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IL-25 Improves IgA Levels During Parenteral Nutrition Through the JAK-STAT Pathway

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Abstract

Introduction—Parenteral nutrition (PN) impairs mucosal immunity and increases the risk of infection in part via lower IgA levels at mucosal surfaces. Transport of IgA across the mucosa to the gut lumen depends on the epithelial transport protein, polymeric immunoglobulin receptor (pIgR), which is reduced during PN. *In vitro* studies demonstrate that IL-4 up-regulates pIgR production via Janus Kinase/Signal Transducers and Activators of Transcription (JAK/STAT) signaling. Since IL-4 stimulates IgA and is reduced during PN, we hypothesized that the suppressed pIgR is a result of decreased JAK-1 and STAT-6 phosphorylation. Since IL-4 is mediated by IL-25, we also hypothesized that PN+IL-25 would restore luminal IgA by increasing phosphorylated JAK-1 and STAT-6, resulting in increased tissue pIgR and luminal IgA.

Method—Experiment 1: 2 days after IV cannulation, male ICR mice were randomized to Chow (n=11) or PN (n=9). Experiment 2: 2 days after IV cannulation, male ICR mice were randomized to Chow (n=12), PN (n=10), or PN + 0.7 µg of exogenous IL-25 (n=11) per day. After 5 days, distal ileum tissue was collected, homogenized and protein extracted for JAK-STAT expression levels using a phospho-specific antibody microarray. Tissue was homogenized to measure pIgR expression via Western blot or fixed in 4% paraformaldehyde to measure pIgR expression via immunohistochemistry. Small intestinal wash fluid was collected and IgA was quantified using ELISA.

Results—Experiment 1: PN significantly reduced phosphorylated JAK-1 and STAT-6 compared to Chow. PN also decreased the tissue levels of the Th2 cytokines, IL-4 and IL-13, as well as pIgR, and luminal IgA compared to Chow. Experiment 2: Exogenous administration of PN + IL-25 increased the phosphorylated JAK-1 and STAT-6 compared to PN alone. IL-25 completely restored expression of IL-13 to Chow levels. IL-4, pIgR, IgA, and phosphorylated JAK-1 were significantly increased with IL-25 treatment compared to PN, but failed to reach levels measured in Chow. STAT-6 was significantly increased with IL-25 treatment compared to Chow and PN.

Conclusion—PN significantly decreases the JAK-STAT pathway by reducing levels of phosphorylated STAT-6 and JAK-1. Consistent with our previous work, sIgA, pIgR, and IL-4 decreased with PN while the addition of IL-25 to PN reversed these decreases and demonstrated the role of the JAK-STAT pathway *in vivo* during PN.

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Keywords

Parenteral Nutrition; small intestine; adaptive immunity; JAK-STAT; IgA; pIgR

Introduction

Parenteral nutrition (PN) is associated with an increased risk of infectious complications in the critically ill compared to enteral feeding (EN)¹⁻⁴. Experimentally, PN alters established mucosal immune defenses by decreasing the total number of lymphocytes throughout the gut associated lymphoid tissue (GALT) including Peyer's Patches, the intraepithelial space, and the lamina propria⁵. PN also reduces levels of two IgA-stimulating Th2 cytokines, IL-4 and IL-10, in the small intestine⁶, and decreases the primary adaptive immune molecule, immunoglobulin A (IgA), which is transported by epithelial cells from the lamina propria onto the mucosal surfaces^{5, 7, 8}. The reduction of IgA levels following PN is, in part, through reduced production of the primary transport protein, polymeric immunoglobulin receptor (pIgR)^{9, 10}. These changes in the mucosal immune system with PN are consistent with the increased risk of infection in hospitalized patients.

The Janus Kinase/Signal Transducers and Activators of Transcription (JAK/STAT) signaling pathway is one of the pleiotrophic cascades employed to transduce many cell signaling events and is activated by hormones, growth factors, and cytokines¹¹⁻¹⁶. JAK/STAT provides the principle intracellular signaling mechanism required for a wide array of cytokines including IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-13, IL-15 and IFN- γ ¹⁶⁻¹⁸. Among other functions, JAK/STAT proteins influence cell development and immune stimulation¹⁹. Specifically, the Th2 cytokines IL-4 and IL-13 stimulate differentiation and maturation of B cells^{20, 21}. Binding of IL-4 and IL-13 to an IL-4 receptor (IL-4R) initiates activation of JAK proteins through phosphorylation. Activated JAK proteins then phosphorylate a tyrosine residue on STAT-6, which otherwise remains latent in the cytoplasm. Uniquely, IL-4 and IL-13 induce tyrosine phosphorylation and activation of STAT-6. *In vitro* work demonstrates that the activated STAT-6 forms dimers, translocates to the nucleus where it binds specific DNA elements and activates transcription of several products, including pIgR²²⁻²⁷. Another Th2 cytokine, IL-25, provides powerful stimulatory effects to promote Th2 immunity by increasing expression of IL-4, IL-9, and IL-13^{28, 29}. Exogenous administration of IL-25 elicits a strong Th2 response *in vivo* and *in vitro*.

Since the lack of EN during PN reduces adaptive immunity by decreasing IgA, pIgR, and Th2 cytokines, we hypothesized that a lack of enteral stimulation during PN would decrease levels of phosphorylated JAK-1 (P-JAK-1) and STAT-6 (P-STAT-6) and impair adaptive immunity. We further hypothesized that administration of IL-25, a known potent stimulator of Th2 responses, during PN feeding would reverse these changes and increase levels of IgA, pIgR and other Th-2 type cytokines through increased levels of P-JAK-1 and P-STAT-6.

MATERIALS AND METHODS

Animals

All protocols were approved by the Animal Care and Use Committee of the University of Wisconsin-Madison, and the William S. Middleton Memorial Veterans Hospital, Madison. 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 with a 12:12 hour light:dark cycle in an American Association for Accreditation of Laboratory Animal Care accredited conventional facility. Animals were fed standard

mouse chow (Rodent Diet 5001; LabDiet, PMI Nutrition International, St. Louis, MO) water *ad libitum* for 1 week prior to initiation of study protocol.

Experimental Design

Experiment 1: PN effects on JAK-STAT signaling, Th2 cytokines, IgA, and pIgR—Male ICR mice, ages 6 to 8 weeks, were randomized to Chow (Chow, n = 9) or parenteral nutrition (PN, n=11). 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 stress³⁰. 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 g³¹.

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). The luminal rinse was centrifuged at $2,000 \times g$ for 10 minutes and supernate aliquots were frozen at -80°C , and IgA was quantified by ELISA. Tissue samples were taken by removing a 3 cm segment of ileum, excluding Peyer's patches. These samples were frozen in liquid N₂ and stored at -80°C until processing.

Experiment 2: Exogenous IL-25 and PN effects on Th2 cytokines, IgA, and pIgR—Male ICR mice, ages 6 to 8 weeks, were cannulated as in experiment 1 and randomized to Chow (Chow, n = 12), PN (n=12), or PN with exogenous intravenous IL-25 (0.7 µg/mouse/day (R&D Systems)) for 5 days (IL-25, n=12). Chow and PN animals also received a saline vehicle control to the IL-25 treatment vehicle. Tissues were harvested as previously mentioned. Ileum tissue was collected and fixed in 4% paraformaldehyde overnight at 4 C for analysis of pIgR expression using immunofluorescence.

JAK-STAT Profiling by the JAK-STAT Antibody Microarray

The Phospho Explorer antibody microarray (Full Moon Biosystems, Inc., Sunnyvale, CA), contains 42 antibodies. Each of the antibodies has six replicates that are printed on coated glass microscope slide, along with multiple positive and negative controls. The antibody array experiment was performed according to established protocol³². In brief, Ileum tissue lysates were biotinylated with Antibody Array Assay Kit. The antibody microarray slides were first blocked in a blocking solution for 30 min at room temperature, rinsed with Milli-Q grade water for 3–5 min, and dried with compressed nitrogen. The slides were then incubated with the biotin-labeled cell lysates (~ 80 µg protein) in coupling solution at room temperature for 2 h. The array slides were washed 5 times with 1X Wash Solution and rinsed extensively with Milli-Q grade water before detection of bound biotinylated proteins

using Cy3-conjugated streptavidin. The slides were scanned on a GenePix 4000 scanner and the images were analyzed with GenePix Pro 6.0 (Molecular Devices, Sunnyvale, CA). The fluorescence signal (I) of each antibody was obtained from the fluorescence intensity of this antibody spot after subtraction of the blank signal (spot in the absence of antibody), and we used the signal of the phosphorylated protein to non-phosphorylated protein.

Analysis of Tissue IL-4 and IL-13

Small intestine segments were homogenized in RIPA lysis buffer (Upstate, Lake Placid, NY) containing 1% protease inhibitor cocktail (P8340, Sigma-Aldrich, St. Louis, MO). The homogenates were incubated 30 minutes on ice before centrifugation at $16,000 \times g$ for 10 minutes at 4°C. All supernatants were stored at -20°C until analyzed. Protein concentrations for each homogenate were determined by Bradford dye binding method using bovine serum albumin as standard.

Concentrations of IL-4 and IL-13 were measured in the small intestinal tissue homogenate using a solid phase sandwich ELISA (BD Biosciences, San Diego, CA). Briefly, 96-well plates (Costar, 9018) were coated in 0.1 M sodium carbonate coating buffer (pH 9.5) with 100 μ L per well containing antimouse IL-4 or IL-13 in a 1:250 dilution. After an overnight incubation at 4°C, the plates were washed three times with wash buffer and blocked with 200 μ L of phosphate-buffered saline (PBS) containing 10% fetal bovine serum (FBS) for 1 hour at room temperature. One hundred μ L of tissue homogenate or cytokine standard (BD Biosciences) were added to respective wells. After incubation for 2 hours at room temperature, plates were washed 5 times. 100 μ L of a 1:250 dilution of secondary antibody was added and incubated 1 hour at room temperature. Plates were washed 5 times before streptavidin-horseradish peroxidase (SAV-HRP) conjugate was added, and the mixture was incubated 30 min at room temperature. Reactions were stopped by adding 50 μ L of 2N H₂SO₄, and the absorbance was read at 450 nm in a Vmax Kinetic Microplate Reader. The mass amounts were determined by plotting sample absorbance values on a 4-parameter logistic fit standard curve, as calculated with SOFTmax PRO software (Molecular Devices).

Analysis of pIgR expression

Experiment 1 - Western Blot—Solubilized protein from small intestinal tissue homogenate was denatured at 95°C for 10 minutes with sodium dodecylsulfate and β -mercaptoethanol, and 20 μ g of protein was separated in a denaturing 10% polyacrylamide gel by electrophoresis at 150 V for 1 hour at room temperature. Proteins were transferred to a PVDF membrane, and western blot was performed as previously described^{9, 33, 34}. Densitometric measurements of protein bands were analyzed and quantified with the NIH Image J software. The combined density of the ~120-kd and ~94-kd bands were determined for the quantitation of the pIgR protein expression in each sample.

Experiment 2 - Immunofluorescence—Since IL-25 increases intestinal smooth muscle protein, a known side effect of this cytokine, analysis of pIgR based on protein standardization was not appropriate. For this reason immunofluorescence, instead of Western Blot, was used in experiment 2 to quantify mucosal pIgR. Immunofluorescence of pIgR was performed by fixed intestinal segments in 4% formalin overnight, processing (Tissue-Tek V.I.P), and embedded in paraffin. Sections were cut (5 microns) and deparaffinized with heat and xylene. Samples were rehydrated and antigen retrieval was performed with HIER (10mM Citrate buffer, pH 6.0) in a DeCloaking Chamber for 2 minutes. Slides were rinsed and blocked with 10% BSA in PBS for 1 hour at room temperature. Sections were incubated with pIgR primary antibody (1:100, Goat anti-mouse polyclonal antibody, AF2800, R&D Systems, Minneapolis, MN) in 1% BSA with PBS for 1 hour at room temperature. Sections were rinsed and incubated with secondary antibody

(1:50, Alexa Fluor 594, Donkey anti-goat, Invitrogen, Grand Island, NY) in 1% BSA with PBS for 30 minutes at room temperature. Slides were rinsed and DAPI (P36935, Invitrogen) was applied to slides to image nuclei before coverslipping. Slides were imaged on a Nikon e600 microscope using an Olympus DP70 camera. Total pIgR expression from 12–15 villi per animal was measured over total epithelial area using NIH ImageJ software.

IgA Antibody Quantitative Analysis

IgA concentration from the SI luminal fluid was measured using a sandwich enzyme-linked immunosorbent assay (ELISA). Methods were identical to those previously published³⁵. Small intestinal luminal IgA concentrations were calculated by plotting absorbance values for an IgA standard curve, which was calculated using a 4-parameter logistic fit with SOFTmax PRO software (Molecular device).

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*. Differences were considered to be statistically significant at $p < 0.05$. All statistical calculations were performed with StatView (Abacus Concepts, Berkeley, CA).

RESULTS

Experiment 1: PN effects on JAK-STAT signaling

Microarray analysis of phosphorylated JAK-1 and STAT-6—Compared to Chow, PN feeding decreased the ratios of phosphorylated to non-phosphorylated STAT-6 (PN: 1.06 ± 0.02 vs Chow: 1.16 ± 0.02 , $p = 0.004$) and phosphorylated to non-phosphorylated JAK-1 (PN: 1.23 ± 0.04 vs Chow: 1.86 ± 0.24 , $p = 0.04$) in intestinal tissue (Figure 1).

Tissue levels of IL-4, IL-13, and pIgR—PN significantly reduced tissue levels of both IL-4 (PN: 23.4 ± 1.6 pg/mg vs Chow: 34.5 ± 2.7 pg/mg, $p = 0.002$), and IL-13 (pg/mg) and IL-13 (PN: 4.4 ± 0.6 pg/mg vs Chow: 8.7 ± 1.5 pg/mg, $p = 0.02$) (Figure 2) compared to Chow feeding. Tissue levels of pIgR (μ g pIgR/40 μ g tissue protein) measured by western blot were significantly depressed in PN mice compared to chow controls (PN: $0.41 \pm .08$ vs 0.75 ± 0.06 , $p = 0.003$) (Figure 3).

Luminal levels of IgA in small intestinal wash fluid—Consistent with our previous reports, PN significantly reduced intestinal fluid IgA compared to Chow (188.1 ± 23.4 vs 387.4 ± 37.9 ng/mL, $p < 0.0001$) (Figure 4).

Experiment 2: IL-25 stimulation of PN

Microarray analysis of phosphorylated STAT-6 and JAK-1—Consistent with the first experiment, PN significantly decreased the ratios of phosphorylated to non-phosphorylated STAT-6 (PN: 0.51 ± 0.02 vs Chow: 0.65 ± 0.04 , $p = 0.04$) (Figure 5A), and of phosphorylated to non-phosphorylated JAK-1 compared to Chow (PN: 1.1 ± 0.04 vs Chow: 1.5 ± 0.04 , $p = 0.0001$) (Figure 5B). PN+IL-25 significantly increased the phosphorylated to non-phosphorylated STAT-6 ratio compared to PN alone (PN+ IL-25: 0.78 ± 0.07 vs. PN: 0.51 ± 0.02 , $p < 0.0001$) and Chow (PN+ IL-25: 0.78 ± 0.07 vs Chow: 0.65 ± 0.04 , $p < 0.05$). PN+IL-25 significantly increased the ratio of phosphorylated to non-phosphorylated JAK-1 compared to PN alone (PN+ IL-25: 1.2 ± 0.03 vs PN: 1.1 ± 0.04 , $p = 0.02$) but the ratio remained below levels observed in Chow (PN+ IL-25: 1.2 ± 0.03 vs Chow: 1.5 ± 0.04 , $p = 0.0004$).

Tissue IL-4 and IL-13 Levels—Consistent with Experiment 1, PN significantly decreased the tissue levels of IL-4 compared to chow fed mice (PN: 22.1 ± 0.9 pg/mg protein vs Chow: 34.5 ± 2.7 pg/mg protein, $p = 0.0003$) (Figure 6A). The addition of IL-25 to PN significantly increased tissue IL-4 compared to PN alone (PN+ IL-25: 28.4 ± 1.8 pg/mg protein vs PN: 22.1 ± 0.9 pg/mg protein, $p = 0.03$), the IL-4 levels remained lower than Chow (PN+ IL-25: 28.4 ± 1.8 pg/mg protein vs Chow: 34.5 ± 2.7 pg/mg protein, $p < 0.05$). PN significantly reduced tissue levels of IL-13 (pg/mg) compared to chow (PN: 8.1 ± 1.0 vs Chow: 15.3 ± 1.8 , $p = 0.002$) (Figure 6B). The addition of IL-25 to PN significantly increased IL-13 tissue levels compared to PN alone (PN+ IL-25: 13.9 ± 1.6 vs PN: 8.1 ± 1.0 , $p = 0.005$) and the IL-13 levels were similar to Chow levels (PN+ IL-25: 15.3 ± 1.8 vs Chow: 15.3 ± 1.6 , $p = 0.5$).

Immunohistochemical analysis of pIgR expression in ileum tissue—PN significantly decreased the expression of pIgR (pIgR/total mucosa area%) as a percentage of total mucosal area in ileal tissue compared to Chow alone (PN: 0.34 ± 0.04 vs Chow: 0.61 ± 0.02 , $p = 0.0002$). The addition of IL-25 to PN significantly increased expression of pIgR in ileal tissue compared to PN alone (PN+ IL-25: 0.51 ± 0.01 vs PN: 0.34 ± 0.04 , $p = 0.003$), but pIgR after IL-25 treatment mice was still significantly depressed compared to chow (PN+ IL-25: 0.51 ± 0.01 vs Chow: 0.61 ± 0.02 , $p = 0.04$) (Figure 7).

IgA concentration in Small intestinal wash fluid—As previously reported, SI luminal fluid IgA concentrations (ng/ml) significantly decreased in PN compared to Chow (PN: 163.4 ± 24.3 vs Chow: 398.1 ± 39.4 , $p < 0.0001$). IL-25 significantly increased SI luminal fluid IgA concentrations (ng/ml) compared to PN (PN+ IL-25: 281.4 ± 29.7 vs PN: 163.4 ± 24.3 , $p = 0.01$) but not to the levels of Chow mice (PN+ IL-25: 281.4 ± 29.7 vs Chow: 398.1 ± 39.4 , $p = 0.01$) (Figure 8).

DISCUSSION

PN provides nutrition to patients unable to be fed enterally. Its use, however, is associated with clinical increases in infectious complications in critically ill patients. Our previous work has defined a cogent explanation for this clinical observation: an impaired adaptive mucosal immune function during PN. This impairment is multi-factorial. Compared to enteral feeding, PN decreases total GALT T & B lymphocytes⁵, Th2 cytokines⁶, IgA at mucosal surfaces^{5, 8}, and the IgA transport protein, pIgR^{9, 36}. The current work expands on our previous observations by examining the involvement of the JAK/STAT pathways.

The JAK/STAT pathways regulate the IgA transport protein, pIgR. STAT6 transcription stimulates production of intestinal pIgR. STAT6 transcription is generated by the Th2 cytokines, IL-4 and IL-13. These cytokines utilize a common receptor and signal solely through phosphorylation of JAK-1 and STAT-6. Since our prior works showed intestinal IL-4 drops during PN, this work examined changes in phosphorylation of tissue JAK-1 and STAT-6 proteins during PN feeding and confirmed a reduction in phosphorylation. We showed PN lead to reductions in phosphorylated JAK-1 and STAT6 compared with enteral feeding. Consistent with our previous work, PN resulted in lower levels of tissue IL-4, pIgR, and luminal IgA. We also observed, for the first time, that PN significantly reduced the Th2 cytokine, IL-13, in the intestinal tissue. These data supported our working hypothesis that PN diminishes established adaptive immunity by decreasing levels of phosphorylated JAK-1 and STAT-6, which are necessary mediators of pIgR transcription. However, these observations did not establish a direct causal relationship between the Th2 cytokines and pIgR-mediated IgA protection.

Our second experiment confirmed the causal relationship between the JAK/STAT pathway and pIgR production by stimulating the STAT6 pathway with exogenous IL-25. IL-25 is a powerful Th2 cytokine that promotes production of IL-4 and IL-13 both *in vitro* and *in vivo*^{28, 29}. Experimentally, IL-25 increased tissue levels of IL-4 and IL-13 as well as expression of the phosphorylated JAK-1 and STAT-6 proteins resulting in increased tissue pIgR and luminal sIgA. Addition of IL-25 to PN increased intestinal IL-4, IL-13, phosphorylated JAK-1, phosphorylated STAT-6, pIgR, and luminal IgA compared to PN alone. These results established a direct relationship between the JAK-STAT signaling pathway with the Th2 cytokines IL-4 and IL-13 and the effector functions of adaptive immunity i.e. IgA levels in the intestinal fluid. Interestingly, IL-25 significantly improved JAK-1, IL-4, IgA, and pIgR compared to PN alone, but not all of these parameters were restored to Chow levels. This partial restoration indicates involvement of other stimuli and signaling pathways in the production of IgA and the epithelial transport protein, pIgR. For example, our prior work showed that IL-10, another Th-2 cytokine important in IgA production, decreases with PN; there is no evidence that IL-25 stimulates release of this cytokine which could have blunted the response in non-Chow fed mice. IL-25 does exert its effects on other pathways separate from the Th2 cytokines. For example, exogenous administration of IL-25 causes proliferation of smooth muscle actin^{37, 38}, an effect demonstrated in our microarray analysis. This mandated the use of different methods for quantification of pIgR in the two experiments, as detailed in the methodology. The IL-25 stimulated smooth muscle hypertrophy elevated the house keeping proteins normally used for Western blot analysis. Therefore, to appropriately quantify pIgR in experiment 2, we used immunofluorescence imaging of the mucosa and measured pIgR expression levels directly. Thus, our partial restoration of adaptive parameters may be a consequence of the actions of IL-25 on other signaling pathways which were not measured in this work.

There are several additional limitations to this study. First, we used whole intestinal tissue for our analysis and did not examine specific cell types in our analysis. Thus, we are unable to attribute the effects to a specific cellular compartment such as lamina propria or intraepithelial cell types. Instead, our results represent changes to our measured parameters for the piece of ileum tissue as a whole. Secondly, we did not examine the IL-25 effects on other mucosal events. For instance IL-25 also stimulates the Th2 cytokine IL-5^{39, 40}, a cytokine that induces eosinophilic responses. In addition, others have shown IL-25 causes increased mucus production and epithelial cell hyperplasia and hypertrophy within the lung and intestinal tissue⁴¹. The additional pathways stimulated by IL-25 could contribute to the partial restoration of IgA we observed in this work. Although IL-25 contributions to other cell types and tissues were beyond the scope of the current investigation, further experiments exploring the ramifications of exogenous IL-25 administration and the effects on other pathways is currently under investigation.

In summary, this study suggests a mechanism for the PN- induced reduction in the IgA transport protein, pIgR. Consistent with our hypothesis, we showed that PN decreases IL-4 and IL-13, the Th2 cytokines which normally stimulate phosphorylation levels of JAK-1 and STAT-6 and documented a reduction in the JAK-STAT pathway activation. We established a causal relationship between the IgA transport system and the JAK-STAT pathway by administering IL-25 to PN fed mice. IL-25 partially restored this transport system by increasing expression of Th2 cytokines, phosphorylated JAK-1 and phosphorylated STAT-6, IgA, and the transport protein pIgR. This work demonstrates that PN-induced vulnerabilities in adaptive mucosal immunity are at least partly driven by Th2 cytokine production and release.

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Figure 1. Ratio of tissue levels of phosphorylated to non-phosphorylated expression for STAT-6 and JAK-1. Parenteral nutrition (PN) significantly decreased the ratio of (A) P-STAT-6:STAT-6 and (B) P-JAK-1:JAK-1 compared to Chow. Data are represented as mean $\pm$ SEM. * $p < 0.02$ vs Chow.

Figure 2. Tissue levels of IL-4 and IL-13. Intravenous parenteral nutrition (PN) significantly decreased tissue levels of (A) IL-4 and (B) IL-13 compared to Chow. Data are presented as mean $\pm$ SEM. * $p < 0.04$ vs Chow.

pIgR expression in Ileal Tissue

Figure 3. Western blot analysis of pIgR expression in Ileum tissue. Parenteral nutrition (PN) significantly decreased the expression of pIgR compared to Chow. Data are presented as mean ± SEM. * $p < 0.003$ vs Chow.

Figure 4. Intestinal fluid immunoglobulin A (IgA) concentration. Parenteral nutrition (PN) significantly suppressed levels of IgA compared to Chow. Data are presented as mean $\pm$ SEM. * $p < 0.0001$ vs Chow.

Figure 5.

Ratio of tissue levels of phosphorylated to non-phosphorylated for STAT-6 and JAK-1 after exogenous IL-25. (A) Parenteral nutrition (PN) significantly decreased the ratio of P-STAT-6:STAT-6 compared to Chow and IL-25. The addition of IL-25 to PN significantly increased the ratio P-STAT-6:STAT-6 compared to Chow and PN. (B) PN significantly decreased the ratio of P-JAK-1:JAK-1 compared to Chow and IL-25. The addition of IL-25 to PN significantly increased the ratio of P-JAK-1:JAK-1 compared to PN, but the ratio was still significantly depressed compared to Chow. Data are represented as mean $\pm$ SEM. * $p < 0.05$ vs Chow. # $p < 0.05$ vs PN.

Figure 6.

Tissue levels of IL-4 and IL-13 after exogenous IL-25. (A) IL-4 was significantly depressed in PN compared to Chow and IL-25. The addition of IL-25 to PN significantly increased IL-4 compared to PN but failed to restore levels back to chow levels. (B) IL-13 was significantly decreased in PN compared to Chow and IL-25. The addition of IL-25 to PN restored IL-13 to Chow levels.. Data are presented as mean $\pm$ SEM. * $p < 0.005$ vs Chow. # $p < 0.03$ vs IL-25.

plgR expression / mucosal area

Figure 7.

Expression of pIgR in Ileum tissue after exogenous IL-25. Parenteral nutrition (PN) reduced the expression of pIgR in ileum tissue compared to Chow and IL-25. The addition of IL-25 to PN significantly increased the expression of pIgR compared to PN, but the levels were still decreased compared to Chow. Data are presented as mean $\pm$ SEM. * $p < 0.05$ vs Chow. # $p < 0.05$ vs IL-25.

Figure 8. Intestinal fluid immunoglobulin A (IgA) concentration. Parenteral nutrition (PN) significantly suppressed levels of IgA compared to chow and IL-25. The addition of IL-25 to PN significantly increased luminal levels of IgA compared to PN, but the levels are significantly decreased compared to chow alone. Data are presented as mean $\pm$ SEM. * $p < 0.05$ vs Chow. # $p < 0.05$ vs IL-25.