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Bombesin Improves Adaptive Immunity of the Salivary Gland During Parenteral Nutrition

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Abstract

Background—The parotid and submandibular salivary glands are gut-associated lymphoid tissues (GALTs) that secrete immune compounds into the oral cavity. Parenteral nutrition (PN) without enteral stimulation decreases GALT function, including intestinal lymphocyte counts and secretory immunoglobulin A (sIgA) levels. Since the neuropeptide bombesin (BBS), a gastrin-releasing peptide analogue, stimulates intestinal function and restores GALT parameters, we hypothesized that PN + BBS would stimulate parotid and salivary gland IgA levels, T lymphocytes, and IgA plasma cell counts compared with PN alone.

Methods—Male (Institute of Cancer Research) ICR mice received intravenous catheters and were randomized to chow with saline, PN, or PN + BBS (15 µg/tid/mouse) for 5 days (8/group), 2 days after cannulation. Salivary glands were weighed and either frozen for IgA and amylase analysis or fixed for histological analysis of acinar cells, IgA+ plasma cells, and T lymphocytes. Small intestinal wash fluid was collected for IgA regression analysis with salivary glands.

Results—PN reduced organ weight, acinar cell size, and amylase activity compared with chow; BBS had no significant effects on these parameters. Compared with chow, PN significantly reduced salivary gland IgA levels, IgA+ plasma cells, and T lymphocytes. PN + BBS significantly elevated IgA and restored cellularity compared with PN. Salivary gland tissue homogenate IgA levels significantly correlated with intestinal fluid IgA levels.

Conclusions—Compared with chow, PN results in atrophy of the salivary glands characterized by reduced amylase, IgA, and immune cellularity. BBS has no effect on acinar cells or amylase activity compared with PN but maintains tissue IgA and plasma cells and T-lymphocyte numbers compared with chow.

Keywords

parenteral nutrition; salivary glands; parotid; submandibular; sIgA; amylase

Introduction

The parotid and submandibular salivary glands produce and secrete saliva into the oral cavity that contains important innate and adaptive immune defense molecules, including lysozyme and secretory immunoglobulin A (sIgA), as well as high levels of digestive enzymes such as amylase produced by acinar cells.¹ Under normal conditions, IgA+ plasma cells in the salivary glands produce greater than 95% of salivary sIgA with minimal contribution from the circulation.² The salivary glands constitute part of the mucosal-associated lymphoid tissue (MALT), and strong evidence suggests a significant proportion of plasma cells in the salivary tissues are induced in gut-associated lymphoid tissue (GALT) sites, such as Peyer's patches, lymphoid follicles, and the appendix.^{2,3} Together, the collective GALT sites—including the salivary glands—produce and release sIgA across the gastrointestinal (GI) mucosa.

Intermittent enteral feeding cycles stimulate GI tract function, mediated in part through the release of enteric nervous system neuropeptides.⁴ The lack of enteral nutrition (EN) results in atrophy of the intestinal mucosa, decreased production of digestive enzymes, and impairment of mucosal immunity.^{5–7} Our established model of intravenous (IV) parenteral nutrition (PN) provided without enteral feeding demonstrates that lack of EN affects GALT immune histology and function, with decreases in Peyer's patches and lamina propria lymphocyte counts; lower Th2 intestinal cytokines; reduced expression of the sIgA epithelial transport protein, polymeric immunoglobulin receptor (pIgR); and ultimately the loss of sIgA release in the intestine and respiratory tract.^{8–10} Other investigators have studied the effects of PN on the parotid salivary gland, documenting decreased organ weight and lower protein and amylase content,¹¹ but none examined PN effects on salivary gland immune parameters.

Neuropeptides released from the enteric nervous system stimulate GI function.¹² We previously administered the gastrin-releasing peptide analogue, bombesin (BBS), during PN and demonstrated that this neuropeptide increases the number of Peyer's patch lymphocytes, restores intestinal Th2 cytokines, and elevates sIgA levels in the intestinal lumen compared with PN alone.^{13,14} In addition, BBS restores established IgA-mediated viral and bacterial defenses lost during PN.^{15,16} BBS has been administered to humans without complication, making this neuropeptide an appealing candidate for future clinical studies.^{17,18} Since BBS stimulates mucosal immunity during PN, the current study investigated whether BBS affects parotid and submandibular gland adaptive immunity in the absence of any enteral stimulus during PN by measuring homogenate IgA content and plasma and T cells numbers in salivary gland tissues. We also addressed changes in salivary gland digestive capacity induced by exogenous BBS by examining acinar cell histology and tissue amylase activity. Finally, we determined the relationship between salivary gland tissue IgA levels with small

intestinal sIgA levels in animals to establish the potential to measure salivary gland immune markers to monitor intestinal immune status.

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. Male Institute of Cancer Research (ICR) mice were purchased from Harlan (Indianapolis, IN) and housed 5 per micro isolator-top cage in a temperature- and humidity-controlled environment with a 12-hour/12-hour light/dark cycle at an Association for Assessment and Accreditation of Laboratory Animal Care–accredited conventional facility. Animals were fed a standard pelleted mouse chow (Rodent Diet 5001; LabDiet, PMI Nutrition International, St Louis, MO) and given ad libitum chow and water for 1 week prior to initiation of the study protocol.

Twenty-four male ICR mice (7–8 weeks old) were anesthetized and randomized to chow with an IV catheter (chow, $n = 8$), PN ($n = 8$), or PN with 45 $\mu\text{g}/\text{mouse}/\text{d}$ BBS (Bombesin; B4272, Sigma-Aldrich, St Louis, MO) delivered IV for 5 days (PN + BBS, $n = 8$). This IV dose was defined in a previous dose-response study and chosen since it consistently restored Peyer's patch cellularity and elevated intestinal sIgA compared with PN feeding alone (unpublished observation). Animals were anesthetized by intramuscular injection, weighed, and underwent placement of silicon rubber catheter (0.012-inch ID/0.025-inch OD; Helix Medical, Carpinteria, CA) in the vena cava through the right external jugular vein. The catheter was tunneled subcutaneously and exited 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 nutrition support and does not produce physical or biochemical evidence of stress.

The catheterized mice were connected to infusion pumps and received saline (0.9%) at 4 mL/d and ad libitum chow (Agway, Syracuse, NY) and water during 48 hours of recovery. After 48 hours, chow mice continued to receive 0.9% saline at 4 mL/d as well as ad libitum chow and water. PN animals received PN solution at rates of 4 mL/d (day 1), 7 mL/d (day 2), and 10 mL/d (days 3–5) 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 nonprotein calorie/nitrogen ratio of 128:1 (Table 1). These values were calculated to meet the nutrient requirements of mice weighing 25–30 g.¹⁹

After 5 days of feeding (7 days postcatheterization), mice were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and acepromazine (10 mg/kg) and exsanguinated via left axillary artery transection. The salivary glands were removed, washed in saline, blotted dry, and weighed. The glands were sectioned and either frozen in liquid N₂ and stored at –80°C until processing or fixed briefly in cold 4% paraformaldehyde, transferred to 70% ethanol, and stored overnight at 4°C for histology. The small intestinal

contents were rinsed with 20 mL of ice-cold Hank's balanced salt solution (HBSS), centrifuged at 2000 g for 10 minutes, and stored at -80°C for IgA analysis.

Measurement of Parotid and Submandibular Protein, DNA, and Amylase Activity

The frozen salivary gland samples were homogenized in ice-cold RIPA lysis buffer (Upstate, Lake Placid, NY) containing 1% protease inhibitor cocktail (P8340; Sigma-Aldrich). The homogenate was kept on ice for 30 minutes prior to centrifugation at 16,000 g for 10 minutes at 4°C . The supernatant was then stored at -20°C until analysis. The protein and DNA concentrations were determined by Bio-Rad (Hercules, CA) assay and a Hoechst reagent fluorimetric method, respectively. Salivary gland amylase activity was measured by the Phadebas blue starch test and normalized to DNA.

Salivary Gland Histology and Immunohistochemistry

The fixed salivary gland tissue sections were processed (Tissue-Tek V.I.P.; Sakura Finetek, Torrance, CA) and embedded in paraffin. The embedded tissue was cut (5 μm thick) and placed on adhesive coated slides (white Aminosilane; Newcomer Supply, Madison, WI), deparaffinized, rehydrated through graded ethanol washes (100% ethanol $\times$ 2, 95% ethanol $\times$ 2, 70% ethanol $\times$ 1, for 2 min each), and rinsed in distilled H_2O . To determine changes in acinar cells, slides were stained with hematoxylin and eosin. Eosin is a fluorescent dye used commonly for bright-field histology analysis of sectioned tissues. However, under fluorescent imaging, eosin-stained tissues emit fluorescence based on the amount of eosin present in the structures. Since acinar cell granules absorb eosin, this method was used to visualize changes in acinar cell granule levels (Figure 1).

To determine changes in IgA+ plasma cells and T cells, we stained for IgA and CD3. Briefly, antigen retrieval was performed by boiling slides in 10 mM sodium citrate buffer (pH 6.0). T lymphocytes were stained by incubating sections with rabbit anti-CD3 γ antibody (cat. 3256-1; Epitomics, Burlingame, CA) overnight in 1% bovine serum albumin (BSA)–phosphate-buffered saline (PBS) at 4°C , quenching endogenous peroxidases with 3% H_2O_2 , incubating ImmPRESS anti-rabbit Ig (MP-7401; Vector Laboratories, Burlingame, CA) for 30 minutes in 1% BSA-PBS at room temperature, and developing with DABI substrate. Slides were counterstained with hematoxylin and imaged (Suppl. Fig. S1). For IgA+ plasma cell staining, sections were incubated with rat anti-mouse IgA conjugated with FITC (11–4204; eBioscience, San Diego, CA), and nuclei were stained by DAPI (P36935; Invitrogen, Carlsbad, CA) (Suppl. Fig. S2). All slides were imaged on a Nikon e600 microscope (Nikon, Tokyo, Japan) using an Olympus DP70 camera (Olympus, Tokyo, Japan). Triplicate fields were imaged for each sample, and cells were normalized to field area (mm^2).

Small Intestinal Wash Fluid IgA Quantitation by Enzyme-Linked Immunosorbent Assay

IgA concentration from the intestinal luminal wash and serum was measured using a sandwich enzyme-linked immunosorbent assay. Methods were identical to those previously

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published.²⁰ Serum and intestinal lumen 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 Devices, Sunnyvale, CA).

Statistical Analysis

The data are expressed as means $\pm$ standard error of the mean. Statistical significance was determined using analysis of variance (ANOVA) with Fisher's protected least significant difference post hoc test or Student *t* test. Differences were considered statistically significant at $P < .05$. Regression analysis was used to compare parotid and submandibular tissue homogenate IgA levels with intestinal luminal wash IgA levels. The slope mean and 95% confidence intervals are displayed in respective graphs. All statistical calculations were performed with StatView (Abacus Concepts, Berkeley, CA).

Results

Salivary Gland Weight, Protein, and DNA

PN significantly decreased parotid gland wet weight compared with chow (Table 1). Addition of BBS to PN further decreased the wet weight, but this was not significant compared with PN alone. Following normalization of parotid wet weight to body weight, PN and PN + BBS remained significantly lower than chow controls. There were no significant differences in parotid protein or DNA concentrations, although the protein-to-DNA ratio was almost significant between chow and PN ($P < .08$), consistent with downregulation of protein synthesis following PN.

PN significantly decreased submandibular gland wet weights compared with chow; however, following normalization of wet weight to body weight, there were no significant differences between groups (Table 2). Addition of BBS to PN had no effect on submandibular wet weights compared with PN alone. There were also no differences in submandibular protein and DNA concentrations or protein-to-DNA ratios.

Salivary Gland Acinar Cell Histology

PN feeding with or without BBS decreased the overall acinar cell size and intensity of granule fluorescence within the parotid and submandibular gland acinar cells compared with chow controls (Figure 1).

Salivary Gland Amylase Activity

To confirm the decreases in the acinar cell granule content observed following PN or PN + BBS compared with chow, we analyzed the level of amylase activity of tissue homogenates (Figure 2) since this protein is one of the most abundant digestive enzymes in saliva. Compared with chow amylase activity (parotid: 14.33 ± 2.5 U/DNA concentration; submandibular: 16.0 ± 3.6), PN feeding resulted in a striking decrease in the level of amylase activity in both salivary glands (parotid: 5.4 ± 2.3 , $P < .02$; submandibular: 4.6 ± 0.5 , $P < .0005$). There was no effect on amylase activity following PN + BBS compared with PN alone, and levels remained significantly decreased compared with chow (parotid: 5.6 ± 2.1 , $P < .02$; submandibular: 3.7 ± 0.2 , $P < .0004$).

Salivary Gland Tissue Homogenate IgA

Compared with chow, PN significantly decreased salivary gland tissue levels of IgA when normalized to either tissue protein (chow parotid: 260.2 ± 21.1 vs PN: 157.9 ± 23.7 IgA ng/mg protein, $P < .005$; chow submandibular: 75.8 ± 3.6 vs PN: 42.0 ± 5.7 , $P < .001$) or DNA (chow parotid: 39.7 ± 5.3 vs PN: 19.7 ± 2.2 IgA ng/DNA, $P < .02$; chow submandibular: 41.9 ± 1.8 vs PN: 18.4 ± 3.6 , $P < .002$). Interestingly, compared with PN alone, addition of BBS to PN significantly increased submandibular gland IgA when normalized to protein (64.3 ± 6.5 , $P < .01$) or DNA (30.3 ± 5.3 , $P < .05$), as well as parotid gland IgA when normalized to protein (230.5 ± 21.3 , $P < .03$), but not DNA (33.7 ± 7.4 , $P = .055$). There was no significant difference between chow and PN + BBS for either gland when normalized to protein or DNA (Figure 3).

Salivary Gland IgA+ Plasma Cells and T Lymphocytes

Since salivary IgA is predominantly produced within the respective salivary glands by endogenous plasma cells, we used immunohistochemistry to semi-quantitatively assess IgA + plasma cell and CD3+ T-lymphocyte numbers (Figure 4). Compared with the IgA+ plasma cells in chow (parotid: 85.5 ± 6.7 cells/mm²; submandibular: 120.2 ± 5.7), there were significantly fewer cells observed following PN (parotid: 47.7 ± 6.6 , $P < .002$; submandibular: 77.3 ± 6.6 , $P < .0001$). Compared with PN alone, addition of BBS to PN significantly increased IgA+ plasma cell numbers (parotid: 79.3 ± 7.0 , $P < .01$; submandibular: 107.3 ± 3.9 , $P < .002$). There was no significant difference between chow and BBS groups (parotid: $P = .52$; Submandibular: $P < .11$).

Compared with chow, PN significantly decreased CD3+ T-lymphocyte numbers in the submandibular gland (13.6 ± 2.0 vs 6.6 ± 1.6 cells/mm², $P < .01$) but not in the parotid gland due to variability between samples (14.6 ± 4.6 vs 6.7 ± 1.9 , $P = .09$). Compared with PN alone, the addition of BBS to PN significantly increased the number of T lymphocytes in the parotid gland (16.2 ± 2.4 , $P < .05$) but not in the submandibular gland, due to sample variability (9.7 ± 1.0 , $P = .14$). There were no significant differences between chow and BBS groups (parotid: $P = .71$; submandibular: $P = .08$).

Small Intestinal Wash Fluid IgA levels

PN significantly decreased IgA levels in the intestinal lumen compared with chow (chow: 159.1 ± 11.6 IgA ng/mL fluid vs 60.7 ± 8.7 , $P < .0001$) (Figure 5). BBS significantly increased intestinal luminal IgA compared with PN alone (111.7 ± 12.2 , $P < .01$), but levels were still significantly lower than chow ($P < .01$).

Regression analysis was used to compare salivary gland tissue levels of IgA and intestinal wash sample IgA levels (Figure 6A,B). Both parotid tissue ($R^2 = 0.319$, $P < .005$) and submandibular gland tissue IgA levels ($R^2 = 0.341$, $P < .005$) were associated with small intestinal wash IgA levels in respective animals, suggesting adaptive immune function in the intestine and salivary glands responses similar to the respective treatments.

Discussion

PN is necessary in patients unable to tolerate enteral feeding. However, the lack of enteral stimulus with PN induces atrophy of digestive organ structure and functions, including the intestine.⁷ Unfortunately, within the intestine, these changes include weakened GALT immune function,^{8,10} and patients on PN are at increased risk of respiratory and intra-abdominal abscess infections compared with enterally fed controls, especially in the critically ill.²¹ Our laboratory previously demonstrated that PN feeding, with no EN, induces significant reductions in lymphocyte counts in the intestinal Peyer's patch and lamina propria compartments²²; lower levels of tissue Th2 IgA-stimulating cytokines, including interleukin (IL)-4, IL-10, and IL-13^{10,23}; decreases in expression of the IgA epithelial transport protein, pIgR²⁴; and lower levels of secretory IgA in the intestinal lumen.²⁵ Since the Peyer's patches serve as important induction sites for the global mucosal immune system, PN also reduces respiratory tract and nasal passage lymphocytes and sIgA levels.²⁶ In our animal model, these alterations lead to increased mortality following *Pseudomonas* bacterial or H1N1 viral challenge.^{27,28} Identifying and administering compounds that stimulate the intestine in the absence of feeding hold promise of clinical value in maintaining GALT function. During normal enteral feeding, efferent vagal nerve fibers and enteric nervous system neurons release gastrin-releasing peptide (GRP), which has broad stimulatory effects in the intestine.²⁹ The GRP analogue, BBS, serves as a ligand to GRP receptors and has been administered to humans without complications, suggesting the potential use of this compound in future clinical studies examining GI immune function during PN.^{17,18} We previously administered BBS during PN feeding in our animal model and demonstrated significant increases in Peyer's patch and lamina propria cellularity, Th2 cytokines, and intestinal and respiratory sIgA levels compared with PN alone.^{13,15,16,30,31} However, no previous work has investigated the effect of PN or BBS on immunity in mucosal immune sites other than the lung and intestine, for example, salivary glands, an important mucosal immune effector site.

The major salivary glands are specialized exocrine tissues that bathe the oral cavity with saliva.¹ These glands release high levels of salivary digestive enzymes produced by gland acini that aid in mastication and digestion, such as amylase that hydrolyses starches and lipase that digests fatty acids.¹ However, a less appreciated role of saliva is that it contains specialized innate and adaptive immune molecules that serve as the first immunoregulatory compounds in the GI tract.² Saliva contributes to direct antimicrobial activity and agglutination of microbes in the upper GI tract and plays important roles in healing mucosal injury.^{2,32} Salivary glands participate in the common mucosal immune system and are therefore considered MALT tissue. Similar to adaptive immunity of the small intestine, salivary glands also release sIgA that is produced by local plasma cells.³³

Most salivary gland plasma cells (90%–98%) are IgA producing, although some (1%–10%) produce IgG and collectively even fewer (<1%) produce IgM, IgD, and IgE.³⁴ Once in the glandular stroma, plasma cells release dimeric and polymeric IgA antibodies stabilized by the J chain.³⁵ The IgA leaves the interstitium, transcytoses across acinar and ductal cells via pIgR, and enters saliva. While it remains undetermined which MALT induction sites are precisely responsible for induction of salivary plasma cells, previous work suggests that a

significant (~40%) proportion of these cells arise from the GALT, including Peyer's patches and lymphoid follicles.^{3,36} Therefore, the measurement of salivary IgA could provide an estimate of intestinal immune function, since plasma cells in both compartments share lymphocyte induction sites. Other contributing induction sites include the Nasal Associated Lymphoid Tissue (NALT), since immunization of the palatine tonsil leads to specific IgA-producing cells in the salivary glands.³⁷ However, tonsillectomy does not significantly decrease salivary gland IgA in adults, suggesting the tonsils are not a primary source of IgA-producing cells.³⁸ Further evidence that plasma cells arise from multiple sites is the distribution of the IgA1 and IgA2 isoforms in human salivary glands. IgA2 is more resistant to bacterial cleavage and is predominantly secreted into the intestine, while IgA1 is less stable and largely found in the lung, but both are found in saliva.³⁹

The current study demonstrates that PN decreases acinar cell size and granule fluorescent intensity, as well as tissue amylase activity, in both the parotid and submandibular glands compared with chow controls. These effects likely explain at least some of the decreased organ wet weight observed in PN and PN + BBS mice, supporting our hypothesis that lack of enteral feeding decreases stimulation of acinar cell granule production. In contrast to previous work that demonstrated trophic effects of BBS on rat parotid gland weight and total tissue protein,⁴⁰ we observed no trophic effects on weight, total protein, or amylase activity in the mouse. In contrast, we observed salivary gland wet weights were reduced further with the addition of BBS, although this did not reach significance. The decrease in wet weights may have resulted from increased salivary secretion rates under regulation of the autonomic nerves during BBS administration, although this is speculation. The degree to which GRP receptors are present and active in the salivary glands is unclear. Previous work using radioimmunoassays in rats positively identified scarce BBS-like expression in some parenchymal salivary gland nerve fibers, and immunohistochemical analysis in the same animals suggested a nonneural form of BBS/GRP may be present in salivary gland ducts, where it may be released in saliva.⁴¹ Other work investigating rat parotid gland extracts was unsuccessful in isolating GRP, despite positively isolating and identifying other neuropeptides, such as substance P, vasoactive intestinal peptide, and calcitonin gene-related peptide.⁴² That work also functionally demonstrated GRP administration had no effect on amylase secretion,⁴² in contrast to the previously mentioned *in vivo* study that showed hypertrophy of the salivary gland and increased amylase secretion rates following BBS administration.⁴⁰ The role of BBS in salivary gland acinar cell regulation remains undetermined.

With regard to immunity, PN reduced the level of IgA and number of IgA-producing plasma cells in both salivary glands investigated. Consistent with the known effects of BBS on GALT immune function—including stimulation of Peyer's patch cellularity and intestinal IgA levels—BBS significantly increased the level of tissue homogenate IgA when normalized to either protein or DNA and elevated the number of IgA-producing plasma cells compared with PN alone. In general, the plasma cell and T-lymphocyte cellularity changes were more dramatic (by percentage) and had larger mean variability in the parotid gland. Since the parotid and submandibular glands were reported to have different CD4:CD8 lymphocyte phenotypes and unequal expression of lymphocyte adhesion molecules,⁴³ these glands may respond differently following PN and neuropeptide stimulation. This may

explain the discrepancy in statistical significance observed in T-lymphocyte numbers between the parotid and submandibular glands following BBS.

Also consistent with previous work that focused on intestinal responses, PN decreased luminal IgA levels in the intestine compared with chow. The addition of BBS during PN significantly increased luminal IgA levels compared with PN alone. The measurement of salivary sIgA as an indicator of immune status has been investigated previously, specifically in models of exercise and stress. Salivary IgA parameters have also been used as a marker of intestinal infections.⁴⁴ To determine if any association existed between adaptive immunity within the intestinal and salivary gland compartments, we measured the intestinal luminal IgA levels and salivary gland tissue homogenate IgA. Our regression analysis demonstrated a very loose association between intestinal fluid concentrations of IgA and tissue levels of IgA within the parotid and submandibular glands. At best, these data suggest similar regulation of these compartments by neuropeptides and enteral stimulation, but further characterization of salivary gland immune parameters—such as salivary IgA—is needed before the salivary glands can be considered reliable markers of intestinal adaptive immune function. From a practical standpoint, saliva IgA levels could be more clinically relevant, but those data could not be obtained in our animal model.

In conclusion, this study demonstrates that adaptive immunity of the parotid and submandibular salivary glands is altered by the route and type of nutrition. Specifically, PN results in decreased salivary gland IgA and IgA+ plasma cell numbers compared with chow. The additional finding that salivary gland IgA and plasma cell numbers are increased with BBS, while acinar cell architecture and tissue amylase activity are not, suggests the immune changes observed in the salivary glands may be reflecting the established GALT changes induced by BBS during PN. While the implications for impaired salivary gland immunity during PN are undetermined, lower IgA levels in the upper GI tract are associated with an increase in upper respiratory tract infection, consistent with the effect of PN in critically ill patients. This study provides further evidence for the role of the salivary gland in GALT immune function. It supports the hypotheses that PN without enteral feeding leads to decreased GALT adaptive immunity and that neuropeptides of the enteric nervous system are capable of reversing these alterations in mucosal immunity during PN.

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Clinical Relevancy Statement

The gastrointestinal (GI) tract is defended by components of the adaptive and innate immune arms—beginning in the oral cavity and extending to the rectum. In the oral cavity, one mechanism of adaptive immune defense is the release of secretory immunoglobulin A (sIgA). Parenteral nutrition (PN) decreases many aspects of GI defense, including sIgA levels. The finding that exogenous administration of the enteric nervous system neuropeptide, bombesin, maintains salivary gland immunity provides further support for the use of neuropeptides for maintaining mucosal defense during PN.

Figure 1.

Parotid and submandibular gland histology. Representative hematoxylin and eosin (H&E) staining of parotid and submandibular salivary gland tissue is displayed for chow (A), parenteral nutrition (PN) (B), and bombesin (BBS) (C). Representative fluorescence of eosin-stained tissue is shown for chow (D), PN (E), and BBS (F). Merged images of a gray-scale H&E and eosin fluorescence are displayed for chow (G), PN (H), and BBS (I).

Figure 2.

Amylase activity in salivary gland tissue. Tissue homogenate amylase activity over DNA levels is displayed for the parotid and submandibular glands, respectively. Compared with chow, parenteral nutrition (PN) ± bombesin (BBS) significantly reduced amylase activity.

* $P < .05$ vs chow.

Figure 3.

Salivary gland tissue homogenate immunoglobulin A (IgA). When normalized to protein, the level of tissue homogenate IgA was significantly lower in both salivary glands in parenteral nutrition (PN) compared with chow; the bombesin (BBS) group had significantly higher IgA levels than PN alone. When normalized to DNA, IgA in the homogenate was also significantly lower in both glands with PN compared with chow. The addition of BBS significantly maintained IgA levels in the submandibular but not parotid gland due to variability. * $P < .01$ vs chow. ** $P < .05$ vs PN.

Figure 4.

Immunoglobulin A+ (IgA+) plasma cells and T lymphocytes in the salivary gland. The number of IgA+ plasma cells was significantly decreased in the parotid (A) and submandibular (B) glands following parenteral nutrition (PN) compared with chow. The addition of bombesin (BBS) to PN significantly increased the number of IgA+ plasma cells compared with PN. The number of CD3+ T lymphocytes was decreased following PN compared with chow. This reached significance in the submandibular (D) but not the parotid (C) gland due to variability. The addition of BBS to PN significantly elevated CD3+ lymphocytes in the parotid but not submandibular gland. * $P < .005$ vs chow. ** $P < .01$ vs PN.

Figure 5.

Small intestinal wash fluid immunoglobulin A (IgA). Consistent with previous work, the level of intestinal wash fluid secretory IgA (ng/mL) was significantly lower in parenteral nutrition (PN) compared with chow. Following bombesin (BBS), the level of IgA was significantly greater than PN alone and not significantly different from chow. * $P < .01$ vs chow. ** $P < .05$ vs PN.

Figure 6.

Regression analysis of small intestinal wash fluid (SIWF) compared with parotid and submandibular gland immunoglobulin A (IgA) levels. (A) Regression analysis compared the level of IgA measured in the SIWF with the IgA levels in parotid gland tissue homogenate. This regression showed an $R^2 = 0.319$, $P < .005$. (B) Regression analysis compared the level of IgA measured in the SIWF with the IgA levels in submandibular gland tissue homogenate. This regression showed an $R^2 = 0.341$, $P < .005$.

Table 1

Parotid Gland Weight, Protein, and DNA.

Characteristic	Chow	PN	BBS
Gland wet weight, mg	63.1 ± 3.3	42.9 ± 5.9 ^a	31.9 ± 3.9 ^a
Gland weight/body weight, mg/g	1.8 ± 0.1	1.3 ± 0.2 ^a	1.0 ± 0.1 ^a
Protein concentration, mg/g weight	343.4 ± 46.7, NS	333.0 ± 48.5, NS	254.5 ± 39.6, NS
DNA concentration, mg/g weight	2.1 ± 0.4, NS	2.7 ± 0.6, NS	2.2 ± 0.3, NS
Protein-to-DNA	164.2 ± 27.9, NS	113.7 ± 10.1, NS	122.0 ± 17.1, NS

BBS, bombesin; NS, not significant; PN, parenteral nutrition.

^a*P* < .05 vs chow.

Table 2

Submandibular Gland Weight, Protein, and DNA.

Characteristic	Chow	PN	BBS
Gland wet weight, mg	162.2 ± 6.2	139.6 ± 9.0 ^a	132.0 ± 7.0 ^a
Gland weight/body weight, mg/g	4.5 ± 0.2	4.4 ± 0.3	4.1 ± 0.2
Protein concentration, mg/g	213.6 ± 21.8, NS	241.3 ± 11.0, NS	250.8 ± 9.2, NS
DNA concentration, µg/g	325.1 ± 36.2, NS	376.2 ± 30.7, NS	377.4 ± 28.6, NS
Protein-to-DNA	482.9 ± 43.8, NS	418.9 ± 27.0, NS	483.0 ± 62.7, NS

BBS, bombesin; NS, not significant; PN, parenteral nutrition.

^a $P < .05$ vs chow.