the cells under each study group are the number of rats with positive translocation of a certain species over total number of rats in the group. The column Total shows the percentage of the rats with translocation of specific bacterial species in the 4 groups of rats.

---

**Fig. 2. Bacterial translocation to mesenteric lymph nodes (MLN) and serum total IgG and anti-LPS IgG levels. A: gram-negative bacterial translocation to MLN, defined as $\geq 10$ colony-forming units per gram tissue. B: serum total IgG measured by ELISA in optical density units. C: serum LPS-specific IgG measured by ELISA in optical density units. Experimental procedures and study groups as outlined in METHODS and Fig. 1. †$P < 0.05$ RX/CON vs. TX/CON and RX/ABX, #$P < 0.05$ vs. all other groups; $N = 9–11$ in each group.**

**Fig. 3. Stool secretory immunoglobulin A (sIgA) concentration over time. Stool samples were collected 1 day prior to surgery and on days 6, 13, and 20 after operation and analyzed for IgA content by ELISA as outlined in METHODS. Operative procedures, diets, antibiotic treatment, and study group abbreviations are as described in METHODS and Fig. 1. *$P < 0.05$ RX/CON and RX/GLN vs. TX/CON and RX/ABX, respectively, at day 13 postoperatively; #$P < 0.05$ RX/GLN vs. all other study groups; $N = 9–11$ in each group.**
ileal cecal valve (ICV). Conflicting data have been published on bacterial translocation in animal models of massive small bowel resection coupled with ICV and/or cecal loss (1–3), a common scenario in human SBS. Our data clearly show that a high incidence of gram-negative bacterial translocation occurs in this massive bowel resection model. We did not culture for other microbes, such as yeast or anaerobes, or examine blood or other organs such as liver for microbial colonization. However, such studies would be of interest to complement our findings, particularly with regard to the potentially beneficial effects of antibiotics and GLN.

Alterations in luminal sIgA or adaptive immune responses to bacterial antigens such as flagellin and LPS following massive bowel resection in animal SBS models have not been previously studied, to our knowledge. Our data indicate that luminal sIgA is markedly increased by 13 days following bowel resection and then declines modestly by day 20. Bacterial translocation with RX occurred temporally with increased total and LPS-specific IgG in serum. This activation of adaptive systemic immunity was likely induced by basolateral exposure to translocated bacterial LPS. Of interest, we did not detect any changes in serum anti-flagellin antibodies in our models (not shown). These differential results suggest that the SBS-associated systemic immune response may target LPS more than flagellin in our rat model. SBBO or other factors, perhaps related to the bowel resection itself, may have contributed to the increase in luminal sIgA observed ~2 wk following RX.

SBS is associated with an increased risk of SBBO in humans and in animal models (3, 7, 19). In addition to causing malabsorption, SBBO may be a factor involved in gut-derived infection as suggested by rat SBS models in which postoperative SBBO was associated with the increased bacterial translocation (31). Oral antibiotics are commonly used on an empiric basis to treat presumed SBBO in SBS patients, but to our knowledge no studies have explored the effects of antibiotics on bacterial translocation or immune responses to bacteria-
Table 3. Expression of tight junction proteins occludin ZO-1 in jejunum and colon

<table>
<thead>
<tr>
<th>Protein-to-Cytokeratin Ratio</th>
<th>TX/CON</th>
<th>RX/CON</th>
<th>RX/ABX</th>
<th>RX/GLN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum occludin</td>
<td>0.040±0.004</td>
<td>0.079±0.028</td>
<td>0.108±0.038</td>
<td>0.105±0.055</td>
</tr>
<tr>
<td>Jejunum ZO-1</td>
<td>0.155±0.010</td>
<td>0.175±0.013</td>
<td>0.172±0.011</td>
<td>0.166±0.009</td>
</tr>
<tr>
<td>Colon occludin</td>
<td>0.384±0.152</td>
<td>0.689±0.105</td>
<td>0.420±0.179</td>
<td>0.344±0.121</td>
</tr>
<tr>
<td>Colon ZO-1</td>
<td>0.186±0.180</td>
<td>0.181±0.017</td>
<td>0.172±0.011</td>
<td>0.165±0.003</td>
</tr>
</tbody>
</table>

Data as means ± SE (N = 6–8/group); zonula occludens-1 (ZO-1) and occludin are corrected for expression of cytokeratin as a loading control. All comparisons are not significant between groups. 

Our findings of potential translational significance for future studies in human SBS and suggest that the observed increase in total and LPS-specific IgG in the circulation may be an adaptive immune response to gram-negative bacterial translocation in this rat model. Of note, oral antibiotics markedly inhibited the increase in luminal total sIgA observed in RX animals not given antibiotics, presumably because of decreased viable bacteria within the lumen. Paradoxically, antibiotic administration significantly increased stool LPS-specific IgA but the reasons for this are unclear at present.

Enteral and parenteral GLN is being studied as a potential therapeutic nutrient in intestinal failure and other catabolic conditions (43). Studies have shown that GLN inhibits bacterial translocation in a number of non-SBS models (6, 8, 11, 15, 43), but the mechanism(s) for this remains unclear. When given intravenously, GLN has been shown to attenuate the decrease of gut mucosal plasma cells and sIgA in rats administered total parenteral nutrition (1, 5). The present study shows for the first time that enteral GLN supplementation upregulates jejunal plasma cell expression of IgA, stool total sIgA, and stool anti-LPS IgA. GLN reduced gram-negative bacterial translocation to MLN by a nonsignificant 29% vs. resected controls; however, the translocation rate was statistically similar to the RX + antibiotics group in which there was no translocation. In addition, GLN supplementation was associated with significantly decreased serum anti-LPS IgG (but not total serum IgG), potentially because of the modest attenuation of gram-negative bacterial translocation. Translocation of other intestinal microorganisms that we did not culture may have occurred, which may be a possible explanation for the maintenance of total serum IgG levels with GLN. Although direct data confirming an effect of sIgA to prevent bacterial translocation in vivo are not available, our findings are also consistent with the notion that GLN may potentially attenuate bacterial translocation specifically through IgA blockade of LPS, which has recently been shown to mediate E. coli transcytosis through interaction with TLR4 in vitro (25).

The mechanism(s) by which enteral GLN increases IgA cell response remains unclear, but studies focused on defining potential underlying mechanisms for this effect are of interest in light of our findings. GLN stimulates both the number and function of gut mucosal plasma cells and sIgA in rat models of total parenteral nutrition (please see Refs. 1, 5 and, when given intravenously, GLN has been shown to attenuate the decrease of gut mucosal plasma cells and sIgA in rat models of total parenteral nutrition; please see Refs. 1, 5). We did not examine whether GLN increases plasma cell proliferation per se, but such information would be of interest. It is also possible that the mucosal IgA response in GLN-treated animals could reflect a differential effect on SBSO such that there may have a higher concentration of small bowel luminal bacteria in RX/GLN compared derived antigens either in human SBS or in animal SBS models. We show here that treatment with a triple antibiotic regimen completely blocked gram-negative bacterial translocation and prevented the increase in serum total IgG and LPS-specific IgG (Fig. 2). We used an oral antibiotic cocktail consisting of neomycin, polymyxin B, and metronidazole because this regimen was previously shown to decontaminate the gut lumen of rodents (please see Ref. 18). All three antibiotics are used clinically (although polymyxin B is currently rarely used and neomycin is infrequently used); neomycin and metronidazole are prescribed as individual oral agents in patients with SBSO. To our knowledge, the specific combination of neomycin, polymyxin B, and metronidazole has not been studied as a selective gut decontamination regimen in humans.
with RX/CON or RX/ABX. Studies to test this hypothesis would be of interest. The measured stool IgA could represent secretion of this immunoglobulin into the lumen as well as that present in cells lost into the lumen; we cannot distinguish these processes with the methods employed in this study. We suspect that the increase in stool anti-LPS IgA with GLN was most likely due to upregulated gut mucosal IgA (Figs. 3 and 4). In contrast, the increase in stool anti-LPS IgA in the RX/ABX rats likely occurred by a different mechanism, because we observed a significant decrease of total fecal sIgA with this treatment. It is possible that gram-negative organisms resistant to the antibiotic cocktail may have stimulated LPS sIgA production.

RX alone or oral antibiotics or dietary GLN combined with RX did not regulate jejunal or colonic expression of ZO-1 or occludin by Western blot. These pilot studies suggests that mechanisms other than steady-state expression of these key tight junction proteins were responsible for the differential effects of RX to increase and antibiotics and GLN to decrease bacterial translocation, respectively. However, it is possible that expression and/or function of these and other apical junctional proteins such as claudins, etc. (22) have played a role in the differential incidence of bacterial translocation in the four study groups. Additional studies on potential regulation of important tight junction and adherence junction proteins, their functional attributes, and cellular localization in response to GLN and oral antibiotics in in vivo SBS models would be of interest.

In summary, our results indicate that gram-negative bacterial translocation as a result of partial small bowel-colonic resection is accompanied by an adaptive luminal and systemic immune response to LPS in rats. Use of oral antibiotics completely prevented gram-negative bacterial translocation and systemic immune responses to LPS in this model. Dietary supplementation with GLN inhibits, but does not prevent, gram-negative bacterial translocation and is associated with significantly decreased LPS-specific IgG in serum. This evidence for improved gut barrier function with enteral GLN may be due, in part, to upregulated production of protective anti-LPS sIgA and an increased total concentration of IgA in the gut lumen over time. Our findings suggest an important role for sIgA as an endogenous factor to prevent postoperative bacterial translocation in SBS and provides a potential mechanism for GLN action in this setting.

ACKNOWLEDGMENTS

We acknowledge Dr. Matam Vijay-Kumar for helpful discussions.

GRANTS

This research was supported, in part, by National Institutes of Health Grants R01 DK55850 (T. R. Ziegler), R01 DK061417 (A. T. Gewirtz), R01DK064730 (I. R. Williams), R01 ES011195 (D. P. Jones), and the Emory Epithelial Pathobiology Research Development Center grant R24 DK064399.

REFERENCES


