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Parenteral nutrition maintains pulmonary IgA antibody transport capacity, but not active transport, following injury

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Abstract

Background—Parenteral nutrition (PN), vs. enteral, increases post-trauma pneumonia. PN impairs murine immunoglobulin A (IgA) airway defense and abrogates a normal IgA increase following injury. PN preserves IgA transport protein (pulmonary polymeric immunoglobulin receptor {pIgR}) levels.

Objectives—Investigate effect of type/route of nutrition on post-injury lung IgA & pIgR.

Methods—Catheterized mice were randomized to 5 days of Chow or PN and sacrificed after no injury (Chow: n=12; PN n=11), or 8 hr post-laparotomy + neck incision (Chow-injury: n=11, PN-injury: n=13). Bronchoalveolar lavage (BAL) and lung IgA levels were analyzed by enzyme-linked immunosorbent assay (ELISA) & lung pIgR by Western blot.

Results—BAL IgA levels increased in Chow-injury vs. PN-injury (p<0.01). Groups displayed no differences in pIgR. PN-injury tissue IgA levels decreased vs. Chow (p<0.01), Chow-injury (p<0.01), and PN (p<0.05).

Conclusion—PN impairs the airway IgA response to injury but is not due to impaired IgA transport capacity/pIgR level.

Keywords

Mucosal Immunology; injury; enteral nutrition; parenteral nutrition; polymeric immunoglobulin receptor

Introduction

The current evidence from randomized clinical trials strongly argues that severely injured patients fed exclusively by the parenteral route experience an increased rate of infectious complications such as pneumonia and abdominal abscess compared to patients fed enterally.^{1–4} Experimentally, parenteral nutrition (PN) in mice impairs established respiratory defenses to both bacterial and viral pathogens through reductions in airway IgA.^{5, 6} Our lab investigates relationships between PN with lack of enteral stimulation and the deterioration of host respiratory and gut immunity⁷ provided by the mucosal immune system..

We recently showed that injury stimulates a mucosal immune system response. Injury generates acute increases in airway IgA levels in both humans and mice.⁸ Experimentally, pre-injury PN impairs this airway IgA response to injury in mice.⁹ Normally, luminal IgA levels increase through active transport of IgA produced by lamina propria T & B cells. This active transport occurs via a specialized transport protein, polymeric immunoglobulin receptor (pIgR) contained in the epithelium.¹⁰ Unstressed PN-fed mice maintain the expression of pIgR in the respiratory tract¹¹ and retain the capacity to transport exogenous polymeric IgA administered intravenously.¹² The current work examines whether PN negatively impacts the IgA transport system in response to injury.

Materials and Methods

Animals

The Animal Care and Use Committee of the University of Wisconsin-Madison, and the Middleton Veterans Administration Hospital, Madison approved all protocols. Male Institute of Cancer Research (ICR) mice were purchased through Harlan (Indianapolis, IN) and housed in an American Association for Accreditation of Laboratory Animal Care-accredited conventional facility. Mice were acclimatized for one week in an environment controlled for temperature and humidity with a 12/12-hour light/dark cycle. Mice were housed 5 per covered/filtered box and fed *ad libitum* chow (LabDiet, PMI Nutrition International, St. Louis, MO) and water for 1 week prior to initiation of study protocol. After entry into study protocol mice were housed individually in metal cages with wire grid floors to prevent coprophagia.

Experimental Protocol

Forty-seven 6–8 week old mice were randomized to diet groups (Chow n= 12, Chow-injury n= 11, PN n= 11, and PN-injury n= 13), anesthetized and cannulated via the external jugular vein (0.012" I.D./0.25" O.D.; Helix Medical, Inc., Carpinteria, CA). Catheters were tunneled subcutaneously over the back and exited mid tail. Mice were partially immobilized by tail fixation to protect the catheter during infusion. This technique has been shown not to induce significant physical or biochemical stress.¹³

After catheterization mice were connected to infusion pumps and recovered for 48 hours while receiving 4 mL of 0.9% saline/day as well as chow and water *ad libitum*. Following the recovery period the different diets were initiated. Chow-fed mice received 0.9% saline at 4 mL/day as well as chow and water *ad libitum* throughout the study. PN-fed mice received solution at 4 mL/day (day 1), 7 mL/day (day 2) and 10 mL/day (days 3–5). The PN solution includes 6.0% amino acids, 34.9% dextrose (6002 kJ/L), electrolytes, and multivitamins, with a non-protein calorie/nitrogen ratio of 535.8 kJ/g Nitrogen. These feedings meet the calculated nutrient requirements of mice weighing 25 to 30 g.

After 5 days of feeding, mice in Chow-injury and PN-injury groups were anesthetized by intramuscular injection of Ketamine 100 mg/kg and Acepromazine 10 mg/kg. A surgical stress (a 3.0 cm laparotomy that included small bowel evisceration and replacement + 1.5 cm neck incision/dissection to trachea) was delivered and incisions immediately closed. Eight hours later mice were again anesthetized and sacrificed by exsanguination which also served to reduce blood contamination of the samples. A midline incision made over the ventral neck superior to the thoracic inlet allowed access to the trachea. Through a tracheotomy, 1 mL saline was slowly injected distally via an 18 gauge angiocatheter attached to a tuberculin syringe. The bronchoalveolar lavage (BAL) was then collected into the tuberculin syringe. Lung tissue was excised and washed in Hank's balanced salt solution (Bio Whittaker, Walkersville, MD). To obtain baseline values, mice in Chow and PN groups were sacrificed without receiving

surgical stress. BAL was stored at -80°C until assayed. The lung tissues were frozen in liquid N_2 and stored at -80°C until processing and analysis.

IgA Antibody Quantitative Analysis

Total IgA in BAL and lung tissue homogenates was measured using a sandwich enzyme-linked immunosorbent assay (ELISA). Briefly, 96-well plates (BD Biosciences, Bedford, MA) were coated with 50 μl of a 10 $\mu\text{g}/\text{mL}$ goat anti-mouse IgA, α -chain specific (Sigma-Aldrich, St Louis, MO) in 0.1 mol/L coating buffer (0.1M carbonate-bicarbonate, pH 9.6), and incubated overnight at 4°C . Plates were washed 3 times and blocked with 100 μl of 1% bovine serum albumin in Tris-buffered saline with 0.5% Tween-20 solution for 1 hour at room temperature. One hundred microliters of samples or IgA standards (seven 2-fold dilutions, from 1,000 $\rightarrow$ 7.8 ng/mL, Sigma-Aldrich, St Louis, MO) were added, and plates were incubated for 1 hour at room temperature. Plates were again washed 3 times and 100 μL of a 1:500 dilution of the secondary antibody, goat anti-mouse IgA, α -chain specific-HRP conjugate (Sigma-Aldrich, St Louis, MO) were added and incubated for 1 hour at room temperature. Plates were again washed 3 times, and 100 μL of the substrate solution (H_2O_2 and *o*-phenylenediamine) were added and incubated for 12 minutes at room temperature. The reaction was stopped by the addition of 50 μL of 2N H_2SO_4 and the absorbance read at 490 nm in a *Vmax* Kinetic Microplate Reader (Molecular Devices, Sunnyvale, CA). Mass amounts of IgA in the samples were calculated by plotting their absorbance values on the IgA standard curve, which was calculated using a 4-parameter logistic fit with SOFTmax PRO software (Molecular Devices, Sunnyvale, CA).

Western blot for pIgR Expression

Solubilized protein extracted from lung was denatured at 95°C for 10 minutes with sodium dodecylsulfate and β -mercaptoethanol, and 20 μg of protein was separated in a denaturing 10% polyacrylamide gel by electrophoresis at 150V for 1 hour at room temperature. The proteins were transferred to a polyvinylidene fluoride membrane using a Tris-glycine buffer plus 20% methanol at 80 V for 50 minutes at 4°C . The membrane was blocked with 5% nonfat dry milk prepared in TBS-Tween for 1 hour at room temperature with constant agitation. Membranes were incubated with the primary antibody, rabbit anti-mouse secretory component (SC) IgG diluted 1: 1,000 for 3 h at room temperature with constant agitation. Membranes were washed and incubated with stabilized goat anti-rabbit-IgG HRP conjugate (Pierce Biotechnology, Rockford, IL) diluted 1: 5,000 for 1.5 h at room temperature with agitation. After the last wash, the membrane was incubated for 5 minutes with the substrate for HRP (Super signal west femto maximum sensitivity substrate, Pierce Biotechnology, Rockford, IL) and bands were detected using photographic film. Densitometric measurements of protein bands were analyzed and quantified with the NIH Image J software. The combined value of the ~ 120 kDa and ~ 94 kDa bands was determined for the quantitation of the pIgR protein expression in each case.^{14–16} pIgR values are expressed as a percentage of the Chow case with the strongest band (100%) on each gel.

Statistical Analysis

All values (including pIgR percentages) are expressed as means $\pm$ SE. Statistical analysis was performed by analysis of variance, followed by the Fisher's protected least significant difference post hoc test.

RESULTS

There were no significant differences in initial body weights between groups. Both PN and PN-injury mice lost significantly more weight during the feeding than both Chow and Chow-injury mice (Table 1). Our previous work shows that much of this difference is accounted for

by the 1.5 g of feces present in chow fed mice while the gastrointestinal tracts of PN-fed mice are empty.

BAL IgA levels increased significantly in Chow-injury (133.1 ± 36.7 ng) compared to PN-injury group (43.0 ± 8.2 ng, $p < 0.01$, Figure 1).

Although pIgR expression both in Chow and Chow-injury groups seemed to decrease compared to the expression both in PN and PN-injury groups, there were no significant differences between groups (Figure 2). The apparent elevation of pIgR expression in the PN group was due to a single outlier and there were no significant differences between the groups.

Lung tissue IgA levels in the PN-injury group (34.0 ± 4.3 ng / mg protein) were significantly decreased compared to Chow (66.8 ± 6.2 , $p < 0.01$), Chow-injury (60.3 ± 7.1 , $p < 0.01$), and PN (54.8 ± 7.1 , $p < 0.05$) groups (Figure 3).

DISCUSSION

This study evaluated IgA transport capacity (as evidenced by pIgR levels), and IgA “availability” (as evidenced by lung tissue IgA levels) in the respiratory tract following injury after manipulating the type & route of nutrition. The work examined levels of pIgR protein present in lung tissue since this represents the only known route for active transport of IgA between the lamina propria and the lumen. The results confirmed our previous observations that PN eliminates the normal increase in airway IgA in response to injury, but shows that levels of the transport protein, pIgR, remain constant after injury. However, an intriguing decrease in tissue IgA occurs shortly after injury in the PN fed mice.

We recently reported that severely injured trauma patients acutely increase airway secretory IgA compared compared to uninjured patients intubated for an elective surgical procedure.⁸ Following this observation, we confirmed that mice respond with a similar acute increase in airway IgA 8 hours after a limited injury of neck and abdominal incisions⁸ and demonstrated that PN pretreatment eliminates this response.⁹ Changes in pIgR protein were hypothesized to be a potential explanation for these observations for several reasons. First, pIgR is the only known mechanism for transport of IgA from production sites in the lamina propria into the lumen. Second, there are known relationships between the stress cytokines and the pIgR mediated IgA transport process.

pIgR is a transmembrane protein consisting of extracellular, transmembrane, and cytoplasmic domains. It is found on the basolateral surfaces of epithelial cells at all mucosal surfaces. pIgR binds dimeric IgA produced by plasma cells in the lamina propria, transporting it from the basal side to the apical side of the cell. After reaching that surface, pIgR is cleaved between its extracellular and transmembrane domains and released into the lumen as secretory IgA to neutralize antigen.^{10, 17} The cleaved extracellular part of pIgR, called secretory component (SC), enhances both stability and effector functions of dimeric IgA, while protecting the secretory IgA from proteolytic degradation.^{18, 19} In addition to the IgA-SC unit, free SC released onto mucosal surfaces after transcytosis of pIgR works as an important component of innate antimicrobial defenses.^{20, 21}

Stress and inflammation mediate pIgR production through the proinflammatory cytokines TNF α and IL-1 β allowing translation of systemic inflammatory signals into mucosal immune responses. *In vitro* data shows that the inflammatory cytokines, TNF- α and IL-1 β up-regulate pIgR through a protein synthesis-dependent mechanism.^{10, 22} Severe trauma stimulates the release of proinflammatory cytokines systemically as well as locally in the airways.²³ We proved experimentally that the airway IgA response involves TNF- α and IL-1 β by administering a pre-injury dose of monoclonal antibody to block TNF- α . Mice exposed to the

blockade failed to generate *any* increase in airway IgA. Likewise, mice pretreated with anti-IL-1 β also trended toward a decreased airway IgA response following the injury.⁹ These results supported the hypothesis that proinflammatory cytokines play a role in this response leading to speculation that stimulation of pIgR expression and/or activity during acute inflammation played an essential role in the mucosal response to injury. However the results of the current experiment show that parenteral feeding fails to impact IgA transport capacity via reduced pIgR levels and this mechanism cannot explain the loss of airway IgA response in the PN-injury group. Possibly, PN with no enteral stimulation affects pIgR function and/or activity but it is unlikely that a blunted cytokine response with PN would explain our findings. In fact, clinical evidence suggests that PN exaggerates the pro-inflammatory response to injury or inflammatory stimulus.^{24–27} If this is the case, the stimulus for pIgR production and/or activity should be exaggerated in PN animals but PN treatment somehow renders them unable to produce a response similar to chow fed animals.

One question raised in this study is why does IgA drop in lungs of the PN-injury group? Pre-injury, both chow and PN animals displayed similar IgA levels in the airways and lung tissue. Following the injury, less IgA was measured in the lung tissue of PN treated animals with no evidence of IgA loss into the airways. The most likely explanation is that PN increased lung permeability which lead to subsequent “wash out” of proteins from the lung interstitium. Tissue IgA measurement provides an estimate of IgA “available” for transport. It captures and measures IgA present in the lamina propria, a small amount of IgA not washed from the lumen and IgA in any serum not washed from the lung. In our models of acute gut ischemia and reperfusion (I/R), we noted that lung permeability in the PN animals increased significantly several hours after a short 15 minute gut I/R insult while no permeability changes occurred in enterally fed mice.²⁸ That work implicated PN- induced ICAM-1 increases in the gut vasculature due to cytokine changes in the lamina propria (reduced IL-4 and IL-10) from lack of enteral stimulation.^{29, 30} The ICAM-1 attracted neutrophils to the gut which primed circulating neutrophils. These “gut primed” neutrophils redistribute to the lung (and liver) and respond to a “second hit” insult with an augmented inflammatory response and increased pulmonary edema as previously described by Moore et al.³¹ Unfortunately, neither wet weight / dry weight ratios nor other markers of permeability were measured in the current experiment.

Parenteral nutrition treatment with lack of enteral stimulation negatively impacts the airway IgA response to injury. We interpret this response to injury as an intentional and beneficial protective mechanism during a time of stress and perceived vulnerability. While nutrition-related alterations in the IgA injury response may not be clinically applicable to the initial response to injury (unless the trauma victim is also a PN patient), it has potential implications for critically ill and critically injured patients during their subsequent hospital course as they respond to subsequent operations (a controlled form of trauma) or other secondary stressors.

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Figure 1. IgA levels in bronchoalveolar lavage

IgA levels significantly increased in Chow-injury compared to parenteral nutrition (PN)-injury group. IgA levels in Chow-injury trend to increase against in Chow with no significant difference ($p=0.115$). Values are means $\pm$ SE. * $p<0.01$ vs. PN-injury.

Figure 2. pIgR in lung

There were no significant differences between groups in lung polymeric immunoglobulin receptor (pIgR) expression. The elevation in parenteral nutrition group was due to a single outlier. Values are means $\pm$ SE.

Figure 3. IgA levels in lung

Lung tissue homogenate IgA levels in parenteral nutrition (PN)-injury group significantly decreased against all of other groups. Values are means $\pm$ SE. *p<0.01 vs. Chow, **p<0.01 vs. Chow-injury, †p<0.05 vs. PN.

Table 1

Mice body weight and weight change.

Group	n	Body weight (g)	Weight change (g)
Chow	12	30.38 ± 1.01	− 0.87 ± 0.44
Chow-injury	11	28.82 ± 0.88	− 0.09 ± 0.43
PN	11	28.49 ± 0.68	− 2.98 ± 0.49 ^{*†}
PN-injury	13	28.98 ± 0.48	− 2.65 ± 0.39 ^{*†}

Values are means ± SE.

*
p < .01 vs. Chow†
p < .01 vs. Chow-injury

PN: Parenteral nutrition