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Impairment of Mucosal Immunity by Total Parenteral Nutrition: Requirement for IgA in Murine Nasotracheal Anti-Influenza Immunity

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Secretory IgA (SIgA) is the primary mucosal Ig and has been shown to mediate nasotracheal (NT) mucosal immunity in normal immune BALB/c mice. This finding has been challenged by a report of NT immunity without IgA in knockout mice, suggesting that IgA may not be necessary for the protection of mucosal surfaces. Although other protective mechanisms may become active in the congenital absence of SIgA, these mechanisms are not the primary means of protection in normal mice. In this paper we show that feeding chemically defined total parenteral nutrition (TPN) to genetically normal, immune ICR mice by the i.v. route results in loss of nasal anti-influenza immunity and a significant drop in influenza-specific SIgA in the upper respiratory tract compared with chow-fed mice (p < 0.005), while the serum influenza-specific IgG titer is unaffected. Loss of upper respiratory tract mucosal immunity is not related to serum Ab, because 10 of 13 TPN-fed mice shed virus into their nasal secretions despite adequate serum anti-influenza IgG titers. The number of IgG Ab-secreting cells in the nasal passages and spleens of TPN-fed mice was unaffected, while both the number and the percentage of splenic IgA-secreting cells were decreased relative to those in chow-fed animals. The loss of immunity is due to the route of nutrition, not the composition of the diet, because TPN solution fed orally via gastrostomy instead of i.v. maintains NT anti-influenza mucosal immunity. We hypothesize that delivery of nutrition via the gut triggers the release of gastrointestinal neuropeptides necessary for maintenance of the mucosal immune system. The Journal of Immunology, 2001, 166: 819–825.

Secretion of IgA by the common mucosal immune system, of which the gut-associated lymphoid tissue (GALT) and bronchus-associated lymphoid tissue are major components, accounts for ~70% of the body’s total Ig production (1). B and T cells sensitized to intraluminal Ags within the Peyer’s patches (PP) of the GALT and the draining lymph nodes of the bronchus-associated lymphoid tissue (2–4) ultimately home to the lamina propria (LP) of both intestinal and extraintestinal sites, where they carry out their mature immune function. IgA produced by LP plasma cells is transported by a secretory component (SC)-mediated process through the mucosal epithelial cells into the mucociliary blanket coating moist mucosal surfaces, such as the respiratory and gastrointestinal (GI) tracts, where it affords protection by neutralizing or otherwise preventing the attachment of viruses, bacteria, and toxins to the mucosal epithelium, thus allowing luminal clearance of these potentially pathogenic agents (5–9). IgA has been shown to be responsible for the protection of the intact murine nose against influenza infection in immune mice (7, 10). Recently, this finding has been challenged by studies in IgA knockout (KO) mice suggesting that other Igs are as effective as IgA in the protection of the murine nose and that IgA may not be necessary for mucosal protection (11).

The KO mouse, while an interesting model, is genetically abnormal. We offer in this report a model for the suppression of mucosal anti-influenza immunity in normal, immune mice. Intravenous feeding with a chemically defined solution (i.v. total parenteral nutrition, TPN) results in a down-regulation of the murine common mucosal immune system with significant reductions in the numbers of both B and T cells present within the PP, LP, and intraepithelial (IE) region of the GI tract (12, 13) and lowered secretory IgA (SIgA) levels throughout the mucosal immune system (12–14). Impaired mucosal immunity is observed in both the upper (URT) and lower (LRT) respiratory tracts of i.v. TPN-fed mice (12, 15, 16), and alterations in GI IL-4 and IL-10 levels have been reported (17). The impairment in immunity is reversed either by 3 days of oral alimentation (14) or by injection of i.v. TPN-fed mice with the neuropeptide bombesin (18, 19), which mimics human gastrin-releasing peptide in stimulating the release of all other GI neuropeptides (20). We propose i.v. TPN feeding as a model for the suppression of mucosal immunity in genetically normal mice.

The purpose of this study, then, is to determine in a model of impaired mucosal immunity in genetically normal immune mice whether serum IgG can substitute for SIgA in mucosal immunity against influenza virus in the murine URT.

Materials and Methods

Animals

Six- to 8-wk-old male ICR mice (Harlan Sprague Dawley, Indianapolis, IN) were housed in an American Association for the Accreditation of Laboratory Animal Care-accredited conventional facility under controlled conditions of temperature and humidity with a 12-h light, 12-h dark cycle.
Before the experiment, mice were fed water and commercial chow ad libitum. During the experiments, the mice were housed in metal, wire-grid bottom metabolism cages to eliminate coprophagia and the ingestion of bedding. All protocols were approved by the institutional animal care and use committee.

**Immunization and challenge**

For immunization, 6- to 8-week old male ICR mice were inoculated intranasally (i.n.) while awake with 20 μl of PBS (pH 7.4) containing 100 mouse 50% infectious doses (MID₅₀) of A/PR8 (H1N1) influenza virus. For challenge, immune mice were inoculated in the same manner. Administration of virus to awake mice leads to infection of the URT only and does not produce fatal pneumonitis.

**Surgical procedures**

Mice randomized into i.v. TPN and chow groups received internal jugular catheters under ketamine/acepromazine anesthesia (100 and 10 mg/kg, respectively, i.p.). As previously described (12), through a right internal jugular approach a silicone catheter (0.012-in. inside diameter by 0.025-in. outside diameter, Baxter, Chicago, IL) was placed into the vena cava. The catheter was tunneled s.c. and exited the tail at its midpoint. The mice were placed into metal metabolism cages, partially immobilized by tail restraint to protect the catheter during infusion, and immediately connected to an infusion apparatus (720 infusion pump, Instech, Plymouth Meeting, PA), and physiologic saline solution was infused at an initial rate of 4 ml/day. This technique is an acceptable method of nutritional support that does not induce physical or biochemical stress (21).

**Intragastric (i.g.) TPN group**

For intragastric feeding, anesthetized mice received gastrostomy tubes as previously described (12). Through a vertical midline incision, the stomach was delivered into the wound. A 7.0 silk purse-string suture was placed on the posterior wall of the stomach, followed by a gastrostomy within the center of the purse-string suture using a 25-gauge needle. The silicone catheter was inserted into the gastrostomy. Omentum was mobilized and fixed to the abdominal wall using 3-0 silk sutures. The proximal end of the catheter was tunneled s.c. over the spine and exited the tail at its midpoint. As with the other two groups, catheters were immediately connected to an infusion pump delivering physiologic saline solution at 4 ml/day.

Mice receiving venous catheters underwent sham laparotomies, and those receiving gastrostomy tubes underwent sham neck incisions. All animals were allowed ad libitum access to chow for 2 days, then were placed on their respective treatment protocols for an additional 5 days unless noted otherwise. The i.v.-fed group received 4 ml of 9% saline i.v. daily in addition to mouse chow and water ad libitum. The i.v.-fed and i.g.-fed TPN groups received a standard TPN solution (4.1% amino acids, 34.3% glucose, electrolytes, and multivitamins with a nonprotein calorie to N ratio of 740 kJ/g Nit), which was initially given at the rate of 4 ml/day, but was increased over the next 24 h to 10 ml/day to meet energy and protein requirements (1619 kJ/kg/day of nonprotein calories and 14 g protein/kg/day as previously published (12)).

**Sample collection**

Nasal washes (NWs) were collected as previously described (7, 10). Briefly, a midline incision was made over the ventral aspect of the trachea slightly anterior to the thoracic inlet. The trachea was clamped off at the thoracic inlet, and 400 μl (Ab assays) or 500 μl (viral assays) of PBS was slowly injected into the tracheal lumen cephalad to the obstruction by using a 25-gauge B bevel needle attached to a tuberculin syringe. Care was taken to avoid blood contamination of the nasal lavages. NWs for viral assay were placed on ice upon collection and immediately assayed for influenza virus. NWs for ELISA analysis were placed upon ice immediately upon collection and stored at 4°C until assay. Blood was collected via cardiac puncture, and the serum was retained for Ab determination.

**Virus**

A/PR8-Mt. Sinai (H1N1) influenza virus was the gift of Parker A. Small, Jr. (University of Florida, Gainesville, FL). To generate a pool, the virus was grown in fertile specific pathogen-free eggs, pooled, filtered through a 0.45-μm pore size filter, aliquoted, and stored at −70°C. The 50% mouse lethal dose for mice was established by the i.n. inoculation of 50 μl of 10-fold serial dilutions of the virus pool into anesthetized mice. The log₅₀ 50% mouse lethal dose of the virus pool was 5.

**ELISA**

A modification of the ELISA described by Renegar and Small (10) was used. A/PR8 (H1N1) influenza vaccine (the gift of Dr. Frank Brandon, Parke Davis, Rochester, MI) in PBS (pH 8.1) at a 1/25 dilution was dried onto 96-well flat-bottom enzyme immunoassay microtiter trays (Linbro/Titertek E.I.A. II microtiter plate, Flow Laboratories, Rockville, MD). Trays were blocked for 1 h with PBS-Tween containing 1% BSA (BSA for ELISA diluent applications from Sigma, St. Louis, MO) and 1% normal rabbit serum. Ab-containing samples were incubated in wells overnight, the plates were washed, and the secondary and tertiary Ab were added and incubated for 1.5 h with appropriate washings between Ab additions. Affinity-purified goat anti-mouse IgA or IgG (Sigma) was the secondary Ab, and the final Ab was rabbit anti-goat IgG conjugated to alkaline phosphatase (Sigma). All Ab solutions were diluted in PBS-Tween, and Ab-containing samples were incubated in wells overnight. The plates were washed with tap water and allowed to dry overnight. Blue spots representing Ab secretion by individual cells were counted with the aid of a dissecting microscope.

**Viral assay**

A modification of the viral assay described by Bender et al. (22) was used. Viral samples were serially diluted (10-fold) in DMEM supplemented with 2% fetal bovine serum and amphotericin B (Sigma), 50 μg/ml gentamicin, and 10% FCS. Triplicate 100-μl samples of each dilution were placed into 96-well round-bottom tissue culture plates. To each well was added 100 μl of a 2 × 10⁶ cells/ml suspension of MDCK cells in supplemented DMEM-10% FCS. The plates were incubated 24 h at 34°C in 5% CO₂. The culture fluid was removed and replaced with DMEM (150 μl/well) containing 2.5 μg/ml amphotericin B, 50 μg/ml gentamicin, and 2 μg/ml trypsin (BD Biosciences, San Diego, CA). The plates were incubated 4 days at 34°C in 5% CO₂. Assay for viral growth was by hemagglutination. To each well was added 50 μl of a 0.5% suspension of chicken RBC. Hemagglutination was read after 1–2 h in the cold. Viral titers were calculated by the method of Reed and Muench (23).

**Spleen and nasal passage (NP) lymphocyte preparation**

Mice were anesthetized with ketamine/acepromazine to effect, exanguminated via the retro-orbital plexus, then decapitated. The facial skin, cheek muscles, and palate were removed, and the NP lymphocytes obtained from the remaining nasal tissues (nasal turbinates, septa, and lateral walls) by a modification of the technique described by Asanuma et al. (24) and Hiroi et al. (25). The NP tissues from each individual mouse were cut into small pieces in culture dishes containing 2 ml of IMEM supplemented with 4 mg/ml collagenase (Sigma), transferred to individual 15-ml centrifuge tubes, and incubated for 30 min at 37°C. After incubation, dissociated cells were filtered and washed twice in DMEM, then resuspended in 2 ml of IMEM containing 75% Percoll (Pharmacia, Uppsala, Sweden). Four milliliters of a 40% Percoll solution were layered on top of the 75% layer, and the interface was collected after centrifugation at 600 × g for 20 min at 25°C. For preparation of splenic lymphocytes, spleens were pressed through a 40-gauge wire mesh, the resultant cells were suspended in 5 ml of IMEM-10% FCS and centrifuged 5 min at 400 × g, and the pellet was resuspended in 10 ml of IMEM-10% FCS. Viable lymphocytes from each source for each individual mouse, as determined by trypan blue exclusion, were suspended in IMEM-10% FCS and assayed for Ab-forming cells (AFCs) by the enzyme-linked immunospot (ELISPOT) assay described below.

**ELISPOT procedure**

The ELISPOT assay was performed as described by Sedgwick and Holt (26). Briefly, 96-well nitrocellulose-bottom plates (Millipore, Bedford, MA) were coated overnight with affinity-purified goat anti-mouse κ and λ Ab (Southern Biotechnological Associates, Birmingham, AL), washed in PBS, and blocked with 1% BSA in PBS. Lympocytes (4 × 10⁴ to 2 × 10⁵/well) suspended in IMEM-10% FCS were added to the wells, and the plates were washed with PBS-Tween and incubated overnight at 4°C with 50 μl of goat anti-mouse IgM, IgG1, IgG2a, IgG2b, IgG3, or IgA conjugated with alkaline phosphatase (Southern Biotechnological Associates). Plates were washed with PBS, and spots representing single Ab-secreting cells were developed with 5-bromo-4-chloro-3-indolyl phosphate (Sigma) in diethanolamine buffer. Optical density was measured at 405 nm at room temperature. The plates were washed with tap water and allowed to dry overnight. Blue spots representing Ab secretion by individual cells were counted with the aid of a dissecting microscope.
Experimental design

Expt. 1: impairment of nasal mucosal immunity by TPN. In a series of experiments, 123 mice were randomized to one of four groups: chow-fed immune (n = 38), i.g. TPN-fed immune (n = 10), i.v. TPN-fed immune (n = 8), or chow-fed nonimmune (n = 24). Mice were instrumented as described above and, after 5 days on their respective feeding protocols, were challenged awake i.n. with 100 MID50 influenza virus. After 48 h, mice were sacrificed by exsanguination under ketamine/xylazine anesthesia, and viral shedding in their nasal secretions was determined. These data represent the pooled results from 17 experiments.

Expt. 2: levels of serum IgG in impaired mice. Twenty-two mice were immunized i.n. with A/PR8 influenza virus. Three weeks later, the mice were randomized to chow-fed, i.g. TPN-fed, or i.v. TPN-fed groups and instrumented as described above. At the time of surgery, serum samples were obtained for determination of influenza-specific serum IgG levels. Mice were allowed to recover for 2 days, then underwent the described feeding protocols. After 5 days on a protocol, they were challenged i.n. awake with 100 MID50 A/PR8 influenza virus, then sacrificed 42 h later, and viral shedding in the nasal secretions was determined. Viral titers were calculated by the method of Reid and Muench (23) and were correlated graphically with preinstrumentation serum influenza-specific IgG levels. For the sake of this correlation, serum IgG-specific Ab levels were taken to be the OD492 nm of a 1/100 dilution of the serum sample.

Expt. 3: effect of TPN on serum IgG levels. Eleven immune mice immunized 3 wk previously were instrumented as described and fed either i.v. saline with ad libitum chow access or i.v. TPN. Serum samples were taken at the time of initial surgery. The mice were allowed to recover from surgery for 2 days, then underwent 5 days of their respective feeding protocols and were challenged i.n. while awake with 100 MID50 A/PR8 influenza virus. After 48 h, the mice were sacrificed under ketamine/xylazine anesthesia and exsanguinated by cardiac puncture, and nasal lavages were performed for viral assay. Serum samples from the time of sacrifice were saved for Ab determination. Serum IgG anti-influenza titer was taken as the serum dilution providing an ELISA OD492 nm of 0.200.

Expt. 4: nasotracheal (NT) influenza-specific SIgA levels. Sixteen immune mice were surgically instrumented as described. Following the 2-day recovery period, they were randomized to one of two groups and fed either chow ad libitum (n = 7) or TPN solution i.v. (n = 8). The chow group also had i.v. lines for continuous saline administration. After 5 days of their respective protocols, mice were sacrificed by exsanguination under ketamine/xylazine anesthesia. NT lavages were performed, and the resulting washes were assayed for influenza-specific IgA by ELISA. NW-specific IgA results are expressed as nanograms of specific IgA per 100 μg of total nasal lavage protein. Our previously described polymeric IgA (pIgA) anti-influenza mAb pool served as the IgA standard (27). The protein concentration of the nasal secretions was determined using a micro protein assay (Bio-Rad, Hercules, CA), and protein and specific IgA levels were normalized to 100 μg of total protein/ml of lavage fluid.

Expt. 5: spleen and nasal mucosal ELISPOT AFCs. Thirteen nonimmune male ICR mice were surgically instrumented as described above and fed chow and i.v. saline for 2 days. They were then randomized to either saline i.v. with chow ad libitum (n = 5) or i.v. TPN (n = 8) groups and were inoculated i.n. while awake with 100 MID50 influenza virus. Following 13 days of feeding on their respective protocols, the mice were sacrificed. NP and spleen cells were obtained as described, and ELISPOT assays for AFCs were performed. These animals were given influenza virus to stimulate proliferation of lymphoid cells within the NPs. Our data are from individual animals, not pooled cells, and we were unable to obtain an adequate number of cells from single animals without on-going immune stimulation. ELISPOT assays were for total AFCs of each Ab class, not for influenza-specific AFCs, because we wanted to determine the effects of TPN on the general AFC population. Day 13 was determined in a pilot study to be the best time point for the enumeration of IgG spot-forming cells by ELISPOT.

Statistics

All data are expressed as the mean ± SE unless otherwise indicated. Non-parametric (Mann-Whitney) and parametric (unpaired two-tailed Student’s t test, ANOVA, and Fisher’s protected least significant difference) analyses were conducted on a Macintosh Performa using StatView 4.2 (Brain Power, Calabasas, CA) software.

Results

Intravenous TPN impairs nasal mucosal immunity

The effects of i.v. TPN feeding on URT mucosal immunity are well established. In a series of 17 experiments (summarized in Fig. 1), chow-fed (n = 38), i.g. TPN-fed (n = 10), and i.v. TPN-fed (n = 51) immune mice or chow-fed (n = 24) nonimmune mice were infected i.n. while awake with 100 MID50 influenza virus. As expected, chow-fed immune mice were protected (2 of 38, or 5.3%, shed virus), while nonimmune mice were not (22 of 24, or 91.6%, shed virus; p < 0.0001 vs chow-fed immune). The i.v. TPN-fed immune mice lost nasal mucosal immunity against influenza virus (30 of 51, or 58.8%, shed virus; p < 0.0001 vs chow-fed immune), while immune mice fed the same diet directly into the GI tract via a gastrostomy (i.g. TPN-fed) maintained their immunity against influenza virus (0 of 10 shed virus; p < 0.4 vs immune; p < 0.005 vs i.v. TPN-fed). These results show that i.v. TPN feeding depresses nasal mucosal immunity against influenza virus and that the loss of immunity is related to the route of feeding, not the composition of the diet, because the same diet fed via gastrostomy maintains immunity. The consistent loss of nasal immunity in TPN-fed mice throughout the series of experiments summarized herein emphasizes the validity of our model as a tool for the investigation of impaired mucosal immunity in genetically normal mice.

Levels of serum IgG in impaired mice

Mbawuike et al. (11) suggested that serum influenza-specific IgG is sufficient to protect the murine nose against influenza infection. To determine whether this was so in our model, 22 immune mice undergoing surgical instrumentation were screened for serum influenza-specific IgG. The catheterized mice were placed on a feeding protocol for 5 days, then challenged i.n. awake with influenza virus. We compared the presurgery anti-influenza IgG ELISA reading of a 1/100 serum dilution with protection against challenge (Fig. 2). All immune chow-fed (n = 8) or i.g. TPN-fed (n = 7) mice were protected against influenza infection, while four of seven (57%) i.v. TPN-fed mice shed virus. The serum
infectious dose. The loss of URT protection against influenza challenge, studies were undertaken to determine whether the loss of URT antiviral mucosal immunity could be due to lowered anti-influenza SIgA Ab. Immune mice were surgically implanted with an i.v. catheter, allowed to recover from surgery, randomized to chow-fed or i.v. TPN-fed groups, placed on a feeding protocol for 5 days, then sacrificed via cardiac exsanguination under anesthesia. Levels of influenza-specific SIgA Ab in the undiluted nasal lavages were assessed via ELISA and expressed as nanograms of influenza-specific IgA Ab per 100 μg of total protein (Table II). Nasal secretions of the chow-fed immune group (n = 7) had a mean influenza-specific IgA Ab level of 2218 ± 794 ng/100 μg protein, while those of i.v. TPN-fed immune mice (n = 8) had a mean value of 388 ± 128 ng/100 μg protein. Nasal influenza-specific SIgA in the TPN-fed group was significantly lower than that in the chow-fed group (p < 0.05). These results suggest that lowered SIgA in the nasal secretions may underlie the immune deficit in i.v. TPN-fed mice.

**Table II. Effect of feeding route on nasotracheal influenza-specific IgA**

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Treatment</th>
<th>Nanograms Influenza-Specific IgA/100 μg Nasal Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chow-fed</td>
<td>2555</td>
</tr>
<tr>
<td>2</td>
<td>Chow-fed</td>
<td>423</td>
</tr>
<tr>
<td>3</td>
<td>Chow-fed</td>
<td>2000</td>
</tr>
<tr>
<td>4</td>
<td>Chow-fed</td>
<td>5000</td>
</tr>
<tr>
<td>5</td>
<td>Chow-fed</td>
<td>63</td>
</tr>
<tr>
<td>6</td>
<td>Chow-fed</td>
<td>486</td>
</tr>
<tr>
<td>7</td>
<td>Chow-fed</td>
<td>5000</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td></td>
<td>2218 ± 794</td>
</tr>
</tbody>
</table>

a Fifteen mice were infected i.n. while awake with influenza virus. Three weeks later, they were surgically instrumented and randomized to i.v.-TPN feeding (n = 8) or chow feeding with saline i.v. (n = 7). Following 5 days on protocol, the mice were sacrificed. Levels of influenza-specific IgA in nasal secretions were determined by ELISA and normalized to ng specific Ab/100 μg nasal protein. Chow vs i.v.-TPN, p < 0.05.
Postprotocol weights and weight changes were similar in both TPN-fed and chow-fed mice, arguing against malnutrition as the cause of the differences. The post-treatment weight of chow-fed mice was 33.6 ± 1.6 g, while that of i.v. TPN mice was 31.4 ± 1.7 g (p < 0.3). Further, there was less than a gram difference in the on-protocol weight changes between the two groups. Over the 5 days of the feeding protocol, chow-fed mice had a marginal weight gain (0.33 ± 0.84 g), while i.v. TPN-fed mice had a marginal weight loss (−0.71 ± 1.4 g). This difference is not significant (p < 0.5).

**ELISPOT AFC in the spleens and NPs of TPN-fed mice**

To determine whether i.v. TPN affects the AFC of the NPs and spleen, 13 nonimmune mice were cannulated as described and randomized to either chow (n = 5) or i.v. TPN (n = 8) groups. To stimulate the NP cells to proliferate to a number detectable in individual animals, 100 MID50 influenza virus was administered i.n. to each mouse. Thirteen days later, mice were sacrificed, NP and spleen cells were isolated, and the number (Fig. 3) and relative percentage (Fig. 4) of AFCs in each animal were determined by ELISPOT analysis.

TPN did not affect the total number of AFCs (Fig. 3) in either the NPs (Fig. 3A; TPN, 89 ± 22 AFC/10^6 cells; chow, 99 ± 31 AFC/10^6 cells; p < 0.8) or spleen (Fig. 3B; TPN, 1612 ± 388 AFC/10^6 cells; chow, 1457 ± 599 AFC/10^6 cells; p < 0.8) of treated mice; however, IgM AFCs were increased marginally, although not significantly, in both the NPs and spleens of TPN-fed mice, and IgA AFCs were decreased. The total number of IgG-producing cells in the spleen (Fig. 3B; TPN, 735 ± 163 AFC/10^6 cells; chow, 715 ± 278 AFC/10^6 cells; p < 0.95) was unaffected by i.v. TPN feeding.

The relative percentages of IgM- and IgA-producing AFC underwent a minor shift with TPN treatment (Fig. 4). In the NPs (Fig. 4A), the percentage of IgA-producing AFCs fell from 40% in chow-fed to 32% in TPN-fed mice (p < 0.6), while the percentage of IgM-producing AFCs rose from 17% in chow-fed to 21% in TPN-fed mice (p < 0.7). Similar changes were seen in the spleen (Fig. 4B), with the percentage of IgA-producing AFCs falling from 14% in chow-fed to 6% in TPN-fed mice (p = 0.11) and the percentage of IgM-producing AFCs rising from 36% in chow-fed to 46% in TPN-fed animals (p = 0.35). The relative percentage of IgG-producing cells rose slightly in the NPs and fell slightly in the spleen with TPN feeding; however, the changes were minimal.

Although the changes in cell number and relative percentages of AFCs of each class are not statistically significant, the changes in the number and percentage of IgA-producing cells, especially in the spleen, are suggestive of a possible effect of TPN. IgG-producing AFCs, especially those in the spleen, do not appear to be adversely affected by TPN feeding.

**Discussion**

Recent work in KO mice has suggested that serum anti-influenza IgG may be adequate to protect mice against influenza challenge. Mbawuike et al. (11) reported that IgA−/− KO mice can be immunized against influenza virus and that pulmonary protection was observed in both IgA+/+ and IgA−/− mice at 24 and 48 h after the infectious challenge. Pulmonary protection in IgA−/− mice, measured at 24 h postchallenge, was also provided by the i.p. administration of all classes of Ab, including IgG and monomeric IgA.
Epstein et al. (28) reported that the lungs of J chain KO mice are, likewise, protected by the i.p. administration of influenza-specific serum Ab. These findings are not remarkable, because Ramphal et al. (29) demonstrated in 1979 that in genetically normal BALB/c mice, protection of the lung from influenza infection is due to serum Ab, i.e., monomeric Ig such as IgG. It is to be expected, therefore, that the lungs of immune IgA−/− or J chain-deficient mice would be protected from fatal influenza infection.

Protection of the URT of normal BALB/c mice has been shown to be due to IgA. In a series of experiments reported in 1991, Renegar and Small (7, 10) demonstrated that influenza-specific plgA mAb administered i.v. to nonimmune mice is preferentially transported into nasal secretions relative to influenza-specific IgG mAb, strongly suggesting a role for SC in plgA transport. Intravenously administered influenza-specific plgA mAb, but not i.v.-administered IgG mAb, was able to protect the noses of these mice against influenza infection. Furthermore, the nasal protection provided by the passively administered plgA could be eliminated by the i.n. administration of anti-α, but not anti-γ, antiserum. In influenza-convalescent mice immune to infection with influenza virus, nasal mucosal immunity could be eliminated by the i.n. instillation of anti-α, but not anti-γ or anti-μ, antiserum. The authors concluded that mucosal protection in the intact noses of normal BALB/c mice is due to SIgA Ab. Epstein et al. (28) did not assess immunity in the noses of J chain KO mice, and the Mbawuike study (11) nasal turbinate and NW passive transport data, while suggesting that Igs other than IgA can mediate protection in the KO mouse nose are somewhat equivocal, because no attempt was made to control for the contamination of the samples by endogenous blood. In passively immunized animals, the high Ab levels reached in the blood (especially of monomeric Ig such as IgG) can contaminate lavages or tissue homogenates and neutralize virus present in the sample, causing false neutralization readings.

The IgA KO mouse provides an excellent model for study of the host defenses that become functional in the absence of IgA and is a good model for those cases in which humans express a selective IgA deficiency. As in those humans, the immune system in this mouse must compensate for the loss of a major component and apparently does so by the production of higher levels of IgG. However, the congenital removal of IgA may have additional, as yet unrecognized consequences for Ab production and transport within the mucosal immune system. Thus, the responses seen in these mice may not be those observed in the normal population, and care must be taken not to overinterpret KO data. Much evidence exists to support the importance of IgA for the mucosal protection of genetically normal individuals. In this report we present a model for the impairment of nasal mucosal immunity in genetically normal mice and show once again the importance of SIgA in the protection of the murine nose against influenza infection.

We have previously reported that feeding of mice parenterally rather than enterally has deleterious effects on the common mucosal immune system. GALT atrophy occurs with associated decreases in PP cells, LP lymphocytes, and IE lymphocytes (IEL) (12); total SIgA levels decrease in the GI (12) and respiratory (14) tracts; and functional mucosal immunity is impaired in both the URT (14, 15, 18) and LRT (16, 19). Anti-influenza mucosal immunity is maintained in the URT of mice fed TPN solution i.g. (Figs. 1 and 2), demonstrating that the chemically defined solution fed is able to provide adequate nutrition for the support of mucosal immunity and that the route by which the solution is fed is of primary importance. Furthermore, as we have shown in studies reported previously, the adverse effects of i.v. TPN on the mucosal immune system can be prevented or reversed by the injection of the neuropeptide bombesin (18, 19), an amphibian analogue of human gastrin-releasing peptide (20), so the effect cannot be attributed to malnutrition. The neuropeptide bombesin stimulates the release of the GI neuropeptides normally released by the presence of food within the GI tract; presumably, chow and the i.g. feeding of TPN solution have the same function.

In this report we show that influenza-specific SIgA in the NT lavages of TPN-fed genetically normal immune mice (Table II) is ~18% that in chow-fed mice and that this decrease mirrors the impairment of protection against i.n. influenza virus infection seen in TPN-fed mice (Fig. 1). In contrast to the KO mouse findings, serum influenza-specific IgG Ab does not compensate for the loss of influenza-specific nasal SIgA in TPN-fed mice (Fig. 2). Intravenous TPN feeding does not suppress influenza-specific IgG, but does depress nasal mucosal immunity. In five of six TPN-fed mice (Table I), the serum IgG anti-influenza titer continued to rise during i.v. feeding, reaching a level comparable to that seen in catherized immune chow-fed mice; however, unlike the chow-fed immune mice, these TPN-fed immune mice were not protected against influenza infection of the nose.

We have shown by ELISPOT analysis that prolonged i.v. TPN feeding has no adverse effect on the number of IgG-producing cells in the spleen (TPN, 735 ± 163 cells/10^6 spleen cells; chow, 715 ± 278 cells/10^6 spleen cells). Both the number and the relative percentage of IgA APC in the spleens of TPN-fed mice are depressed relative to those in chow-fed mice (Figs. 3 and 4), however, this depression, although not statistically significant (number, p = 0.18; percentage, p = 0.11), is highly suggestive and is consistent with the reduction in systemic IgA production previously reported in TPN-fed mice (12, 14), agreeing with the hypothesis that IgG-mediated humoral immunity remains intact in TPN-fed mice, while IgA-mediated immunity is impaired. The 8% drop in the relative percentage of IgA AFCs in the NP of TPN-fed mice compared with chow-fed mice also supports this hypothesis. The presence of a normal number of IgG- and IgM-producing cells in the NP of TPN-fed mice further demonstrates that IgG and IgM do not substitute for SIgA in the genetically intact mouse, because these cells are presumably also present and producing Ab in the LP of immunologically impaired mice, but protection against URT influenza infection is lost.

The mechanism for the loss of IgA-mediated mucosal immunity in TPN-fed mice has not yet been fully elucidated; however, modulation of both the production and transport of mucosal Ab is likely to be involved. We have previously shown a drop in GALT PP, IEL, and LP lymphocyte yields in TPN-fed mice, with both B and T cells being affected (8). The LP CD4+/CD8+ T cell ratio decreased in TPN-fed mice, primarily due to a drop in CD4+ cells, although the PP and IEL ratios remained unchanged. The B cell yield in all three GALT compartments of TPN-fed mice was reduced to ~60% that of chow-fed animals, as were the levels of IgA within gut and serum. It is possible that the factors leading to the atrophy of the GALT in TPN-fed mice could also exert an influence on the IgA AFCs of the NPs.

We have shown previously (27) that the selective transport of i.v. administered plgA is lowered by ~75% in TPN-fed mice compared with that in chow-fed mice, suggesting that impaired transport of IgA into secretions makes a major contribution to the lowered influenza-specific SIgA levels and decreased nasal anti-influenza mucosal immunity seen in TPN-fed animals. Furthermore, we have shown that protection can be restored if the defective transport system is saturated by i.v. administered plgA anti-influenza mAb (27), suggesting that lowered production of IgA Ab in the LP, discussed above, also contributes to the defect in nasal mucosal immunity, because IgA-mediated protection can be restored if adequate amounts of Ab are available for transport.
The impaired transport of pIgA (27) suggests that expression of SC may be depressed in TPN-fed animals. This is not unexpected, because SC expression is known to be under cytokine control. SC expression in intestinal and uterine epithelial cells is regulated by IFN-γ (30–32), the expression of membrane SC is enhanced in a synergistic fashion by IFN-γ and IL-4 (33), and SC production, surface expression, and release can be up-regulated in colonic cell lines by TNF-α (34). We have shown (17) that although the level of IFN-γ remains unchanged by diet, the levels of IL-4 and IL-10 in the intestinal tracts of TPN-fed mice decrease significantly, with this decrease correlating to a decline in intestinal IgA levels. We hypothesize that in TPN-fed animals IL-4:IFN-γ synergism is lacking, so a lowered level of SC expression is to be expected. In enterally nourished chow-fed mice, normal IL-4:IFN-γ interaction occurs, allowing for normal SC expression. Further studies are required to substantiate this hypothesis.

In conclusion, the data reported herein support the previous body of work demonstrating the importance of IgA in nasal anti-influenza mucosal immunity (7, 10) and reiterate the importance of vaccination strategies that trigger Ab production at mucosal surfaces. In addition to its obvious importance for the study of the effects of GI neuropeptides on mucosal immunity, our model has significant clinical implications. Nosocomial respiratory tract infections, such as bacterial or viral pneumonia, account for 100,000 deaths annually in the United States alone and are the second most common hospital-acquired infection (35–37). Clinically, severely injured patients fed via the GI tract have a significantly lower incidence of pneumonia than patients fed i.v. (38–40). It may be that the clinical use of pharmacologic agents such as the neuropeptide bombesin, which has been shown to restore TPN-impaired mucosal immunity in mice (13, 18, 19), will lead to a decrease in the morbidity and mortality due to nosocomial pneumonia in severely injured patients. In addition, the respiratory mucosal surfaces of patients undergoing prolonged TPN may be potential candidates for protection by the passive administration of protective pIgA Ab.

References