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Recovery of Gut-Associated Lymphoid Tissue and Upper Respiratory Tract Immunity After Parenteral Nutrition

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Objective

The authors characterize the recovery of parenteral nutrition-induced changes in gut-associated lymphoid tissue (GALT) and upper respiratory tract immunity with enteral nutrition and provide further information defining the effects of enteral feeding on mucosal immunity.

Summary Background Data

The small intestine plays a prominent role in development and maintenance of mucosal immunity, both intestinal and extraintestinal, primarily through immunoglobulin A (IgA)-mediated mechanisms. Prior research has shown that mice fed total parenteral nutrition (TPN) have reduced GALT T and B cells, the cells responsible for IgA production, as well as impaired upper respiratory tract immunity to viral challenge of previously immunized animals. The recovery of TPN-induced changes in GALT and upper respiratory tract immunity after enteral refeeding is studied.

Methods

Male Institute of Cancer Research mice received 5 days of TPN followed by 0 to 4 days of chow. Small intestinal GALT was characterized by flow cytometry. In a second experiment, animals were immunized intranasally with moused-adapted influenza virus. Three weeks later, one group received a 5-day course of TPN followed by enteral refeeding for 5 days. A second group received TPN alone. Both groups were challenged with intranasal virus and killed 40 hours postchallenge to determine viral shedding from the upper respiratory tract.

Results

Animals fed TPN only had significantly fewer GALT lymphocytes compared with those chow-fed control subjects. Peyer's patch counts increased after a single day of refeeding, returning to normal levels by 48 hours. Lamina propria counts remained significantly depressed after 24 hours of refeeding, but also returned to normal after 48 hours of refeeding. The T-cell and B-cell populations mimicked total cell patterns. Lamina propria CD4+/CD8+ ratio returned to normal only after 72 hours of refeeding. None of the 9 animals refed enterally for 5 days were positive for viral shedding, compared with 8 of 12 matched TPN-fed animals.

Conclusions

Enteral refeeding after TPN is associated with rapid repletion of GALT cellularity, initially within Peyer's patches and subsequently within the lamina propria. Refeeding corrects the impairment of IgA-mediated upper respiratory tract antiviral immunity occurring with TPN administration. This work further enhances the authors' knowledge of the underlying immunologic differences influenced by routes of nutrition.

Total parenteral nutrition (TPN) can play a vital role in maintaining the nutritional status of critically ill patients; however, recent evidence suggests that this form of nutritional support may not support all immunologic defenses, particularly the mucosal immune system.[1-7] The enteral route appears to prevent such deficits, as reflected by a number of experimental and clinical trials showing a reduction in infectious complications when comparing enteral to parenteral feeding.[7-12] Although the mechanism behind this difference remains unclear, lack of enteral nutrition may impair the gastrointestinal mucosal barrier and depress its function as an organ and source of immunity.

Prior research has focused on mucosal immunity, investigating the effect that route and type of nutrition has on the gut-associated lymphoid tissue (GALT) and the association of these changes with extraintestinal immunity. Animals fed TPN have significantly fewer lamina propria, intraepithelial, and Peyer's patch lymphocytes and reduced levels of intestinal immunoglobulin A (IgA) compared with chow-fed animals or mice fed complex enteral diets via gastrostomy.[5] Additionally, TPN administration impairs established IgA-mediated upper respiratory tract immunity to influenza virus in previously immunized animals.[6] The purpose of this study was to characterize the recovery of GALT and upper respiratory tract immunity occurring with reinitiation of an enteral diet after a course of parenteral nutrition.

MATERIALS AND METHODS

Animals

These studies conform to the guidelines for the care and use of laboratory animals established by the Animal Care and Use Committee of The University of Tennessee, and protocols were approved by that committee. Male Institute of Cancer Research mice (Harlan, Indianapolis, IN) were housed in an American Association for the Accreditation of Laboratory Animal Care-accredited conventional facility under controlled conditions of temperature and humidity with a 12:12 light:dark cycle. Mice were fed commercial mouse chow with water *ad libitum* for 2 weeks before protocol entry. During the experiments, the mice were housed in metal metabolism cages with wire-grid bottoms to eliminate coprophagia and bedding ingestion.

Experimental Design and Formulas

In experiment 1, all mice underwent placement of catheters for intravenous infusion after an intraperitoneal injection of ketamine (100 mg/kg per body weight) and acepromazine maleate (10 mg/kg per body weight) mixture. A silicone rubber catheter (0.012-in intradermal × 0.025-in O.D. Baxter, Chicago, IL) was inserted into the vena cava through the right jugular vein. The distal end of the catheter was tunneled subcutaneously and exited at the midpoint of the tail. The mice were immobilized partially by tail restraint to protect the catheter during infusion. This infusion technique has proved to be an acceptable method of nutritional support and does not produce physical or biochemical evidence of stress in the mouse.[13] Catheterized mice were connected immediately to an infusion apparatus, and saline was infused at an initial rate of 4 mL/day. All mice were allowed *ad libitum* access to chow (Agway, Syracuse, NY) for the first 2 days to recover from surgery and then were randomized to either the chow-fed control group (chow, n = 9) or to one of five experimental groups, all of which received 5 days of TPN. These TPN groups received a standard TPN solution intravenously as described previously (prepared in the hospital pharmacy).[5] The TPN solution provided 5597 kJ with a total nonprotein calorie/nitrogen ratio of 731 kJ:1. The chow group received an infusion of physiologic saline in addition to standard laboratory mouse diet and water *ad libitum.* After postoperative chow feeding, the TPN infusion rates were increased over a 48-hour period to 10 mL/day and were continued at those rates for the 5 days of parenteral feeding. This provided approximately 54.6 kJ energy and 67 mg nitrogen, meeting the calculated requirements for mice weighing 25 to 30 g.[14]

After 5 days of TPN, mice in one experimental group (day 0, n = 8) were killed immediately and served as the TPN-only group. Animals in the remaining four experimental groups were fed chow *ad libitum* and were killed

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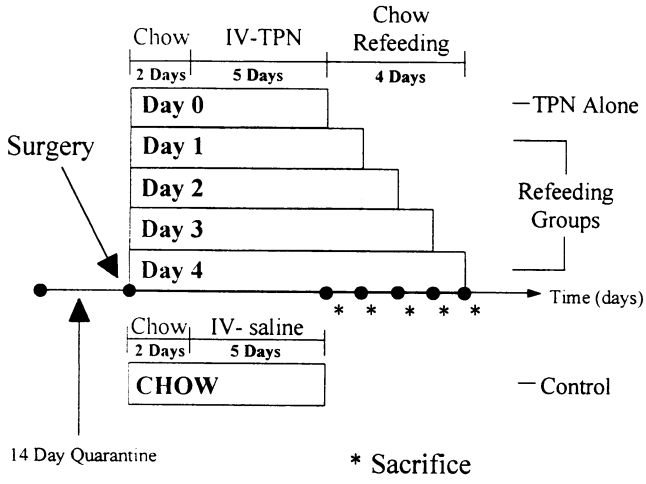


Figure 1. Protocol for recovery study.

at 24 hours (day 1, n = 9), 48 hours (day 2, n = 9), 72 hours (day 3, n = 6), and 96 hours (day 4, n = 9) after onset of chow refeeding (Fig. 1). Mice were weighed and anesthetized with the ketamine–acepromazine maleate mixture. The thoracic and abdominal cavities were opened aseptically, and the animals were exsanguinated by cardiac puncture. Lymphocyte preparations then were harvested from the entire small intestine.

In experiment 2, all mice were administered A/PR8 (H1N1), a mouse-adapted influenza virus, intranasally while awake (a 20-μL volume containing 10^5 mouse 50% lethal doses). After 3 weeks to allow for establishment of mucosal immunity, the mice were given an intraperitoneal injection of ketamine (100 mg/kg per body weight) and acepromazine maleate (10 mg/kg per body weight) mixture and underwent placement of catheters for intravenous infusion. These mice (refed, n = 9) then received 2 days of chow *ad libitum* for 5 days and paired with a cohort of similarly immunized mice (TPN, n = 12) that had

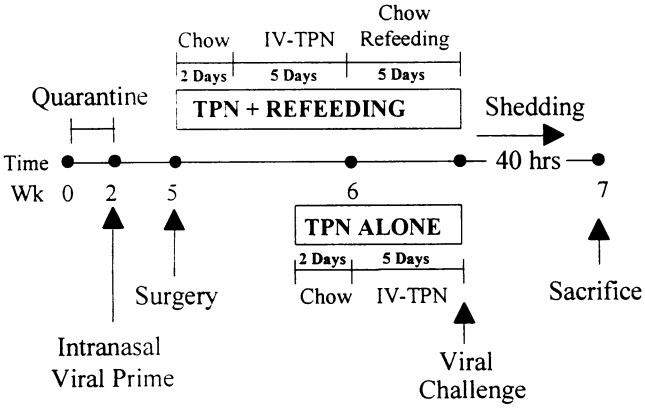
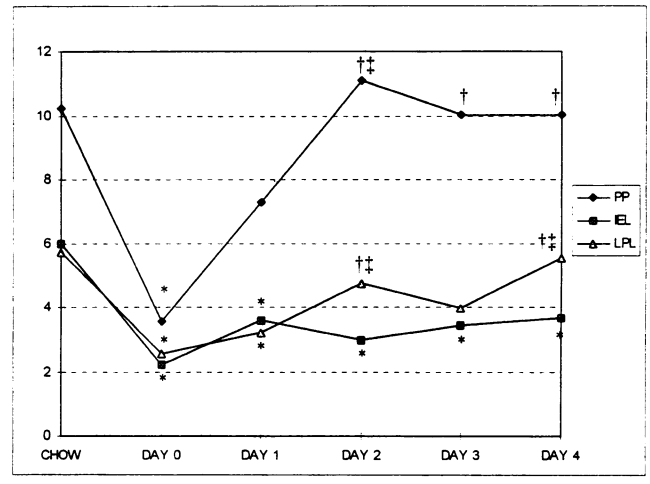


Figure 2. Protocol for viral challenge study.



Cell Counts: PP (x 10^6) IEL (x 10^5) LPL (x 10^6)

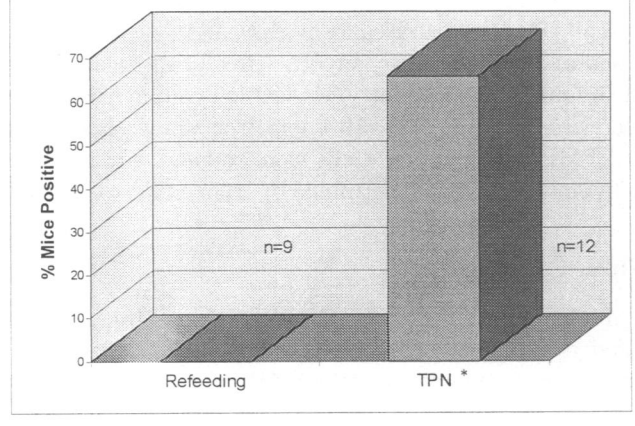
* p<0.05 vs. CHOW: † p<0.05 vs. Day 0: ‡ p<0.05 vs. Day 1

Figure 3. Absolute cell counts from the Peyer's patches, intraepithelial space, and lamina propria. Recovery was first noted within the Peyer's patches, followed by the lamina propria. Intraepithelial lymphocytes failed to improve.

undergone similar catheter placement, 2 days of chow-fed recovery, and TPN for 5 days. Both groups were challenged on the same day, intranasally while awake, with influenza virus and allowed to continue their respective diets for an additional 40 hours (Fig. 2). Both groups were killed simultaneously to determine viral shedding from the upper respiratory tract; normal convalescent mice shed no virus because of intact IgA-mediated mechanisms.

Cell Isolations

Lymphocyte isolations from the Peyer's patches were performed as described by Deitch et al.[15] The Peyer's



* p=0.005 vs. Refeeding

Figure 4. Five days of chow refeeding completely reversed the TPN-induced impairment in respiratory immunity.

patches were excised from the serosal side of the intestine and teased apart with 18-gauge needles. The fragments were treated with type 1 collagenase (Sigma, St. Louis, MO) (50 units/mL) in modified essential medium for 60 minutes at 37 C with constant rocking. After collagenase digestion, the cell suspensions were passed through nylon filters.

Lamina propria lymphocytes and intraepithelial lymphocytes were isolated as follows. The small intestine was removed, flushed with Hanks balanced salt solution (HBSS) to remove intestinal contents, measured, and weighed. After excision of the Peyer's patches, the intestine was opened lengthwise and cut into 5-mm pieces. The pieces were incubated three times, 30 minutes each time, with prewarmed (37 C) calcium and magnesium-free HBSS containing 5 mM ethylene diaminetetraacetic acid (EDTA) (Sigma, St. Louis, MO) in a flask on a magnetic stirrer at 20 rpm at 37 C. The supernatants containing sloughed epithelial and intraepithelial cells from each incubation period were pooled and stored on ice for further purification.

To block residual EDTA activity, the remaining tissue pieces were incubated for 30 minutes at 37 C with RPMI-1640 (Gibco, Gaithersburg, MD) containing 5% heat-inactivated fetal bovine serum. The RPMI-1640 was decanted and 30 mL RPMI-1640 containing 40 units/mL collagenase (type I, 30 units/mL; type III, 10 units/mL) and 5% inactivated fetal bovine serum was added to the flask, which then was incubated on a magnetic stirrer (100 rpm) at 37 C. Released cells were decanted from the tissue fragments. Fresh enzyme-containing media was added, and the process was repeated twice (30 minutes each time) for a total time of 90 minutes. After the third extraction, pooled cells were mixed gently and placed on ice for 10 to 15 minutes to let larger debris settle.

Supernatants containing lymphocytes, debris, and dead cells were filtered through a glass-wool column. The suspensions were centrifuged, pellets resuspended in 40% Percoll (Pharmacia, Piscataway, NJ), and the cell suspensions overlaid on 70% Percoll. After centrifugation for 20 minutes at 600 g at 4 C, viable lymphocytes were recovered from the 40%/70% interface and washed twice in RPMI-1640 medium. Cells were counted, and viability of lymphocytes was determined by trypan blue exclusion.

Flow Cytometry

To determine the phenotypes of the lymphocytes isolated from the Peyer's patches and lamina propria, 10^5 cells were suspended in 50 μL HBSS containing either fluorescein-conjugated (FITC) anti-CD3 (clone 145-2C11; Pharmigen, San Diego, CA) or phycoerythrin-conjugated (PE) goat anti-mouse immunoglobulin (Southern Biotechnology Associates, Birmingham, AL) to identify T cells and B cells, respectively, or in FITC-anti-CD4 (clone RM4-5) and PE-anti-CD8 (clone 53–67; Pharmigen) to identify the two T-cell subsets. All antibodies were diluted to 2.5 μg/mL in HBSS containing 0.1% azide; incubations were for 30 minutes on ice. After staining, the cells were washed twice in HBSS and were fixed in 1% paraformaldehyde (Sigma, St. Louis, MO). Flow cytometry analysis was performed on a Profile I counter (Coulter, Hileah, IL).

Virus Preparation

A/PR8-Mt. Sinai (H1N1) influenza virus was the gift of Parker A. Small, Jr., M.D. (University of Florida, Gainesville). To generate a pool, the virus was grown in specific pathogen-free, fertile eggs, pooled, filtered through a 0.45-μm filter, aliquoted, and stored at −70 C. The 50% lethal dose for mice was established by the intranasal inoculation of 50 μL of tenfold serial dilutions of the virus pool into anesthetized mice. The $\log_{10}$ 50% mouse lethal dose of the virus pool was 10^5.

Nasal Secretion Collection (Experiment 2)

Nasal secretions were collected as follows. The mouse was placed in dorsal recumbency, and the trachea was clamped at the thoracic inlet through a midline neck incision. Six hundred microliters of cold Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and antibiotics, as detailed below, was injected slowly into the tracheal lumen cephalad to the obstruction. The wash fluid draining from the nostrils was collected in a microcentrifuge tube and placed on ice, and the viral assays were conducted immediately.

Viral Assay

Viral samples were diluted serially (tenfold) in Dulbecco's modified Eagle's medium supplemented with 2.5 μg/mL amphotericin B, 50 μg/mL gentamicin, and 10% fetal bovine serum. Triplicate 100-μL samples of each dilution were placed in 96-well round-bottom tissue culture plates. To each well, 100 μL of a 2×10^5 cells/mL suspension of Madin–Darby canine kidney cells in antibiotic-supplemented Dulbecco's modified Eagle's medium–10% fetal bovine serum was added. Plates were incubated at 5% carbon dioxide and 37 C. Culture fluid was removed 24 hours later and replaced with Dulbecco's modified Eagle's medium containing 2.5 μg/mL gentamicin, 2.5 μg/mL amphotericin B, and 2 μg/mL trypsin. Plates were incubated 4 days longer. Assay for viral growth was determined by hemagglutination. To each well, 50 μL of a 0.5% suspension of chicken erythrocytes was added. Hemagglutination was read after 1 to 2 hours in the cold.

Table 1. TOTAL CELL YIELD

	Days of Chow Refeeding After Total Parenteral Nutrition					
	Chow	Day 0	Day 1	Day 2	Day 3	Day 4
Peyer's patch ($\times 10^6$)	10.2 ± 1.5	3.5 ± 1.0*	7.3 ± 1.3	11.1 ± 1.6†	10.0 ± 1.3†	10.0 ± 1.4†
Intraepithelial ($\times 10^5$)	6.0 ± 0.4	2.2 ± 0.5*	3.6 ± 0.6*†	3.0 ± 0.5*	3.4 ± 0.5*	3.7 ± 0.3*†
Lamina propria ($\times 10^6$)	5.7 ± 0.6	2.5 ± 0.7*	3.2 ± 0.3*	4.7 ± 0.4†‡	4.0 ± 0.5*	5.5 ± 0.6†‡

* $p < 0.05$ *vs.* chow.
† $p < 0.05$ *vs.* day 0.
‡ $p < 0.05$ *vs.* day 1.

Statistical Analysis

All data were expressed as the mean ± the standard error of the mean. Statistical analysis was carried out by analysis of variance and Scheffe's multiple comparison and simple linear regression procedure, using Statview (Brain Power, Calabasas, CA) software. In experiment 2, the Fisher's exact test was used for analysis of virus shedding. A p value of 0.05 or less was considered significant.

RESULTS

Body Weight

In experiment 1, body weight of animals at the time of they were killed in the group receiving TPN alone (day 0) was lower than that of the chow-fed control subjects, but this did not reach statistical significance (day 0, 26.1 ± 1.4 g *vs.* chow, 29.7 ± 1.8 g). Animals in groups that received chow refeeding all had greater body weight than did those in the group receiving TPN alone but were similar to chow-fed animals (day 1, 30.9 ± 1.1 g; day 2, 31 ± 1.2 g; day 3, 35.4 ± 1.2 g; day 4, 32.5 ± 1.2 g; all p = not significant *vs.* chow; $p > 0.05$ *vs.* TPN alone). In experiment 2, there were no statistically significant postexperiment body weight differences between the group receiving TPN alone and the group chow refed after TPN (30.6 ± 1.2 g *vs.* 33.7 ± 1.5 g, p = not significant).

Total Cell Yields

The TPN alone (day 0 mice) significantly lowered total cell yield in the Peyer's patches, the intraepithelial layer, and lamina propria compared with chow-fed control levels (Table 1) ($p < 0.05$). Peyer's patch total cells approached chow control levels after 24 hours of refeeding (day 1 *vs.* chow, p = not significant), although they did not reach a statistically significant difference from TPN alone levels (day 1 *vs.* day 0; p = 0.13). Peyer's patch total cells were significantly improved from TPN alone levels after 48 hours of refeeding (Fig. 3). Intraepithelial cell yield remained significantly lower than did chow control levels throughout the refeeding period. Lamina propria cell yield remained significantly depressed compared with control chow levels after 1 day of chow refeeding but returned to baseline levels by day 2 and remained statistically significantly higher than TPN alone levels, except for day 3.

T-Cell and B-Cell Yields

Within the study, T cells outnumbered B cells in the lamina propria and B cells outnumbered T cells in the Peyer's patches. Both Peyer's patch B-cell and T-cell counts were decreased in the TPN alone group compared with those in the chow control group (Table 2) ($p < 0.05$). Peyer's patch B-cell numbers were essentially midway corrected after 24 hours of refeeding (day 1, $p < 0.05$ *vs.* day 0 and day 2), returning to control chow-fed levels after 48 hours of refeeding. Peyer's patch T-cell numbers followed a similar trend after 24 hours (day 1 *vs.* day 0 and day 2, $p < 0.05$) and were at chow control levels by day 2. Both lamina propria B-cell and T-cell counts remained significantly depressed on day 1 but returned to chow control levels by day 2. These counts fell on day 3 but again returned to control levels on day 4.

T-Cell Subsets

Within the Peyer's patches, the number of CD4+ and CD8+ cells significantly decreased in the TPN alone group (day 0) compared with that of the chow control group (Table 3) ($p < 0.05$). Peyer's patch CD4+ counts significantly increased, and CD8+ counts nearly reached statistical significance (CD8+ p = 0.07 *vs.* day 0) by day 1. Both were significantly improved from TPN alone levels by day 2. Within the lamina propria, the CD4+ subset was significantly depressed in the TPN alone (day 0) group and on day 1 compared with that of the chow group ($p < 0.05$). These counts increased on day 2 ($p < 0.05$ *vs.* day 0). Day 3 levels trended downward from day

Table 2. B CELL AND T CELL YIELD ($\times 10^6$)

	Days of Chow Refeeding After Total Parenteral Nutrition					
	Chow	Day 0	Day 1	Day 2	Day 3	Day 4
Peyer's patch						
B cell	6.0 ± 0.9	2.1 ± 0.6*	4.2 ± 0.7	6.3 ± 1.0†	5.8 ± 0.8†	5.6 ± 0.8†
T cell	3.3 ± 0.5	1.1 ± 0.3*	2.5 ± 0.5†	3.8 ± 0.5†‡	3.3 ± 0.3†	3.4 ± 0.5†
Lamina propria						
B cell	1.2 ± 0.2	0.5 ± 0.1*	0.6 ± 0.1*	1.0 ± 0.1†‡	0.8 ± 0.1	1.1 ± 0.2†‡
T cell	2.8 ± 0.3	1.2 ± 0.3*	1.7 ± 0.2*	2.3 ± 0.4†	1.8 ± 0.2*	2.8 ± 0.4†‡

* $p < 0.05$ *vs.* chow.
† $p < 0.05$ *vs.* day 0.
‡ $p < 0.05$ *vs.* day 1.

2, reaching significant difference from chow levels, but rebounded on day 4. The CD8+ subset showed much less change, but was significantly depressed in the TPN alone (day 0) group compared with that of the chow group and had a similar pattern of recovery from day 0 levels on day 2 and day 4.

There were no significant differences in the CD4+/CD8+ ratio within Peyer's patch cells between the TPN alone and chow groups (Table 4). With refeeding, the ratio trended upward to day 2, reaching statistical significance on day 2 compared with that of both the TPN alone and chow groups ($p < 0.05$), then leveling off. This contrasted with findings within the lamina propria, in which the CD4+/CD8+ ratio was significantly depressed in the TPN alone group compared with that of the chow group ($p < 0.05$), and this remained so until day 3 of refeeding ($p < 0.05$ *vs.* day 0, day 1, and day 2) when the ratio returned to baseline levels.

Viral Shedding

After 5 days of TPN and subsequent refeeding, no mouse in the refed group (0/9) shed virus on rechallenge. In contrast, 66% of the group that received 5 days of TPN alone shed virus (8/12). This reached statistical significance at $p = 0.005$ (Fig. 4).

DISCUSSION

Mucosal immunity provides a major defense against systemic invasion by the external pathogens. Amid a constant barrage of pathogens and toxins within the oropharynx, respiratory tree, and gastrointestinal tract, the body secretes >70% of the body's entire immunoglobulin production in the form of IgA at mucosal surfaces, preventing attachment and adherence of potentially dangerous agents.[16–19] Breakdown of this mucosal barrier may create a cascade of events that ultimately leads to the demise of the organism as a whole; therefore, understanding of this complex barrier system is important, particularly in the care of patients who are critically ill and at risk of compromised mucosal defenses.

This complex mucosal immunologic barrier appears to be influenced by nutrient administration, as shown by the growing body of literature examining route and type of

Table 3. T CELL SUBSETS ($\times 10^6$)

	Days of Chow Refeeding After Total Parenteral Nutrition					
	Chow	Day 0	Day 1	Day 2	Day 3	Day 4
Peyer's patch						
CD4	2.8 ± 0.4	0.9 ± 0.3*	2.1 ± 0.4†	3.2 ± 0.4†‡	2.7 ± 0.3†	2.9 ± 0.4†
CD8	0.8 ± 0.1	0.2 ± 0.1*	0.5 ± 0.1	0.7 ± 0.1†	0.7 ± 0.1†	0.8 ± 0.1†
Lamina propria						
CD4	2.0 ± 0.2	0.7 ± 0.2*	1.0 ± 0.1*	1.5 ± 0.2†	1.3 ± 0.2*	1.9 ± 0.3†‡
CD8	0.9 ± 0.1	0.5 ± 0.1*	0.7 ± 0.1	0.9 ± 0.1†	0.6 ± 0.1	0.9 ± 0.1†

* $p < 0.05$ *vs.* chow.
† $p < 0.05$ *vs.* day 0.
‡ $p < 0.05$ *vs.* day 1.

Table 4. CD4/CD8 RATIO

	Days of Chow Refeeding After Total Parenteral Nutrition					
	Chow	Day 0	Day 1	Day 2	Day 3	Day 4
Peyer's patch	3.8 ± 0.1	3.7 ± 0.2	3.9 ± 0.2	4.4 ± 0.2*†	4.1 ± 0.3	4.0 ± 0.2
Lamina propria	2.4 ± 0.2	1.4 ± 0.1*	1.5 ± 0.1*	1.8 ± 0.1*	2.3 ± 0.3†‡	2.2 ± 0.1†‡

* $p < 0.05$ *vs.* chow.
† $p < 0.05$ *vs.* day 0.
‡ $p < 0.05$ *vs.* day 1.

nutrition and its effect on immunity.[1-5,20-22] After an intraperitoneal septic challenge, animals have improved survival, presumably through improved host defenses, when receiving enteral diet compared with those receiving similar amounts of nutrients via parenteral routes.[8,9] Enteral feeding produced a more brisk cytokine response within the peritoneal cavity and was associated with more rapid killing of intraperitoneal bacteria with less systemic spillover of cytokines into the systemic circulation.[23] Route of nutrition is clinically important as well. Early enteral nutrition reduces the incidence of pneumonia (a mucosal epithelial infection) and intra-abdominal abscess in patients who are critically injured compared with patients fed parenterally.[10-12] Our working hypothesis is that although both enteral and parenteral nutrition can nourish the body cell mass, enteral nutrition maintains the mucosal barrier more effectively, preserving both gastrointestinal and extraintestinal immunity.

The gastrointestinal tract plays the central role in coordinating and maintaining this mucosal defense.[24] Antigen within the gastrointestinal lumen is taken up by M cells overlying the Peyer's patches of the small intestine, the lymphoid aggregates where antigenic presentation and processing occur.[25] Activated B- and T-cell lymphocytes then travel to mesenteric lymph nodes where further processing and amplification occur. Sensitized lymphocytes, the majority of which are precursors to IgA production, seed the systemic circulation via the thoracic duct. These lymphocytes localize by regional blood flow to both intestinal and extraintestinal mucosal sites influenced in the intestine.[26-30] Once within the intestinal lamina propria, these sensitized cells produce antigen-specific IgA, which is secreted onto the mucosal surface after transport through the overlying mucosal epithelial cells, thus providing a specific barrier to attachment and adherence of that agent.[31-33]

Our laboratory has focused recently on route of nutrition and its specific effects on the GALT, that cellular component of gastrointestinal immunity most directly responsible for antigen processing and IgA production.[17,25,31,32] Previously, we demonstrated that intragastric or intravenous TPN reduces small intestinal GALT mass relative to chow-fed animals; depletes Peyer's patch, intraepithelial, and lamina propria lymphocyte populations; reduces intestinal IgA levels; and depresses the CD4+/CD8+ ratio within the lamina propria.[5] Additionally, we found that TPN decreases established upper respiratory tract immunity to A/PR8 (H1N1), a mouse-adapted influenza virus, in previously immunized mice.[6] We used this virus as a model testing the integrity of a purely IgA-mediated defense. Nonimmune mice inoculated intranasally with A/PR8 (H1N1) virus shed the virus in their nasal secretions unless passively immunized with influenza-specific polymeric IgA.[34] Solid immunity is established within 3 weeks of initial upper respiratory tract infection, and convalescent immune mice challenged intranasally with influenza virus do not shed virus in their nasal secretions 24 hours post-inoculation unless anti-IgA antibodies are given simultaneously. If, during challenge, virus is administered with antibody against immunoglobulins, only those mice given anti-IgA antibodies (and not those given anti-IgG or anti-IgM antibodies) fail to clear the virus,[18] indicating that mucosal immunity to influenza virus in the intact murine nose is IgA mediated. Administration of IV-TPN has a detrimental effect on this mucosal defense in the respiratory tract, implicating the GALT as a key component of the TPN-induced depression of mucosal immunity.[6]

In this experiment, we examined the ability of enteral refeeding to reverse the TPN-induced changes in gastrointestinal immune cellularity and extraintestinal immunity. As before,[5] mice receiving a 5-day course of parenteral nutrition had significantly depressed GALT cell counts in the lamina propria, intraepithelial space, and Peyer's patches. After a single day of enteral refeeding, cell counts within the Peyer's patches rapidly returned to near-baseline levels. Cell counts within the lamina propria, the primary site of IgA production, failed to improve until after 48 hours of refeeding. These changes after refeeding correspond with previously published work suggesting that cells released from Peyer's patches seed the lamina propria within 24 hours.[29,35-37] Cells within the intraepi-

thelial space, the function of which remains obscure, remained depressed throughout the refeeding period.

When these cellular changes were examined by subtype, both B-cell and T-cell populations within Peyer's patches and lamina propria were found to be decreased in association with total cell count drop. Consistent with our prior work,[5] both the Peyer's patches and the lamina propria CD4+ and CD8+ cell populations dropped, although the ratio of CD4+ to CD8+ cells within the Peyer's patches remained stable throughout the refeeding period. In contrast, the ratio within the lamina propria was significantly depressed after TPN. This depressed ratio of CD4+ helper-type cells to CD8+ suppressor-type cells in the lamina propria, hypothesized to be associated with cytokine regulation of lymphocyte function,[38] returned to normal after 72 hours of refeeding, which may be related to the lower intestinal IgA levels documented in our previous work.[5]

Enteral refeeding returned the antiviral immunity in the upper respiratory tract of TPN-fed mice to normal within 5 days. Thus, despite temporary impairment with TPN,[6] memory to previous antigenic challenge remains intact. We arbitrarily chose a period of 5 days for this experiment to match the time of TPN administration, but it is likely that the upper respiratory tract anti-influenza immunity returned to normal at an earlier point in time, as suggested by GALT recovery within 2 to 3 days.

These findings provide groundwork for further investigations into GALT cellular control mechanisms, specialty nutrients, or neuroendocrine interactions and suggest closer scrutiny of the changes occurring during the first 24 to 48 hours of refeeding within either the lymphocyte nuclear regulatory proteins or the cytokine milieu. For example, the balance between Th1-type cytokines, which upregulate IgA production, and Th2-type IgA inhibitory cytokines[38,39] could be disturbed by the changes in CD4+ cell populations, but this has not been tested. Recently, we showed that supplementing TPN with glutamine,[7] an important respiratory fuel of small bowel enterocytes and lymphocytes, or bombesin,[40] a neuropeptide trophic to the gastrointestinal tract, prevents many of the immune defects associated with parenteral nutrition from occurring at both intestinal and extraintestinal sites. Use of these agents may lead to cellular recovery in TPN-fed states and serves as a possible therapeutic intervention to prevent defects in this barrier from occurring.

In conclusion, enteral feeding after TPN is associated with rapid return of GALT cellularity in an order similar to that of antigen processing by the gastrointestinal immune system (*i.e.,* an initial response within the Peyer's patches followed by a lamina propria response). Refeeding also corrects the impairment of upper respiratory tract immunity to a viral challenge known to occur with TPN administration. This work provides further understanding of the mechanism underlying the immunologic differences between routes of nutrition, investigating one reason why early enteral nutrition improves the outcome in patients who are critically ill.

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Discussion

DR. STEVEN M. STEINBERG (New Orleans, Louisiana): Dr. Hanks, Dr. Copeland, Members, and Guests. I would like to congratulate Dr. Kudsk and his group for another fine study that expands on the information that he presented last year at this meeting. I have one comment and three questions, however. The comment is first.

Dr. Kudsk is to be commended and, I believe, admired for attacking a difficult issue in both the clinical and basic science laboratories over the course of his career, and that is the relationship between the gut, the route of nutrition, and critical illness. I think they are right to investigate the effect of nutrition on the immune function of the gut, and the secondary effects that the gut has on other organs, rather than trying to blame bacterial translocation for all of our intensive care unit woes.

Although one can look crosswise at a rodent and cause bacterial translocation, there is little, and some might say, no direct evidence that bacterial translocation occurs in a pathologic sense in humans. The few human studies that purport to demonstrate bacterial translocation are in patients with primary gastrointestinal tract disease such as small bowel obstruction. Even in those patients, there is no correlation between translocation and any complication or mortality. So, again, I congratulate Dr. Kudsk for being bold enough to investigate this problem from a different angle.

Now, I will pose the questions. First, you demonstrated that refeeding the animals enterally restored the cell lymphocyte yield in Peyer's patches and lamina propria but not in the intraepithelial site. Given that you were able to reverse the immune consequences that you were looking for, what do you think the significance of this finding is?

Second, you hypothesized in your present manuscript and also in last year's presentation that enteral feeding increased immunoglobulin A secretion, and that is what blocked the viral infection. I'll ask the same question this year that I asked last year. Do you have any direct evidence of IgA production in this model?

And finally, given the fact that most intensive care unit-acquired infections are bacterial in nature and probably not viral, what is the clinical significance of being able to prevent viral infection by enteral feedings? Is there a connection between the immune response of the gut and preventing viral and bacterial infections?

Thank you.

DR. JOHN A. MANNICK (Boston, Massachusetts): I, too, enjoyed this latest chapter in the ongoing work from Dr. Kudsk's laboratory. One thing I think is important to remember is that mucosal immunity may be relevant clinically.

If you look at the progression of organ systems failing in severely injured patients, for example, the lung is the first to go. The defense of the lung is, at least in part, through the mucosal immune system and probably through its secretion of