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Parenteral Nutrition Decreases Paneth cell function and Intestinal Bactericidal Activity while Increasing Susceptibility to Bacterial Enteroinvasion

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Abstract

Introduction—Parenteral nutrition (PN) increases the risk of infection in patients with contraindication to enteral feeding. Paneth cells produce and secrete antimicrobial products that protect the mucosa from pathogens. Their loss is associated with increased host-pathogen interactions, mucosal inflammation, and altered microbiome composition.

Hypothesis—We hypothesized that PN reduces Paneth cell product expression, and these changes would reduce bactericidal properties of tissue secretions following cholinergic stimulation, increase mucosal enteroinvasion, and shift the intestinal microbiome.

Method—*Expt 1*: Male ICR mice were randomized to Chow (n=8) or PN (n=8). Ileum tissue was collected for Paneth cell antimicrobial expression using RT-PCR, stimulated with a cholinergic agonist degranulate Paneth cells bactericidal activity, or used to assess bacterial enteroinvasion in EVISC. *Expt 2*. Mice were randomized to Chow (n=11) or PN (n=8) and ileum washing was collected for 16s pyrosequencing analysis.

Results—Compared to Chow, PN decreased tissue expression of REGIII- γ ($p < 0.002$), lysozyme ($p < 0.002$), and cryptdin-4 ($p < 0.03$). At the phylum level, PN decreased total *Firmicutes* but increased total *Bacteroidetes*, and *Proteobacteria*. Functionally, secretions from PN tissue was less bactericidal ($p < 0.03$) and demonstrated increased susceptibility to enteroinvasion by *E. coli* ($p < 0.02$).

Conclusion—PN without enteral nutrition impairs innate mucosal immune function. Tissue expression of Paneth cell antimicrobial proteins decreases associated with compositional shifts to the microbiome, decreased bactericidal activity of mucosal secretions and greater susceptibility of to enteroinvasion by *E. coli*. These changes may explain in-part the increased risk of infection in parenterally fed patients.

Keywords

Parenteral Nutrition; Paneth cells; small intestine; innate immunity; cryptdins; microbiome

Introduction

Parenteral nutrition (PN) prevents progressive malnutrition in patients unable to feed enterally. Unfortunately, PN is associated with an increased probability of infectious complications compared with enteral nutrition in critically ill patients¹⁻⁴. The intestinal mucosal barrier normally limits direct pathogenic host-microbiome invasion through the use of tight-junction proteins between epithelial cells, mucin glycoproteins secreted by goblet cells^{5, 6}, antimicrobial molecules released from Paneth cells⁷⁻¹⁰, and the release of immunoglobulin-A (IgA) by the epithelium¹¹⁻¹³. PN without enteral stimulation impairs multiple component of host defense, including reduced sIgA produced by acquired (adaptive) immunity¹⁴⁻²².

We recently demonstrated that PN reduces the Paneth cell antimicrobial molecule, secretory phospholipase A₂ (sPLA₂) within the intestinal tissue and lumen²³ as well as the bactericidal activity of *ex vivo* intestinal tissue secretions in mice compared with chow feeding²⁴. This suggested PN without enteral feeding diminished innate mucosal immune defense. Further investigations demonstrated an sPLA₂ inhibition reduced some - but not all - bactericidal activity of intestinal secretions against a gram-negative pathogen, suggesting the role and significance of the other antimicrobial products secreted by the epithelium.

Paneth cells are the primary source of antimicrobial compounds for epithelial protection releasing multiple molecules in addition to sPLA₂, including lysozyme, RegIIIγ, ang4, and cryptidins²⁵⁻²⁹. The release of these products from Paneth cell granules occurs by cholinergic stimulation^{27, 30}. These agents influence the growth and composition of the intestinal microbiome³¹⁻³³. Antimicrobial compounds generally exert their activity by disrupting the integrity of the bacterial cell membrane or wall. Cryptdin-4 create pores in the membrane and are bactericidal activity against gram-negative and gram-positive bacteria³⁴. REGIII-α (murine REGIII-γ) binds peptidoglycans in bacterial cell membranes and is bactericidal against gram-positive species, including *Listeria monocytogenes*³⁵⁻³⁷. Lysozyme hydrolyzes peptidoglycan in bacterial cells and knockout studies demonstrate this enzyme is required to prevents accumulation of peptidoglycan in tissues that otherwise induces inflammatory responses³⁸. The loss of Paneth cell function completely results in translocation of pathogenic as well as commensal bacterial strains from the intestinal tract^{32, 36}.

The intestinal microbiome is comprised of an enormous heterogeneous population of bacteria that have been enumerated at maximal total levels of 10¹⁴ colony forming units

(CFUs) and exist in a symbiotic relationship with the host under normal circumstances. The microbiome plays essential roles in host immune stimulation and maintenance, digestion of nutrients, and the production of short chain fatty acids and vitamins. In return, the host provides the microbiome a stable habitat and nutrient source. The importance of the microbiome is demonstrated in germ free mice, which demonstrate an increased risk of infection and susceptibility to enteric pathogens³³.

Since PN reduces sPLA₂ in the small intestine tissue and lumen, we hypothesized that PN would decrease expression of the other Paneth cell products, lysozyme, RegIII γ , and cryptidin4, and that these changes would be associated with compositional changes in the intestinal microbiome. We utilized *E. coli* and *P. aeruginosa* in our studies since they are the predominant organisms in intra-abdominal infections.³⁹ Functionally, we hypothesized that PN feeding decreases the bactericidal activity of tissue secretions following cholinergic stimulation and results in an increase in mucosal enteroinvasion of *Escherichia coli* in our *ex vivo* intestinal segment culture (EVISC) model.

MATERIALS AND METHODS

Animals

All protocols were approved by the Animal Care and Use Committee of the William S. Middleton Memorial Veterans Hospital-Madison and the University of Wisconsin-Madison, and. Male Institute of Cancer Research (ICR) mice were purchased from Harlan (Indianapolis, IN) and housed 5 per covered/filtered box under controlled temperature and humidity conditions. Animals were fed standard mouse chow (Rodent Diet 5001; LabDiet, PMI Nutrition International, St. Louis, MO) and water *ad libitum* for an acclimation period of 1 week prior to initiation of study protocol.

Experimental Design

Experiment 1: PN effects on Paneth cell product expression and bactericidal activity—Male ICR mice, ages 6 to 8 weeks, were randomized to Chow (Chow, n = 8) or parenteral nutrition (PN, n=8). Animals were anesthetized by intramuscular injection, weighed, and underwent placement of silicon rubber catheter (0.012-inch I.D./0.025-inch O.D.; Helix Medical, Inc., Carpinteria, CA) in the vena cava through the right external jugular vein. The catheter was tunneled subcutaneously and existed at the midpoint of the tail. The animals were housed individually in metabolic cages with wire floors to prevent coprophagia and bedding ingestion and partially immobilized by tail restraint to protect the catheter during infusion. This technique has proven to be an acceptable method of nutritional support and does not produce physical or biochemical evidence of stress⁴⁰. The catheterized mice were connected to infusion pumps and received saline (0.9%) at 4 mL/day and *ad libitum* chow and water during 48 hours of recovery. After 48 hours, Chow mice continued to receive 0.9% saline at 4 mL/day as well as *ad libitum* chow and water. PN animals received PN solution at rates 4 mL/day (day 1), 7 mL/day (day 2) and 10 mL/day (day 3 to 5), because a graded infusion period was demonstrated to be necessary for the mice to adapt to the glucose and fluid loads. The PN solution contained 6.0% amino acids, 35.6% dextrose, electrolytes, and multivitamins, containing 1440 kcal/L and a non-protein calories/

nitrogen ratio of 128:1. These values were calculated to meet the nutrient requirements of mice weighting 25 to 30 g⁴¹

After 5 days of feeding (7 days post-catheterization), mice were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and acepromazine (10 mg/kg), and exsanguinated via left axillary artery transection. The small intestine was removed and the lumen rinsed with 20 mL Hanks Balanced Saline Solution (HBSS, Bio Whittaker, Walkersville, MD). Tissue samples were taken by removing a 3 cm segment of ileum, excluding Peyer's patches. These samples were either frozen in liquid N₂ and stored at -80°C until processing for PCR analysis, used immediately and stimulated with bethanechol to access bactericidal activity of the resultant sections, or stored in cold RPMI prior to initiating the ex vivo intestinal segment culture (EVISC) detailed below.

Experiment 2: PN effects on the ileal microbiome—Male ICR mice, ages 6 to 8 weeks, were cannulated as in experiment 1 and randomized to Chow (Chow, n = 11) or PN (n = 8). Tissues were harvested as previously mentioned. The distal 1 cm of ileum tissue was flushed with 1 mL Hank's balanced salt solution and used to isolate RNA for 16s pyrosequencing.

RNA extraction, Reverse transcription and PCR for Paneth Cell products

2 cm of distal ileum was collected and stored in RNeasy (Cat # R0901, Sigma, St. Louis) until further use. Using directions from the manufacturer, the SV Total RNA isolation kit (V3100, Promega, Madison WI) was used to isolate RNA from tissue samples stored in RNeasy. A nanodrop spectrometer was used to ensure samples were pure and of appropriate concentration. After RNA isolation, cDNA was made using the random primers and the instructions from the manufacturer and the ImPromII Reverse transcription kit (Cat# A3800, Promega, Madison WI). Primers were designed for lysozyme cryptdin-4, and RegIII-g and 1 micrograms of DNA was run in each reaction with b-actin used as a control. PCR's were performed with a recombinant Taq DNA polymerase (Cat# Z3100, Invitrogen). Each 20 µL PCR mixture contained 10 X Taq buffer, 50 mM MgCl₂, 10 mM of each deoxynucleoside triphosphate (dNTP), 1 U of recombinant Taq DNA polymerase, 1 µM of each primer, and 1.5 µL of cDNA. The samples were amplified in a Hybaid PCR express system with the following program: initial denaturation at 94 °C for 3 minutes, 25 thermal cycles of 94 °C for 1 minute, 56 °C for 30 s and 72 °C for 45 s with final extension at 72 °C for 10 minutes. The size and the amount of PCR products were confirmed by analyzing all reaction product on a 3 % agarose gel (w/v) electrophoresis and stained with ethidium bromide.

Sample collection and DNA isolation

1 cm-long distal ileum was dissected from mice receiving either chow (n = 11) or IVPN (n = 8). The wash fluid was collected after flushing the ileal tube with 1 ml of Hank's balanced salt solution. Bacterial DNA was isolated from 0.2 ml of each sample using DNeasy blood and tissue Kit (Qiagen Sciences, MD) and the same amount of DNA was pooled within the same treatment group.

16S rRNA pyrosequencing

The partial 16S rRNA genes were determined by Roche-454 GS FLX technology at University of Nebraska-Lincoln, Core for Applied Genomics and Ecology (CAGE) as previously described ⁴². Briefly, the V1-V2 region was amplified using bar-coded fusion primers with the Roche-454 A or B titanium sequencing adapters (in italics), followed by a unique 8-base barcode sequence (B) and finally the 3' ends of primer A-8FM (5' - *CCA TCT CAT CCC TGC GTG TCT CCG ACT CAG* BBB BBB BBA GAG TTT GAT CMT GGC TCA G - 3') and of primer B-357R (5' - *CCT ATC CCC TGT GTG CCT TGG CAG TCT CAG* BBB BBB BBC TGC TGC CTY CCG TA - 3'). All PCR reactions were quality-controlled for amplicon saturation by gel electrophoresis; band intensity was quantified against standards using GeneTools software (Syngene). The amplicons from all reactions were pooled in equal amounts and gel purified. The resulting products were quantified using PicoGreen (Cat# P7581, Invitrogen) and a Qubit fluorometer (Invitrogen) before sequencing. The data processing pipeline removed low-quality reads (i) that did not completely match the PCR primer and barcode, (ii) that were shorter than 200 bp or longer than 500 bp in length, (iii) that contained more than two undetermined nucleotides (N), and (iv) had an average quality score over 20. After filtering, each read was trimmed to remove 5' adapter and primer sequences and was parsed by a barcode. The taxonomic status (phylum and genus) was assigned to each read using a parallelized version CLASSIFIER ⁴³. At the standard threshold of 0.8, reads were classified down to the lowest level until the score < 0.8, at which point reads were classified as "unclassified" at the next-higher taxonomic rank. There were 10,540 and 7,290 reads obtained from CHOW and PN groups, respectively. Percentage of each phylum and genus was used to create a bar chart in Excel.

Bacteria Preparation and Bactericidal Assay—To determine bacterial activity, *Pseudomonas aeruginosa* (*P. aeruginosa*) was cultured as previously described. Briefly, culture was grown overnight in a lysogeny broth (LB) (Fisher Scientific, Fair Lawn, NJ) for 24 hr at 37 °C. After washing and resuspending in CMF-HBSS, the bacterial suspension was adjusted to 2×10^3 CFU /mL with CMF-HBSS. 10 µL (~100 CFU) of bacterial stock was combined with 200 µL culture media after stimulation with bethanechol or CMF-HBSS (Control) and incubated for 1 hr at 37 °C. Surviving CFUs were determined in duplicate by plating on LB agar plates (Fisher Scientific, Pittsburgh, PA) under aerobic condition at 37 °C overnight in triplicate. CFUs were normalized to control plates in respective experiments and results were expressed as bactericidal activity as measured by the reduction in % CFUs.

Bacterial Preparation and Ex Vivo Intestinal Segment Culture—*Escherichia coli* 5011-Lux containing ampicillin resistance were grown in 40 mL medium (LB) for 48 hours at 37 °C under 5% CO₂ and passed to a new 40 mL LB broth and grown for 24 hours at 37°C under 5 % CO₂. Bacteria were spun down at 1780 x g for 11 min, the supernatant was poured off, and the pellet was re-suspended in 40 mL LB to wash twice, and then re-suspended in 1 mL DPBS at 4 °C as a bacteria stock solution. A 1:100 dilution of the bacterial stock solution was made and read on a spectrophotometer (DU640B, Beckman) at 450 nm wavelength. Bacterial concentrations were adjusted based on growth curves previously established.

Intestinal Segments were placed on a sterile surface covered in a light layer of RPMI and carefully opened apical side up. Tissue glue (Dermabond, Ethicon, Cornelia, GA) was lightly covered on one side of a plastic tissue ring (9 mm aperture) and lowered over the intestinal segment. A second tissue disc was lightly covered with tissue glue and placed on the serosal side of the intestinal segment. Once the second tissue disc was adherent, a light layer of tissue glue was applied to the bottom of the serosal disc and the intestinal segment sandwiched between tissue discs were lowered into a cell culture insert (Cat 3292, 3.0 μ M pore, 12 well format, BD bioscience, NJ). Gentle pressure was applied to ensure adherence of the bottom tissue disc to the cell culture insert. Cell culture inserts were placed into 12 well plates prefilled with 1 mL RPMI + Ampicillin per well.

400 μ L of bacterial inoculums (1×10^8 Colony Forming Units (CFU)/mL) in RPMI + Ampicillin was placed into the wells for 1 hour at 37°C. Then the bacterial inoculum was collected, centrifuged at 14,000g for 2 minutes to pellet bacteria, and the supernatant stored for analysis of mucosal secretions. The wells were rinsed gently with 500 μ L of DPBS 3 times. 600 μ L RPMI + Gentamicin was added to each well for 1 hour at 37°C. This was done to kill any remaining bacteria in the well or adherent to the mucosal surface. RPMI + Gentamicin were then removed and the wash step repeated. Then 500 μ L of 0.1% Triton-X in PBS was added to each well. The 12 well plates were placed on an orbital shaker and agitated (175 rpm; New Brunswick Scientific Classic Series C1 Shaker) for 30 minutes at room temperature. Serial dilutions (10^1 – 10^7) of the cell lysate in DPBS were plated on LB + Ampicillin agar plates and grown for 18 hours at 37°C. Enteroinvasion was assessed by counting CFUs. The RPMI + Ampicillin added to the 12 well plate was also plated to detect cell culture insert leaks. If leaks were detected from the apical to serosal side, the well was discarded from the experiment.

Statistical analysis—The data are expressed as means $\pm$ standard error of the mean. Statistical significance was determined using ANOVA with Fisher's protected least significant difference *post hoc test* or Student's *t*-test. Differences were considered to be statistically significant at $p < 0.05$. All statistical calculations were performed with StatView (Abacus Concepts, Berkeley, CA).

RESULTS

Analysis of Tissue Paneth Cell Products

The relative expression of Cryptidin4/ β -actin within the small intestinal tissue was significantly lower in PN compared with Chow (PN: 0.530 ± 0.025 vs. Chow: 0.646 ± 0.035 , $p < 0.03$) (Figure 1a). The relative expression of REGIII- γ / β -actin within the small intestinal tissue was also significantly lower in PN compared to chow (PN: 0.445 ± 0.044 vs. Chow: 1.46 ± 0.207 , $p < 0.002$) (Figure 1b). Lysozyme/ β -actin expression was also significantly decreased in PN tissue compared to chow tissue (PN: 0.541 ± 0.024 vs. Chow: 0.715 ± 0.029 , $p < 0.002$) (Figure 1c).

16sRNA pyrosequencing of mouse ileum digesta

To assess the influence of PN and Chow feeding on the composition of the ileum microbiota 16S rRNA pyrosequencing was conducted. A total of 17,830 filtered reads were obtained from PN (7,290) and Chow (10,540). After the taxonomic status of each read was assigned by CLASSIFIER, there were 10 phyla, 16 classes, 26 orders, 45 families, and 67 genera identified overall. The microbial composition of predominant phyla and genera in the chow and PN groups are displayed in Figures 2A and 2B respectively. The microbial composition of all bacteria detected at the phyla and genera level are shown in Supplemental Table 1. CLASSIFIER was able to make assignments for 14,535 reads at the phylum level and 7,111 reads at the genus level. Changes in the composition of the ileum microbiota are apparent at the phylum level (Figure 2, Panel A), with the microbiota in the PN mice having a lower percentage of Firmicutes (PN: 48.0% vs. Chow: 80.6%) and higher levels of Bacteroidetes (PN: 32.1% vs. Chow: 14.8%) and Proteobacteria (PN: 14.5% vs. Chow: 1.8%), relative to the chow fed mice. The genus level alterations are presented in (Figure 2 Panel B). The PN ileum microbiota contained significantly greater biodiversity at the genus level than the chow ileum microbiota with nine genera being present at 2% prevalence or greater, while the chow ileum microbiota had only 2 genera present at greater than 2% prevalence. The numerically largest change was observed in the prevalence of *Lactobacillus*, which comprised 92.4% of the classified chow feed ileum microbiota versus 41.5% of the classified PN ileum microbiota.

Bactericidal activity of culture medium

The bactericidal activity against *Pseudomonas* (expressed in % reduction in CFUs compared with controls) was significantly lower in culture media stimulated with bethanechol from PN fed animals compared to Chow animals (PN: 12.9 ± 3.2 vs. Chow: 25.3 ± 3.6 CFUs/well, $p < 0.03$) (Figure 3).

Bacterial Invasion after EVISC

Consistent with previous reports using our ex vivo intestinal segment culture, ileum tissue segments from PN mice were significantly more susceptible to bacterial invasion (expressed as CFUs/segment) than Chow segments (PN: $1.32 \times 10^5 \pm 3.75 \times 10^4$ vs Chow: $2.09 \times 10^4 \pm 4 \times 10^3$ CFUs/segment, $p < 0.02$) (Figure 4).

DISCUSSION

Parenteral Nutrition (PN) is necessary in patients with contraindications to enteral feeding to prevent malnutrition. Unfortunately, lack of enteral nutrition during PN is associated with an increased risk of infectious complications that are likely due-at least in part- to alterations in the gut and respiratory tract mucosal defenses. The intestinal mucosa is protected immunologically by the adaptive (acquired) and innate immune arms of the small intestine. With a total surface area reaching 300 meters², these mechanisms normally prevent bacteria from breaching the mucosa while allowing symbiosis with commensal bacteria and digestion and absorption of nutrients⁵. The primary consequence of PN upon the adaptive immune system is the loss of sIgA production and secretion at mucosal surfaces. Since sIgA is the primary defense molecule released at mucosal surfaces, this results in increased host-

pathogen interactions and increased mucosal inflammation. The innate immune system provides non-specific compounds secreted at the mucosal surface, including antimicrobial Paneth cell molecules, mucin glycoprotein release by goblet cells, and the release of lower levels of antimicrobial compounds by enterocytes.

Paneth cells reside at the base of the intestinal crypts where they produce, store and secrete an array of antimicrobial products, including sPLA₂, lysozyme, and the defensins, that provide a chemical defense against luminal microorganisms⁴⁴⁻⁴⁶. These compounds provide protection to the host from the large collection of pathogenic bacteria by direct effects upon the bacteria itself or by influencing the growth and composition of the intestinal microbiome. sPLA₂ is a Paneth cell product that attacks Gram negative bacterial cell membranes by cleaving phospholipids and causing cell lysis via disruption of the bacterial membrane⁴⁶. Other Paneth cell products that exert anti-bacterial effects include lysozyme, RegIII- γ , and α -defensins (cryptdins in mice). Lysozyme hydrolyzes peptidoglycan, a bacterial cell wall product. RegIII- γ binds peptidoglycan and demonstrates bactericidal activity against Gram positive bacteria. Cryptdin-4 exert bactericidal activity against both Gram positive and Gram negative bacteria. The importance of these mechanisms is demonstrated in mice lacking Paneth cell products; they display impaired clearance of infectious organisms and a frequently lethal colitis⁴⁷.

We previously demonstrated that PN feeding decreases the luminal and tissue levels of the Paneth cell antimicrobial compound, sPLA₂^{23, 48, 49} as well as the luminal levels of the goblet cell mucin MUC2. The current study investigated whether PN induces alterations in other Paneth cell molecules, if changes in microbiome structure occur, and functionally how bactericidal activity and resistance to enteroinvasive bacteria are altered. We demonstrated that compared with Chow, PN results in significant reductions in tissue expression of lysozyme, cryptdin-4, and RegIII- γ , consistent with the loss of sPLA₂ in previous work. These results are also similar to a recent report by Hodin *et al*⁵⁰ that demonstrated 48 hours of fasting reduced Paneth cell antimicrobial expression and concentration in ileal tissues.

To assess functional changes in host defense during PN, this work also examined the bactericidal activity of secretions from small intestinal segments following stimulation with the cholinergic agonist bethanechol. This compound induces the degranulation of Paneth cell granules to the apical surface of the mucosa, releasing antimicrobial products stored in the tissue. Using this method, we demonstrated that secretions from PN tissue were half as bactericidal as secretion after chow feeding, suggesting a defect in innate immune responsiveness. This result is consistent with previous work showing decreased bactericidal activity in secretions collected from PN tissue and further suggests other antimicrobial peptides may be involved in this process. Partitioning the effects of the various antimicrobial peptides is currently under investigation.

In addition, we used an *ex vivo* intestinal segment culture model to quantify bacterial enteroinvasion in tissue from Chow and PN fed mice. Consistent with impaired Paneth cell antimicrobial peptide production and decreased bactericidal activity in PN fed mice, small intestinal segments from the PN mice had significantly greater enteroinvasion of bacteria compared to chow. The decreased expression of antimicrobial peptides and decreased

bactericidal activity of stimulated secretions implicate the role of Paneth cell products in protecting the host from harmful bacteria within the intestine.

Since PN without enteral nutrition alters the luminal environment of the intestine characterized by the loss of ingested nutrients and reduced levels of endogenous glycan secretion by the epithelium, we expected to find dramatic shifts in the intestinal microbiome using 16s pyrosequencing. Our results revealed compositional changes between Chow and PN at both the phylum and genus levels. Prior work defined the microbiome changes that occurred in mice after parenteral nutrition.⁵¹ Our work confirmed those results showing that the microbiome of PN fed mice exhibit reduced levels of the phylum *Firmicutes* and higher levels of the phylum *Bacteroidetes* and *Proteobacteria* compared to the Chow fed mice. Interestingly, these compositional changes led to inflammation that was dependent on Myd88, a signaling molecule centrally involved in sensing and responding to microbial pathogens. At the genera level, PN feeding resulted in decreased levels of *Lactobacillus* and increased levels of *Serratia*, *Parabacteroides*, *Streptococcus*, *Bacterioides*, and *Staphylococcus* compared with Chow. Interestingly, similar changes in microbiome composition are observed in knockout mice that lack specific Paneth cell alpha-defensins, with decreased *Firmicutes* and increased *Bacteroidetes*.⁵² These changes indicate PN feeding with the lack of enteral stimulation is correlated with dramatic changes in the composition, and likely function, of the microbiome that may be related in-part with the loss of antimicrobial expression.

The primary limitation of this study is we cannot draw causative conclusions on the associated changes in Paneth cell tissue expression and the altered microbiome composition. Since the loss of enteral nutrients results in consequences upon the host and the microbiome, impairment of Paneth cell expression in the presence of enteral intake as well as the stimulation of Paneth cell expression during PN are needed to elucidate the interactions between enteral intake, tissue antimicrobial expression, and microbiome composition. Additionally, we focused on tissue expression in this study while the release of these molecules into the lumen is also important and currently under investigation.

In summary, this study supports the hypothesis that PN with the lack of enteral stimulation induces dysfunctional mucosal barrier responses, as measured through the loss of expression of the Paneth cell products lysozyme, RegIII- γ , and cryptdin-4. These alterations were associated with dramatic changes in intestinal microbiome composition. Functionally, PN feeding resulted in less bactericidal activity after cholinergic stimulation and an increase in the susceptibility of enteroinvasion by a *E. coli* using our *ex vivo* intestinal segment culture model. These intestinal alterations are consistent with the increased risk of infection in PN patients who are unable to feed enterally.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Clinical Relevancy Statement

The intestinal mucosal barrier is multifactorial and includes contributions from goblet cells, which produce and secrete mucous, Paneth cells, which produce and secrete antimicrobial proteins and peptides, as well as the compositional structure and function of the commensal bacterial population within the intestine. This work examines the role of Paneth cell antimicrobial production in barrier function and associated changes to the commensal microbiota after administration of parenteral nutrition – providing further mechanistic understanding of immune changes that may increase infectious risk in patients receiving parenteral nutrition.

Figure 1.

Tissue RT-PCR analysis of Paneth cell products. Parenteral nutrition (PN) significantly decreases tissue expression of (A) Cryptdin-4 (B) RegIII- γ and (C) lysozyme compared to chow. Data are represented as mean $\pm$ SEM. * $p < 0.02$ vs Chow.

Figure 2. Pyrosequencing analysis of ileal wash samples with chow or parenteral nutrition. (A) Representation of bacteria at the phylum level. (B) Representation of bacteria at the genera level.

Figure 3.

Bactericidal activity of bethanechol stimulated ileal tissue. Parenteral nutrition significantly decreases the bactericidal killing activity of cholinergic stimulated tissue compared to chow feeding. Data are presented as mean $\pm$ SEM. * $p < 0.02$ vs Chow.

Figure 4. Recovered CFU's after ex vivo intestinal segment culture (EVISC). Parenteral nutrition significantly increases the recovered CFU's compared to Chow. Data are represented as mean $\pm$ SEM. * $p < 0.03$ vs Chow.