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Small Intestine Mucosal Immune System Response to Injury and the Impact of Parenteral Nutrition

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Abstract

Background—Both humans and mice increase airway IgA after injury. This protective response is associated with TNF- α , IL-1 β , and IL-6 airway increases and in mice is dependent upon these cytokines as well as enteral feeding. Parenteral nutrition (PN) with decreased enteral stimulation (DES) alters gut barrier function, decreases intestinal IgA, and decreases the principal IgA transport protein pIgR. We investigated the small intestine (SI) IgA response to injury and the role of TNF- α , IL-1 β , IL-6, and PN/DES.

Methods—**Expt 1:** Murine kinetics of SI washing fluid (SIWF) IgA; SI, SIWF and serum TNF- α , IL-1 β , and IL-6, was determined from 0 to 8 hours by ELISA after a limited surgical stress injury (laparotomy and neck incisions). **Expt 2:** Mice received chow or PN/DES before injury and SIWF IgA and SI pIgR levels determined at 0 and 8 hours. **Expt 3:** Mice received PBS, TNF- α antibody, or IL-1 β antibody 30 minutes before injury to measure effects on the SIWF IgA response. **Expt 4:** Mice received injury or exogenous TNF- α , IL-1 β , and IL-6 to measure effects on the SIWF IgA response.

Results—**Expt 1:** SIWF IgA levels increased significantly by 2 hours after injury without associated increases in TNF- α or IL-1 β while IL-6 was only increased at 1 hour after injury. **Expt 2:** PN/DES significantly reduced baseline SIWF IgA and SI pIgR and eliminated their increase after injury seen in Chow mice. **Expt 3:** TNF- α & IL-1 β blockade did not affect the SIWF IgA increase after injury. **Expt 4:** Exogenous TNF- α , IL-1 β , & IL-6 increased SIWF IgA similarly to injury.

Conclusions—The SI mucosal immune responds to injury or exogenous TNF- α , IL-1 β , & IL-6 with an increase in lumen IgA, although it does not rely on local SI increases in TNF- α or IL-1 β as it does in the lung. Similar to the lung, the IgA response is eliminated with PN/DES.

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INTRODUCTION

Parenteral nutrition prevents progressive malnutrition and provides lifesaving therapy in patients with prolonged inability to receive enteral nutrition (EN). However, when parenteral feeding is given to critically ill patients capable of being fed enterally, its use increases infection rates, particularly pneumonia compared to enterally fed patients.^{1, 2} The gut functions as both a site of nutrient absorption and as a primary immune organ which contains 70-80% of the body's lymphoid tissue.³ This gut lymphoid tissue constitutes a substantial amount of mucosal immunity (MI) dispersed at mucosal sites throughout the body.⁴ The strategic molecule of MI resides in secretory immunoglobulin A (sIgA), a dimeric IgA bound to secretory component (SC). SC is a remnant of polymeric immunoglobulin receptor (pIgR) that transports IgA across the epithelium onto the mucosal surface where the main function of IgA is immune exclusion by binding to pathogens and preventing tissue invasion and subsequent infection.^{5, 6} In the gut, sIgA also functions in antigen recognition and processing, control of inflammation (by preventing complement activation and inflammatory responses to nonpathogenic antigens), and control of commensal bacteria (by influencing gene expression).^{7, 8} Gut sIgA protects against infection by various pathogenic bacteria and viruses.⁹

While sIgA protects and regulates immune defenses at mucosal surfaces under normal conditions, it also plays an important role during stress. Our group recently observed that humans increase airway levels of sIgA after severe trauma, presumably as a protective mechanism to prevent infection in the lung.¹⁰ A limited surgical injury reproduces this airway stress response in mice resulting in a sIgA increase 8 hours after injury with a return to baseline levels by 24 hours.¹⁰ This airway sIgA response to injury involves the pro-inflammatory cytokines tumor necrosis factor alpha (TNF- α), interleukin-1beta (IL-1 β), and interleukin 6 (IL-6), each of which is found in both human and murine airway samples after injury. The airway levels of TNF- α , IL-1 β , and IL-6 greatly exceed systemic levels in both human and murine specimens implying a local, rather than a systemic response.¹¹ In our murine model these elevations occurred in a distinct bimodal pattern peaking at 3 and 8 hours after injury.¹¹ Experimentally, we showed that monoclonal antibodies neutralizing TNF- α and IL-1 β either eliminate (TNF- α) or reduce (IL-1 β) the airway sIgA increase after injury and discovered that exogenous administration of TNF- α , IL-1 β , and IL-6 together (but not individually or in pairs) elicits a sIgA airway response similar to injury.^{11, 12} The exact mechanism needs further defining although it is known that TNF- α and IL-1 β stimulate pIgR transcription *in vitro* while IL-6 stimulates B-cell differentiation into IgA-secreting plasma cells.¹³⁻¹⁶ The fact that we found no change in lung pIgR levels following injury despite increases in airway sIgA indicates an increase in pIgR production after injury since pIgR is consumed 1:1 during IgA transcytosis.^{17, 18}

The protective airway sIgA response also depends on enteral stimulation. Parenteral nutrition with decreased enteral stimulation (PN/DES) decreases both airway baseline sIgA levels and eliminates the airway sIgA increase after injury compared to EN fed mice.¹² Experimentally, PN/DES down-regulates multiple components of MI including cell entry and distribution of cells resulting in functional loss of established immunity to respiratory pathogens.¹⁹⁻²¹ We believe that these changes provide a cogent explanation for the higher rate of pneumonia in critically ill patients receiving parenteral rather than enteral nutrition.¹

Our previous work focused on the pulmonary response due to the human clinical response we observed but stresses such as trauma, hemorrhagic shock, burns as well as lack of enteral feeding also compromise the gut mucosal barrier.^{22, 23} PN/DES reduces intestinal sIgA levels by reducing the number of lamina propria cells, levels of Th-2 type IgA-stimulating cytokines and expression of the transport protein pIgR.²⁴⁻²⁶ However, the sIgA response in

the SI to stress or injury and the effect of route of nutrition on this response remains unexplored. The gut and lung develop embryologically from the same endoderm lined primitive gut and have similar mucosal immune mechanisms. Mucosal immune T&B cells residing in both the lung and gut are initially sensitized in Peyer's patches prior to distribution to their sites of function via the thoracic duct and circulatory system.^{7, 21} Because of the common origins, we hypothesized in this series of experiments that the gut sIgA responses to injury would be similar to airway responses implicating important roles for TNF- α , IL-1 β , IL-6 and increases in pIgR in the gut. We also hypothesized that PN/DES would reduce or eliminate this gut response as in the airway.

MATERIALS AND METHODS

Animals

Male five-to-seven-week-old Institute of Cancer Research mice were purchased from Harlan (Indianapolis, IN) and housed in the Animal Research Facility of the William S. Middleton Memorial Veterans Hospital, an American Association for Accreditation of Laboratory Animal Care accredited conventional facility. Mice were allowed to acclimatize for 1 week with free access to standard chow diet (PMI Nutritional International, St. Louis, MO) and water, under controlled conditions of temperature and humidity with a 12:12 hour light:dark cycle.

Experiment 1: Post-injury kinetics of sIgA in small intestine washing fluid (SIWF) and of TNF- α , IL-1 β & IL-6 in SIWF, small intestine (SI), and serum

Animals were anesthetized with an intraperitoneal ketamine (100 mg/kg) and acepromazine (5 mg/kg) mixture. The skin was disinfected using 75% ethanol and 2 wounds were created. First, a 3.0-cm celiotomy incision was made and the small intestine was gently eviscerated and immediately returned to the peritoneal cavity. The wound was closed in 2 layers with 3 simple interrupted 4-0 silk sutures per layer. Second, a 1.5-cm ventral neck incision was made and blunt dissection carried down to the pretracheal plane. This wound was closed with a single layer of 2 simple interrupted 4-0 silk sutures. This same injury was used in our prior studies of the airway sIgA response to injury because it is highly reproducible, causes no mortality, and used the same incisions previously approved by the Animal Care and Use Committee for venous or gastric cannulation.^{11, 27}

Animals were sacrificed at 1, 2, 3, 5, and 8 hours after injury ($n = 8$ for 1, 2, 3, and 8 h; $n = 7$ for 5 h) by exsanguination from a left axillary artery transection. Prior to sacrifice, awake animals received additional anesthesia (up to half of the original dose) until the righting reflex was lost. One group of animals ($n = 8$) was sacrificed without injury to provide baseline IgA and cytokine values (0 h).

Blood was collected from the left axillary artery transection site for the serum sample. The celiotomy incision was then reopened and the small intestine was removed from just distal to the pylorus to the ileocecal valve by dissecting off the mesenteric fat. Twenty milliliters of Hanks' balanced salt solution (HBSS; Bio Whittaker, Walkersville, MD) was irrigated through the intestinal lumen to obtain the SIWF sample. SIWF samples were then spun at 3000 rpm for 10 minutes and the supernatant collected and stored for analysis.²⁸ The washed small intestine was divided into proximal, middle, and distal sections and 3-cm sections from each were taken together for tissue homogenates.

Tissue homogenate preparation

Small intestine tissues were homogenized in RIPA lysis buffer (Upstate, Lake Placid, NY) containing 1% protease inhibitor cocktail (Sigma-Aldrich). The homogenates were

incubated 30 minutes on ice and centrifuged at $16,000 \times g$ for 10 minutes at 4°C , and the supernatants were stored at -20°C until assayed. Protein concentration of each preparation was determined by the Coomassie dye-binding method using bovine serum albumin as standard.

IgA quantitative analysis

Total IgA in the SIWF samples was measured using a sandwich ELISA (Enzyme-Linked Immunosorbent Assay). 96-well plates (BD Biosciences, Bedford, MA) were coated with 50 μL of α -chain-specific goat anti-mouse IgA (Sigma-Aldrich, St. Louis, MO) 10 $\mu\text{g}/\text{mL}$ in 0.1 M carbonate-bicarbonate coating buffer (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 0.05% Tween-20 solution (TBS-Tween) for 1 h at room temperature. One hundred μL of SIWF (diluted 1:100), or IgA standards (seven two-fold dilutions, from 1,000–7.8 ng/mL; Sigma-Aldrich, St. Louis, MO) were added, and the plates were incubated for 1 h at room temperature. The diluent was 5% non-fat dry milk in TBS-Tween. The plates were washed 3 times, and 100 μL of a 1:500 dilution of the secondary antibody, goat anti-mouse IgA, α -chain-specific-horseradish peroxidase conjugate (Sigma-Aldrich, St. Louis, MO), was added, after which, the mixture was incubated for 1 h at room temperature. Plates were washed five times, and 100 μL of the substrate solution (H_2O_2 and o-phenylenediamine) was added; the mixture was then incubated for 12 min at room temperature. The reaction was stopped by the addition of 50 μL of 2N H_2SO_4 , and absorbance was read at 490 nm in a *Vmax* Kinetic Microplate Reader (Molecular Devices). The mass amounts of IgA in the samples were calculated by plotting their absorbance values on the IgA standard curve, which was calculated using a four-parameter logistic fit with SOFTmax PRO software (Molecular Devices).

TNF- α , IL-1 β , and IL-6 quantitative analysis

Concentrations in pg/mL of TNF- α , IL-1 β , and IL-6 were measured in SIWF, SI tissue and serum using solid phase sandwich ELISA for the respective cytokines (BD Biosciences, Bedford, MA). Briefly, separate 96-well plates were coated with 100 μL per well of either the anti-mouse TNF- α , IL-1 β , or IL-6 in a 1:250 dilution in 0.1 M sodium carbonate coating buffer (pH 9.5) and incubated overnight at 4°C . Plates were washed 3 times and blocked with 200 μL of Phosphate-Buffered Saline (PBS) with 10% Fetal Bovine Serum (FBS) for 1 h at room temperature. One hundred microliters of SIWF, SI tissue homogenate, serum or cytokine standard (BD Biosciences, Bedford, MA) were added, and the plates were incubated for 2 h at room temperature. The diluent was PBS with 10% FBS. Plates were washed 5 times, and 100 μL of a 1:250 dilution of the secondary antibody, either biotinylated anti-mouse TNF- α or IL-1 β was added and incubated 1 h at room temperature. After washing 5 times, Streptavidin-horseradish peroxidase (SAv-HRP) conjugate was added, and the mixture incubated 30 min at room temperature. For IL-6, a 1:250 dilution of the secondary antibody was also used; however, this was mixed with the SAv-HRP, done in one step, and allowed to incubate for 1 h. Plates were then washed 7 times, and 100 μL of the substrate solution (tetramethylbenzidine and hydrogen peroxide) was added; the mixture was then incubated for 30 minutes at room temperature in the dark. The reaction was stopped by adding 50 μL of 2N H_2SO_4 , and the absorbance was read at 450 nm in a *Vmax* Kinetic Microplate Reader (Molecular Devices, Sunnyvale, CA). The mass amounts of TNF- α , IL-1 β , or IL-6 were calculated by plotting their absorbance values on their respective standard curves, which was calculated using a four-parameter logistic fit with SOFTmax PRO software (Molecular Devices, Sunnyvale, CA).

Experiment 2: Effect of nutrition on SI sIgA and pIgR following injury

Twenty-nine mice were randomized to diet groups (chow, n = 14; PN, n = 15), anesthetized with an intraperitoneal ketamine (100 mg/kg) and acepromazine (5 mg/kg) mixture and cannulated via the right external jugular vein (0.012-in ID/0.25-in OD; Helix Medical, Inc, Carpinteria, CA). Catheters were tunneled subcutaneously over the back and exited midtail. Mice were immobilized by the tail, which has been shown not to induce significant physical or biochemical stress.

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*. After the recovery period, the two different diets were initiated. Chow-fed animals received 0.9% saline at 4 mL/d, as well as chow and water *ad libitum* throughout the study. Parenterally fed mice received solution at 4 mL/d (day 1), 7mL/d (day 2) and 10 mL/d (days 3-5) with access to water *ad libitum*. The PN solution contained 6.0% amino acids, 35.6% dextrose, electrolytes, and multivitamins, with a non-protein calorie/nitrogen ratio 127.68 Kcal/g nitrogen. The feedings met the calculated nutrient requirements of mice weighing 25-30 g.

After 5 days of feeding, mice were randomized to receive a controlled surgical stress injury identical to that in experiment 1 (Chow, n = 7; PN, n = 8) or sacrifice without injury (Chow, n = 7; PN, n = 7). In these studies, the model is a double stress model: once during cannulation and again after nutritional manipulation. Animals receiving injury were sacrificed 8 hours later by exsanguination from a left axillary artery transection. As in experiment 1, SIWF was collected and analyzed for IgA by ELISA technique.

Additionally, the SI was removed, irrigated, and then homogenized as previously described. Solubilized protein as well as mouse pIgR antibody standard (R & D systems, Minneapolis, MN) was then denatured at 95°C for 10 min with sodium dodecylsulfate and β -mercaptoethanol and protein in each specimen (40 μ g) was separated in a denaturing 10% polyacrylamide gel by electrophoresis at 150 V for 1 hour at room temperature. A total of 0.015 μ g of pIgR standard was run on each gel. The proteins were transferred to a polyvinylidene fluoride membrane using Tris-glycine buffer plus 20% methanol at 80 V for 50 min at room temperature. The membrane was blocked with blotto for 1 hour at room temperature with constant agitation. Membranes were incubated with primary antibody, rabbit anti-mouse secretory component (SC) IgG diluted in blotto (1:20,000) for 3 hours at RT with constant agitation. Membranes were then washed and incubated with stabilized goat anti-rabbit-IgG HRP conjugate (Pierce Biotechnology, Rockford, IL) diluted 1:5,000 for 1.5 hour at RT with constant agitation. After a final wash, the membrane was incubated for 5 min with the substrate for HRP (Supersignal West Femto Maximum Sensitivity Substrate; Pierce Biotechnology, Rockford, IL) and bands were detected using photographic film. The anti-SC antibody used in this Western blot detected two bands at ~120 kDa and ~94kDa representing pIgR and free SC, respectively.^{29, 30} The combined value of these bands was determined for the quantification of the pIgR expression in each case and compared to the pIgR standard to determine the actual protein amount.

Experiment 3: Effect of TNF- α or IL-1 β blockade on SI sIgA response to injury

Mice were randomized to treatment groups and underwent intra-peritoneal (IP) injection of either phosphate-buffered saline (PBS) (n = 12), 100 μ g of antagonistic TNF- α monoclonal antibody (TN3-19.12; Santa Cruz Biotechnology, Santa Cruz, CA; n = 12), or 50 μ g of antagonistic IL-1 β (B122; Santa Cruz Biotechnology, n = 12). These doses were identical to those used in our previous studies of the effect of TNF- α and IL-1 β blockade on the airway sIgA response to injury; doses were determined in a series of pilot experiments.¹² All treatment groups received equal injection volumes (200 μ L). Thirty minutes later, mice were

anesthetized by IP injection of the ketamine (100 mg/kg) and acepromazine (5 mg/kg) mixture. Surgical stress identical to that in experiments 1 and 2 was then performed. After 8 hours, mice were sacrificed and SIWF was collected for analysis of IgA by ELISA.

Experiment 4: Effect of exogenous TNF- α , IL-1 β , and IL-6 injection on SI IgA levels

Animals were randomized to receive injury (n = 12) via surgical stress identical to experiments 1-3 or an intraperitoneal injection of recombinant TNF- α , IL-1 β , and IL-6 (n = 12). Uninjured animals serving as controls (n = 12) provided baseline values. For the IP cytokine injection, recombinant mouse TNF- α , IL-1 β and IL-6 (Sigma-Aldrich, St. Louis, MO) solutions reconstituted in distilled water were prepared. All 3 cytokines were used together and a 2-hour time point was chosen for sacrifice due to our data indicating all 3 were required to elicit an IgA response at 2 hours in the airway.¹¹

Animals were anesthetized with an intraperitoneal injection of a ketamine (100 mg/kg) and acepromazine (5 mg/kg) mixture. Following anesthesia, animals received injury as in experiment 1 or an IP injection consisting of TNF- α (2 μ g), IL-1 β (1 μ g), and IL-6 (1 μ g) (n = 8). Two hours later animals were sacrificed as in experiments 1-3 while the uninjured animals were sacrificed to provide baseline values (0h). SIWF was collected for analysis of IgA by ELISA.

Statistical analysis

TNF- α , IL-1 β , IL-6, IgA, and pIgR data from treatment groups were compared using analysis of variance (ANOVA) with a post hoc analysis using Fisher protected least significance difference (PLSD) test, with $\alpha = 0.05$ (Statview 5.0.1, SAS, Cary, NC). Numerical results are presented as mean $\pm$ standard error of the mean.

RESULTS

Experiment 1: Post-injury kinetics of IgA in SIWF and of TNF- α , IL-1 β & IL-6 in SIWF, SI, and serum

Injury resulted in a significant increase in SIWF IgA by 2 hours compared to baseline control (328.7 ± 38.7 vs 134.6 ± 124.9 μ g, $p < 0.01$). Levels of IgA continued to increase and remained significantly elevated 8 hours after injury compared to control (477.1 ± 492.1 , $p < 0.0001$). The increase in IgA between 2 hours after injury to 8 hours after injury was also significant ($p < 0.05$) (Figure 1).

Injury resulted in no significant changes in concentrations of TNF- α in SIWF, SI, or serum (Figure 2). No significant changes in IL-1 β concentrations occurred in SIWF or SI; serum levels were nondetectable at all times (Figure 3). A large significant increase in serum concentration of IL-6 occurred by 5 hours after injury compared to control (673.8 ± 138.2 vs 0.0 ± 0.0 pg/mL, $p < 0.0001$) and remained elevated at 8 hours (738.2 ± 155.2 pg/mL, $p < 0.0001$). SI concentrations of IL-6 increased significantly by 5 hours after injury (10.2 ± 1.8 vs 5.6 ± 1.7 pg/mL, $p < 0.02$), but was not significantly elevated at 8 hours. SIWF concentrations of IL-6 peaked at 1 hour after injury compared to control (13.2 ± 1.5 vs 8.7 ± 1.1 pg/mL, $p = 0.02$), but decreased by 2 hours after injury and remained similar to control through 8 hours (Figure 4).

Experiment 2: Effect of nutrition on SI IgA and pIgR following injury

Parenterally-fed animals had significantly more weight loss compared to Chow animals over the 7-day course of the study (-4.4 g $\pm$ 0.4 vs -1.2 ± 0.5 , $p < 0.05$).

Uninjured (0h) parenterally-fed animals had significantly lower levels of SIWF IgA compared to uninjured (0h) chow-fed animals (92.2 ± 15.6 vs 329.0 ± 44.2 μg , $p < 0.005$). Injury (8h) resulted in a significant increase in SIWF IgA in chow-fed animals (546.1 ± 96.4 vs 329.0 ± 44.2 μg , $p < 0.01$) with no significant increase in SIWF IgA in parenterally-fed animals (Figure 5).

Uninjured (0h) parenterally-fed animals had significantly lower levels of SI pIgR compared to uninjured (0h) chow-fed animals (33.3 ± 11.3 vs 86.7 ± 13.5 μg , $p < 0.002$). Injury (8h) resulted in a significant increase in SI pIgR in chow-fed animals (125.3 ± 4.0 vs 86.7 ± 13.5 μg , $p < 0.02$) with no increases in parenterally-fed animals (Figure 6).

Experiment 3: Effect of TNF- α or IL-1 β blockade on SI sIgA response to injury

All 3 groups receiving an IP injection and subsequent injury significantly increased SIWF IgA from uninjured control levels (PBS: 639.2 ± 24.5 vs 433.3 ± 40.3 μg , $p < 0.0001$; TNF- α antibody: 606.9 ± 23.2 , $p = 0.0003$; IL-1 β antibody: 628.2 ± 34.5 , $p < 0.0001$). Neither blockade of TNF- α nor IL-1 β affected SIWF IgA compared to the PBS injection group (Figure 7).

Experiment 4: Effect of exogenous TNF- α , IL-1 β , and IL-6 injection on SI sIgA levels in chow-fed mice

Injection of the combination of TNF- α , IL-1 β , and IL-6 cytokines significantly increased SIWF IgA compared to control (615.2 ± 31.3 vs 447.7 ± 28.2 μg , $p = 0.0002$). Injury also significantly increased SIWF IgA compared to control (594.7 ± 25.1 μg , $p = 0.0009$). There was no difference between the cytokine injection group and the injury group (Figure 8).

DISCUSSION

Following severe injury or during prolonged critical illness, both the respiratory and intestinal tracts must withstand and react to unusual challenges. Aspiration, prolonged intubation, poor pulmonary toilet, increased secretions and bacterial colonization challenge the upper and lower respiratory tracts.^{31, 32} Ileus, bacterial overgrowth, bacterial contamination with ICU organisms, altered perfusion/permeability and antibiotic pressure (which disrupts normal bacterial flora) challenges the GI tract.³³⁻³⁵ Rapid and effective resuscitation, control of injuries, and judicious use of antibiotic affect outcome.^{36, 37} But under these conditions early decisions regarding nutritional support- in particular the route of nutrition - affect the susceptibility of the body to infectious complications. There is substantial, if not overwhelming evidence, that delivery of enteral feeding- when clinically feasible- improves outcome by reducing infections.^{1, 38-40} Our prior work on the effect of route and type of nutrition on mucosal immunity provides a cogent explanation for reduced infectious complications associated with enteral feeding and our recent work on inflammation and injury provides insight onto the effects of route of feeding on the inflammatory response of the mucosal immune system.

Recently, we identified an acute airway sIgA mucosal immune response to injury occurring in both critically injured patients and injured mice and studied both the mechanisms of this response and the impact of nutrition-related alterations in airway responses of injured mice.^{10, 27} The current work explores the intestinal mucosal immunologic responses to that injury examining the similarities and differences between the lung and the intestine. While one would expect similarities in function of the lung and GI tract MI due to their commonality in formation, organization and maintenance, the results show that while both the lung and intestine increase release of sIgA onto their surfaces after injury, the mechanisms differ in

the two organs. In addition, we showed that PN/DES impairs this immunologic response in both sites.

The pulmonary and gastrointestinal tracts represent the largest surface areas of the body in contact with the external environment. While the lower respiratory tract remains largely sterile in health through multiple mechanisms which clean inspired air, the intestine requires maintenance of a constant barrier against the large quantities of intra-luminal bacteria potentially capable of causing infection.³ The submucosal areas of both organs contain immune cells which provide specific immune protection via the major strategic immune molecule, sIgA.⁴¹ The production of sIgA normally occurs through a regulated process common to both the intestine and the respiratory tract.⁴² Peyer's patches take up intra-luminal antigens through specialized M cells for processing by dendritic cells and sensitization of naïve T&B cells. The T&B cells which enter the Peyer's patches are destined for mucosal immune function. The sensitized cells migrate to the mesenteric lymph nodes and through the thoracic duct to the systemic circulation for distribution to intestinal and to extra-intestinal sites such as the lung.²¹ Localization occurs under the direction of specialized adhesion molecules expressed on endothelial surfaces and integrins expressed on the sensitized lymphocytes.⁴³ In the specific site of function, B-lymphocytes under the direction and stimulation of helper T-cells and various cytokines (IL-4, 5, 6, 10) produce IgA in its dimeric form.⁴ The dimeric IgA binds to pIgR on the basolateral surface of the epithelium for transport to the apical surface via transcytosis. Enzymatic cleavage of the pIgR-IgA complex releases the IgA molecule attached to a pIgR remnant (known as secretory component) in the form of sIgA.¹⁸ A main function of MI is immune exclusion whereby sIgA binds gut or respiratory pathogens preventing their adherence to the epithelium and subsequent infection.^{5, 6} In the gut sIgA also plays a role in immune homeostasis whereby sIgA attenuates responses to commensal bacteria at least in part due to lack of an inflammatory reaction to binding of its Fc domain.^{8, 44} Under non-inflammatory states, sIgA functions as a non-inflammatory immunoregulatory molecule maintaining homeostasis between gut bacteria and the host.⁷

The current work confirms that the intestine responds with an IgA increase after injury just like the lung.¹⁰ We interpret this as an innate and adaptive defense mechanism to prevent infection and inflammation after injury. The mechanisms are similar between the two sites but with several notable differences. Clearly systemic cytokine release after injury can trigger the lung and intestinal immune response; both sites increased sIgA after intra-peritoneal injection with the cytokine cocktail of TNF- α , IL-1 β , and IL-6 (all three were used since prior work demonstrated that the respiratory response required all 3¹¹), but blockade of TNF- α or IL-1 β with specific monoclonal antibodies had no effect in the intestine at doses which effectively blocked or reduced the lung response.¹² In the mouse, airway levels of TNF- α , IL-1 β , and IL-6 increased in a distinct bimodal pattern with peaks at 3 and 8 hours after injury, with the 8 hour peak corresponding to increases in sIgA.¹¹ Levels of these cytokines in the airway far exceeded serum levels indicating a local rather than a systemically-driven airway cytokine response.¹¹ In both the intestinal fluid and intestinal tissues, however, IL-6, but not TNF- α nor IL-1 β , increased. Mechanistically, pIgR increased significantly in the intestine of Chow mice while pIgR levels remained constant in the airway. Because pIgR is consumed 1:1 with IgA transport into the lumen and sIgA release increased in both the airway and the intestine, production of pIgR must be up-regulated at both sites following injury to meet the demands of IgA transport.¹⁸ TNF- α and IL-1 β increase pIgR production at a molecular level^{13, 14}, which at least partly explains the increased transport of IgA into the lumen at both sites but not the observation that pIgR levels increased from baseline in the intestine after injury but not in the lung.

The intestinal pIgR pool appears labile compared to the lung levels, a fact clearly observed in our prior comparisons of enteral and parenteral feeding and the PN studies in this work.²⁴ We demonstrated that PN/DES reduced levels of intestinal pIgR without altering levels in the lung and decreased respiratory and intestinal IgA levels. The current work shows an effect of PN/DES on the response to injury. Within the intestine, PN eliminated the increase in intestinal pIgR seen in chow fed mice after injury (as well as reducing baseline pIgR levels) and eliminated the increase in intestinal levels of sIgA following injury.

Several factors likely contributed to failed gut sIgA response in PN animals including this inability to increase pIgR levels perhaps through inducing unresponsiveness to TNF- α and IL-1 β stimulation. Our prior work also showed that PN/DES significantly impairs the machinery for IgA production by reducing the absolute numbers of immune cells in both the lungs and gut, significantly decreasing the cytokines which normally stimulate IgA production (the T_H2 cytokines), and reducing pIgR to ultimately limit IgA production and transport capacity.^{21, 24} As a result, less intra-luminal sIgA is available to support the mucosal barrier defense in a scenario of gut stasis, injury and increasing bacterial virulence. Diebel et al. noted that *in vitro* sIgA levels blunt the release of pro-inflammatory cytokines and reduce polymorphonuclear chemotaxis.⁴⁵ Therefore, inability to mount an sIgA response to injury provides further evidence that PN renders the host more susceptible to infection and augments the inflammatory response following injury.

The mucosal immune system plays an important role in defending mucosal borders throughout the body from a variety of pathogens. It represents an innate defense mechanism that is adaptable to specifically target pathogens through the expression of sIgA. Enteral nutrition or stimulation maintains this defense in both the airway and the gut mucosal sites and support an innate post-injury response at both sites. The mechanisms underlying these responses differ between the airway and the gut. Local pro-inflammatory release of TNF- α and IL-1 β do not appear to play as critical a role in the gut response but are important in the lung response. Experimentally, PN/DES adversely affects MI and alters inflammatory responses and defenses against infectious challenges posed by systemic stresses. Increased understanding of nutrition-induced alterations in mucosal immune integrity after injury may suggest novel clinical therapies to minimize complications under conditions when enteral feeding is not possible.

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Figure 1. sIgA in small intestine washing fluid after injury (* $p < 0.05$ versus 0 hour, † $p < 0.05$ versus 2 hour).

Figure 2. TNF- α in small intestine, small intestine washing fluid, and serum after injury. No significant changes occurred.

Figure 3.
IL-1 β in small intestine and small intestine washing fluid after injury. No significant changes occurred. Serum levels were nondetectable at all times.

Figure 4. IL-6 in small intestine, small intestine washing fluid, and serum after injury (* $p < 0.05$ versus 0 hour).

Figure 5.
sIgA in small intestine washing fluid with chow and parenteral feeding and after injury (*
 $p < 0.05$ versus Chow 0 hour).

Figure 6.
pIgR in small intestine tissue with chow and parenteral feeding and after injury (* $p < 0.05$ versus Chow 0 hour).

Figure 7. sIgA in small intestine washing fluid 8 hours after injury preceded by intraperitoneal injection of either PBS, anti-TNF- α , or anti-IL-1 β (* $p < 0.05$ versus 0 hour).

Figure 8. sIgA in small intestine washing fluid 8 hours after either injury or intraperitoneal injection of TNF- α , IL-1 β , and IL-6 combination (* $p < 0.05$ versus 0 hour).