Published in final edited form as:

Curr Probl Surg. 2016 January ; 53(1): 11–47. doi:10.1067/j.cpsurg.2015.10.005.

The gastrointestinal immune system: Implications for the surgical patient[☆]

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Nutritional status, infection, and nutrition support: The clinical problem

Approximately one-half million uncontrolled infections occur per year, costing the United States annual health care budget \$10–20 billion; sepsis remains the leading cause of death.^{1–3} In these patients, the mortality rate averages 30% and ranges from 20% to 60% depending upon the study. Over the past 3 decades, current medical therapies have not reduced the mortality from sepsis. Prevention is the most effective means to reduce morbidity and mortality; the administration of nutrition, in particular enteral feedings (ENTs), decreases the incidence of sepsis.

Advanced medical and surgical care prolongs life but at the price of longer hospital stays and increased cost to society. An aging American population compounds these issues. Postoperative complications remain a major reason for the increase in health care expenditures and the etiology of these complications is multifactorial. Each operation carries its own risk of complications, ranging from low-risk procedures such as an inguinal hernia repair to major risks such as a pancreaticoduodenectomy or esophagectomy. However, a patient's inherent ability to heal remains a critical factor in overall outcome. It has long been recognized that a deteriorating or a poor preoperative nutritional state increases the risk of postoperative complications.^{4,5} Patients are commonly admitted with preexisting weight loss, compromised protein compartments and energy stores, and a weakened immune status. Unfortunately, with a shift to outpatient evaluation and same day surgery over the past 3 decades, preoperative evaluation frequently neglects even a cursory nutritional assessment. At our institution, 99.6% of operative patients underwent nutritional assessment with approximately 80% of assessments performed postoperatively on the first day precluding any opportunity to reverse nutritional deficiencies and reduce nutrition-related postoperative

[☆]This research was supported in-part by Award no. I01BX001672 from the Biomedical Laboratory Research & Development Service of the VA Office of Research and Development. The contents of this article do not represent the views of the Department of Veterans Affairs or the United States Government. This work was also supported in-part by for the National Institute of Health (NIH) Grant no. F32 DK105728-01A1 and T32 CA090217, United States Grant no. R01GM53439.

risk.⁶ The few patients that received assessments preoperatively had been admitted for cardiovascular evaluation, management of significant comorbidities, or complex diagnostic testing. However, impaired nutritional status develops in several additional ways other than preoperatively. Many patients admitted with reasonable nutritional status sustain progressive deterioration of their body composition postoperatively due to delayed return of gastrointestinal (GI) tract function, development of infectious or noninfectious complications, or aggravation of existing comorbidities. Additionally, well-nourished patients admitted following significant blunt or penetrating trauma experience hypermetabolism, increased oxygen consumption, and a profound metabolic mobilization of the protein compartment. If uncontrolled, these metabolic factors result in progressive weakening and increased susceptibility to infectious and noninfectious complications. Simultaneously, routine accepted care designed to prevent complications frequently aggravates the susceptibility of patients by altering host defenses: antibiotics alter the normally protective microbiome; airway intubation, nasogastric tubes, drains, incisions, IV lines and stomas violate natural host defenses; drugs inhibit gastric acidity to reduce the risk of GI hemorrhage; and vasopressor administration redistributes the blood flow to vital organs while starving the gut and other tissues of oxygen and nutrients. Although each therapy might reverse an immediately critical problem, the price of the therapies includes increased patient vulnerability to subsequent nutrition-related complications.

For many of the reasons noted above, nutrition support may occasionally be instituted preoperatively, but more commonly, it is administered postoperatively. In the 1960s, Dudrick et al⁷ first demonstrated that puppies could be raised with parenteral nutrition (PN) alone. This observation rapidly found its way into clinical care. Countless individuals who would otherwise have died from starvation survived their injuries and illnesses and healed their wounds with PN. However, PN is one of the most, if not the most complicated therapies used in patient care. Due to its complexity and the vulnerable population in which it is used, PN carries significant risks associated with everything from vascular access to the metabolic complications associated with administration of the highly concentrated parenteral formula. In the early years after more widespread adoption of PN, its use was broadened to include not only malnourished patients with loss of GI tract function but also patients who received it “prophylactically” but who did not require this therapy. This approach violates a basic tenet important in patient care: If one administers therapy to patients lacking the clinical issue for which the therapy is designed to treat, the patient may suffer the risks of therapy with no hope of benefit. However, if therapy is restricted to patients exhibiting the problem(s) for which the therapy is effective, complications may still occur but patients can benefit from treatment.

This important concept was confirmed in the landmark Veterans Administration (VA) Cooperative study in which 395 patients in various states of malnutrition were randomized to receive 85% or more of their calculated nutrient goals intravenously for 7–15 days preoperatively (as well as ad lib oral intake) and compared to a control population receiving neither total PN nor forced ENT preoperatively or for the first 72 hours postoperatively.⁸ The control group was allowed oral ad lib intake. The incidence of major infectious complications in patients randomized to PN was more than double that of the control group with no significant improvement in major noninfectious complications, such as wound

dehiscence or an anastomotic dehiscence. Although the overall results demonstrated a negative effect of PN in the entire patient population, stratification of patients by degree of malnutrition provided insights into the appropriate indications for preoperative PN. At that time, the Nutrition Risk Index (NRI) was commonly used to quantify the nutritional status patients in an equation using weight loss and preoperative serum albumin: $NRI = 1.519 \times \text{serum albumin (gm/L)} + .417 \times (\text{current weight/usual weight}) \times 100$. If the NRI was 100 or less, the patient was considered malnourished.⁹ This calculation allowed stratification of patients according to degree of malnutrition. A second criterion for malnutrition used in the trial included whether a patient exhibited any 2 of the following: a current weight $\leq 95\%$ of the ideal body weight; a serum albumin ≤ 39.2 g/L; or a serum prealbumin ≤ 186 mg/L.

When patients were stratified into borderline, mild, and severe preoperative malnutrition, the investigators noted no significant increase in infectious complications in severely malnourished patients given preoperative PN. The increase in infectious complications occurred in patients with borderline or mild degrees of malnutrition, a population (which today) would not be expected to benefit from the use of nutrition support. The PN and control patients with mild and borderline malnutrition also experienced no differences in noninfectious complications. However, severely malnourished patients in the control group sustained noninfectious complications at a rate almost twice as high as those receiving preoperative PN (Fig 1).

This breakthrough study demonstrated 2 important concepts. Firstly, specialized nutrition support—especially PN—should not be used in patients who are unlikely to benefit from the therapy (ie, if patients are not malnourished, the therapy produces only complications with no benefits). Secondly, the administration of PN to malnourished patients appears to benefit healing, rather than infectious complications, by reducing wound dehiscence and anastomotic failures. Unfortunately, although identification of at-risk patients and institution of effective nutrition therapy improves clinical outcomes, no strict definition, measurement, defining feature of nutritional status, and malnutrition currently exists. Malnutrition must be considered a continuum or spectrum. A survey of experts highlighted the discrepancies working within the field,¹⁰ including definitions and review of adequate energy intake, adequate protein intake, changes in fat mass based on involuntary weight loss, altered body mass index, decrease or lack of nutritional intake, and features of acute disease, malabsorption, and age.

The situation is different in patients sustaining severe blunt or penetrating trauma. Although preexisting malnutrition may or may not be a feature in an individual patient (usually it is not), complications arise during recovery resulting in malnutrition. Scoring systems can predict the risk for developing complications. One scoring tool, the Injury Severity Score (ISS) identifies the 3 most severely injured regions of the body of a total of 6 individual regions: the head and neck, the face, the thorax, the abdomen and pelvic contents, the extremities, and external wounds.¹¹ The ISS score is tabulated to quantify risk by assigning a score for each injury and identifying the 3 regions with the most severe injuries. A score ≥ 20 is considered severe and appears to correlate well with mortality rate following trauma. The ISS appears best suited for blunt injury, which frequently involves multiple regions. However, the ISS has 2 major shortcomings. First, following trauma, a single body region

may contain multiple injuries while other regions are spared, a fact ignored by the ISS. Secondly, a specially trained individual, usually a nurse, usually performs analysis and calculates the score well after the admission so that initial management decisions cannot be based on this score.

Since the ISS falls short in victims of penetrating trauma where multiple organs within a region are injured and the majority of trauma patients requiring an operative procedure have injuries to the intraabdominal cavity, the Abdominal Trauma Index (ATI) provides a means to predict complications, particularly septic complications.^{12–14} This tool defines the predictive risks of each intraabdominal organ which, when injured, results in septic complications. The moderate and high-risk intraabdominal organs include the pancreas, the vascular tree, the duodenum, liver, colon, spleen, and stomach, whereas the bladder, kidneys and ureter are low-risk organs. Each organ is evaluated for the severity of injury to that organ and the total adjusted taxable income (ATI) is a sum of each injured organ's risk multiplied by its severity of injury. As the ATI increases, the risk of postoperative sepsis increases as well. In general, an ATI of ≥ 25 predicts a high rate of septic complications. By using both the ISS and the ATI, clinicians can readily identify the patients most likely to develop complications and institute therapy designed to reduce those complications. Nutrition support—particularly enteral nutrition—is 1 therapy shown to affect these complications.

In the late 1970s, experimental work from Sheldon's laboratory examined the influence of nutritional status and malnutrition on animal survival after septic peritonitis. In all, 70% of well-nourished rats survived the septic challenge, but survival dropped to 10% in malnourished animals.¹⁵ Malnourished animals refed with chow had their survival rate return to that of well-nourished animals. However, 2 additional groups of well-nourished animals randomized to receive PN, both with and without fat, sustained the lowest survival of all groups, at less than 5%. It was initially felt that some essential nutritional component(s) were lacking in the parenteral formula leading to the poor survival. A follow-up study, however, compared animals that drank the PN to a pair-fed group that received an identical volume intravenously. Surprisingly, animals that drank the PN formula survived like well-nourished animals, whereas intravenously fed animals survived like malnourished animals. There appeared to be no problem with the parenteral formula itself but rather the route of nutrition was the critical factor in recovery from the infectious challenge.^{16,17} Subsequently, a number of clinical trials, first performed on trauma patients determined to be at risk for septic complications by ISS and ATI scoring confirmed the clinical relevance of these experimental results. In the initial trauma studies, individuals randomized to ENT sustained significantly fewer episodes of pneumonia or intraabdominal abscess and less multiple organ dysfunction when fed via the GI tract compared to patients either starved for the first 7 days postoperatively, or randomized to PN.^{18–20} Subsequent trials also included general surgery patients and they also demonstrated the benefit of ENT.²¹ From this body of research, clinicians learned that (1) the ISS and ATI were useful in predicting patients likely to develop infectious and noninfectious complications, (2) this information—in particular the ATI—can identify at-risk patients at the time of the initial operation so that the nutrition plan can be initiated during the operation by obtaining enteral access if appropriate and indicated, (3) the more severely injured the patient, the more benefit the patient gets from

ENT, and (4) beneficial effects of ENT are independent of prior nutritional status but dependent on the type and magnitude of injuries sustained by the patient.

Although both clinical and experimental evidence confirm that route of nutrition affects clinical outcome, the intriguing intellectual issue is why GI feeding should reduce infectious complications while parenteral feeding does not. When investigators performed the early clinical trials, no cogent explanation existed to rationalize these results. Subsequently, investigators embarked on studies of GI permeability changes and bacterial translocation where luminal bacteria “translocate” from the gut lumen across the mucosa either to lymphatics or to the blood stream. These phenomena were tested in animal models of starvation, malnutrition, alterations in diet (complexity of diet, fiber, and PN), hemorrhagic shock, ischemia or reperfusion, or a host of other factors. However, confirmation of a clinical relevance of bacterial translocation could not be confirmed in clinical trials.^{22,23} Although bacterial translocation very likely occurs under certain conditions, it fails to correlate with the development of extraintestinal infections such as intraabdominal abscess formation, pneumonia, or septicemia.

What is the GI immune system?

In the early 1990s, our work began to focus on the immunologic mucosal defenses, which protect the GI tract in addition to the upper and lower respiratory tract. The primary strategic and adaptive immunologic defensive molecule protecting these epithelial sites (as well as the genitourinary system and the ducts of the mammary gland) is IgA produced and released by an abundance of lamina propria (LP) cells located just below the mucosa. The common variable across these sites is that the preponderance of cells producing IgA are sensitized and programmed for IgA production with the Peyer’s patches (PP) of the small intestine (Fig 2). Thus, the initial work focused on the GI tract changes in mucosal immunity with different routes and types of nutrition.

In addition to being the largest digestive and endocrine organ, the GI tract is the largest immune organ in the body. From the oral cavity to the rectum, the intestinal tract accomplishes the dual tasks of digesting and absorbing nutrients along with the roles of sensing the presence of microbes and environmental infectious and toxin agents, and defending against them. To achieve these tasks, multiple layers of protective mechanisms function in the intestine under normal conditions. This intestinal boundary between host tissues and the environmental threats maintain host physiology and homeostasis and consist of anatomical barriers, secreted chemical barriers, and an adaptive immune layer. In health, these defenses maintain and support a normal intestinal microbiome. Together, these layers of defense orchestrate normal digestive function while protecting more than 400 m² of estimated intestinal surface area from foreign antigens and more than 10 trillion microorganisms—including those from the kingdoms of prokaryotes, eukaryotes, archaea, as well as viruses.

Cellular and chemical components of the mucosa barrier

The most basic level of defense in the intestine is the physical cellular and secreted chemical barrier that separates the host from the intestinal milieu. Although a relatively thick layer of

squamous cells line the oral cavity and esophagus, a single continuous monolayer of columnar epithelial cells line the GI tract distal to the lower esophageal sphincter lining the stomach and the small and large intestine. This epithelial layer derives completely from Lgr5+ stem cells that reside at the base of the intestinal crypts. These Lgr5+ stem cells subsequently differentiate into enterocytes, Paneth cells, goblet cells, enteroendocrine cells, or specialized lymphoid associated microfold cells (M-cells).²⁴ Tight-junction proteins including occludins, zonulins, and claudins, bind the epithelial cells together, preventing the extracellular and paracellular movement of luminal compounds into host tissues.²⁵ Although largely inhibiting macromolecule movement, respective tight junctions permit the free movement of cations, anions, and water, helping regulate luminal water content.²⁵ The vast majority of differentiating epithelial cells—more than 90%—develops into absorptive enterocytes, which have the fundamental roles of nutrient uptake, bile acid reabsorption, and electrolyte balance.²⁴ Enterocytes also produce and release low levels of antimicrobial products, including β -defensins and RegIII γ , in response to the presence of bacteria. These antimicrobial peptides therefore contribute to defense against microorganisms.^{26,27} The remaining 10% of epithelial cells differentiate into the less numerous but specialized populations of Paneth cells, goblet cells, enteroendocrine cells, or M-cells.²⁴

With the exception of Paneth cells and M-cells, the absorptive enterocytes and secretory goblet and enteroendocrine cells migrate from the crypts to the villus tips and slough into the intestinal lumen every 3–5 days, through a process called anoikis. The mucosa maintains a constant renewal process that allows adaptation of the host to changing diet and immunologic challenges.²⁸ Goblet cells produce mucous, predominantly releasing mucin glycoprotein molecules onto the intestinal luminal surface. Although 18 mucin genes are identified in humans, mucin2 (MUC2) is the most abundant mucin glycoprotein expressed in the intestine.²⁹ Mucins are classified as either secreted or membrane bound. For instance, MUC2 is released from goblet cells while other smaller membrane-bound glycoproteins cover the surface of enterocytes and other epithelial cells. Together, these glycoproteins provide a viscoelastic protective layer through the assemblage of large polymerized and highly glycosylated structures that expand to contain up to 95% water following release from the goblet cell theca.

The mucin layer provides a physical, defensive layer over the entire epithelial surface that aids in the passage of digestive bulk. An important characteristic of mucin glycoprotein includes the large quantities of anionically charged residues—namely, sialic acid or sulfate groups at the N-terminus—that facilitate concentration of secreted cationic immune molecules within this layer. These cationic molecules provide a chemical layer of defense.^{30,31} Characteristics of the mucous layer differ depending on location. In the small intestine, the mucous layer remains loose and colonized by mucosal-associated microbial organisms. In contrast, the large intestine contains 2 mucous layers, an inner compact layer that remains sterile and a loose outer layer that is colonized by microorganisms.³² As an additional function, the highly glycosylated mucin structure appears to play an important role in feeding and maintaining non-pathogenic species of the endogenous microbiome. One such example is the mucin degrading gram-negative bacteria, *Akkermansia muciniphila*.³³ In return, *A. muciniphila* stimulates epithelial barrier function, which benefits the host.³³

Additionally, goblet cells produce and release other molecules, such as resistin-like molecule beta (RELM β), which has antimicrobial properties, and trefoil factor 3, thought to play important roles in epithelial defense and repair processes following injury.²⁹ Mucosal cytokines, specifically the T-helper 2 (Th2) cytokines, interleukin (IL)-4, IL-13, and IL-25 influence the regulation of goblet cell expression.³⁴ Lower than normal levels of IL-4 or IL-13 result in a lack of goblet cell expansion in response to intestinal parasites and prolonged disease. The release of goblet cell products is influenced by hormones, neuropeptides, inflammatory mediators, and bacterial cell products. The Th 1 (Th1) cytokines, IL-1, IL-6, and TNF each stimulate a rapid release of mucin from the goblet cell into the intestinal lumen while hyper-inflammatory conditions can lead to goblet cell depletion.³⁵

Enteroendocrine cells constitute a class of hormone- and peptide-producing cells that comprise less than 1% of total epithelial cells. In toto, they collectively qualify the intestine as the largest endocrine organ in the body. More than 18 distinct peptide hormones are produced, including serotonin, vasoactive intestinal polypeptide, gastrin, peptide YY (PY), glucagon-like peptide-2, gastric inhibitory polypeptide, glucagon-like peptide, cholecystokinin, neurotensin, somatostatin, and secretin, among others. Various luminal nutrients, microbial antigens, and bile acids stimulate their release into the vascular system.³⁶ These compounds play important roles in total body and intestinal physiology, satiety, insulin signaling, and mood. However, relative to surrounding epithelial cells, enteroendocrine cells also express high levels of toll-like receptors and respond to bacterial ligands with the release of the chemokines CXCL-1, CXCL-3, and the cytokine IL-32, suggesting roles in innate immune function,³⁷ although the role of these cells in GI immunity remains largely underappreciated. Recent work also demonstrates important immune roles for GLP-2 in regulating intestinal Paneth cell function. Animals lacking GLP-2 receptors exhibit impaired Paneth cell antimicrobial expression, sustain significantly greater intestinal bacterial colonization, and suffer significantly greater morbidity and mortality following bacterial enteritis challenge.³⁸ Other enteroendocrine peptide hormones may also have other unrecognized roles in regulating immune function and immune cell cross-talk.

Compared with the 3–5 day life cycle of most epithelial cells, Paneth cells survive for a longer time and reside at the base of the intestinal crypts for 20–30 days.³⁹ Paneth cells contain many antimicrobial products that are released into the intestinal lumen in response to bacterial ligands or cholinergic stimulation through parasympathetic nervous system innervation. Remarkably, the concentrations of certain Paneth cell cryptdins reach 15–100 mg/mL within the intestinal crypts, more than 1000 times greater than the concentrations required to inhibit bacterial growth.⁴⁰ Therefore, the Paneth cells appear to maintain sterility near the rapidly dividing small intestinal Lgr5+ stem cells at the base of the crypts. Indeed, the loss of antimicrobial products from Paneth cells results in enteritis apparently related to increased bacterial attachment to the small intestinal mucosa.^{39,41} The colon, however, normally lacks Paneth cells. Therefore it appears that protection of stem cells at the base of colonic crypts involve the greater number of goblet cells and greater general epithelial release of antimicrobial peptides. Antimicrobial compounds released from small bowel Paneth cells include lysozyme, secretory phospholipase-A₂ (sPLA₂), angiogenin4, RegIII γ ,

and α -defensins (cryptdins in mice). These compounds clearly localize to the mucin layer once released, delivering and adding a chemical defense mechanism to the mucin layer.³¹ The Th2 mucosal cytokines IL-4, IL-9, and IL-13 stimulate Paneth cell expression of antimicrobial products, similar to goblet cells.⁴²

The sundry Paneth cell antimicrobial products each exhibit a unique antibacterial function. sPLA₂ functions catalytically to cleave fatty acids from the glycerol backbones of phospholipids imbedded in the cell membranes of microorganisms. Negatively charged bacterial cell membranes within the gut lumen attract sPLA₂ with its cationic charge. sPLA₂ then induces membrane permeability and cell lysis. sPLA₂ promotes the lysis of Gram-positive and Gram-negative bacterial strains in vitro and appears to preferentially attack membranes sites involved in growth.^{43,44} A second antimicrobial peptide, lysozyme, functions by degrading bacterial cell walls, conveying direct antimicrobial functions against bacteria. In that process, lysozyme provides an important signaling role by specifically releasing the pathogen-associated molecular pattern muramyl dipeptide from peptidoglycan residues found in bacterial cell walls. Muramyl dipeptide then serves as the ligand for the pathogen recognition receptor intracellular nucleotide-oligomerization domain 2 (NOD2), which subsequently stimulates further production and secretion of Paneth cell products. This sensing mechanism is conserved across a broad range of species, highlighting the importance of these pathogen-associated molecular pattern receptors in host defense. However, certain bacteria take advantage of the conserved host recognition by modifying their peptidoglycan walls.⁴⁵ Furthermore, the importance of NOD2 is now recognized in Crohn disease, where mutations in the NOD2 gene are associated with the risk of inflammatory bowel disease in the presence of certain microbial pathobionts. These mutations are associated with greater levels of Actinobacteria and Proteobacteria.⁴⁶ Lastly, the small cationic peptides released by Paneth cells, α -defensins in humans and cryptdins in rodents, are pore-forming molecules that attack bacterial membranes and induce lysis through membrane disruption.⁴⁷ RegIII- γ maintains a physical separation zone of 50 μ m between the mucosal cell layer and bacteria to regulate bacterial association.⁴⁸ RegIII- γ is released as a compensatory response by the intestinal mucosa to maintain this zone during changes in the luminal environment.^{49,50} Collectively, Paneth cell compounds influence the intestinal microbiome and provide the host with an array of direct antimicrobial defense molecules for host defense.

Finally, specialized cells called M-cells cover the surface of intestinal PP and lymphoid aggregates. In contrast to other epithelial cells, the porous M-cells actively sample the intestinal luminal contents and transport antigen across the epithelium to underlying immune cells to subsequently generate adaptive immune responses.⁵¹ This process is discussed in greater detail in the following section. M-cells play important roles in barrier function, since they regulate the amount of luminal contents allowed to pass across the epithelial barrier.⁵² These exposed cells reside unprotected by microvilli or the mucin glycoprotein layer to facilitate more rapid sampling of luminal antigens.

The adaptive mucosal immune layer—The gut-associated lymphoid tissue

The adaptive mucosal immune compartment plays a fundamental role in mucosal vigilance. This acquired immune defense system of the intestinal (and extraintestinal) sites depends upon the production and release of immunoglobulins (Igs), namely, secretory IgA (sIgA), into the lumen.⁵³ Beneath the physical and chemical barrier of the intestinal epithelium lie millions of adaptive immune lymphocytes residing in the LP, intraepithelial (IE) spaces, PP and lymphoid aggregates.⁵⁴ The LP constitutes the space within the villous structures that also contains the vascular supply, nerve innervation of both the autonomic and enteric nervous systems (ENS), and lymphatics. Lymphocytes in the IE space are tightly associated with the epithelial cells and play fundamental roles in epithelial biology. These collective lymphoid cells in the LP and IE compartments along with PP and lymphoid aggregate cells are known as the gut-associated lymphoid tissue (GALT). More than 60% of total body immune cells participate in the mucosal immune system in mice and humans.⁵⁵ The production and release of antigen-specific sIgA provides the most important function of GALT: production of IgA as the primary adaptive defense molecule against microorganisms at mucosal surfaces. This includes targeted removal of pathogenic bacteria and viruses when required.

The GALT is compartmentalized into induction sites, maturation sites, and effector sites. PP and lymphoid follicles serve as induction sites in humans and mice, where M-cells sample luminal antigens from the intestine by transporting them apically for release to underlying antigen-processing cells basolaterally through endocytosis.⁵⁵ Lymphocytes destined for mucosal immunity express unique integrins on their surfaces, including L-selectin and $\alpha_4\beta_7$.⁵⁶ Naïve T and B cells express high levels of L-selectin and moderate levels of $\alpha_4\beta_7$ encourage adherence to the receptor mucosal addressin adhesion molecule-1 (MAdCAM-1), constitutively expressed on the high-endothelial venules of the intestinal PP and lymphoid aggregates. Lymphotoxin β receptor (LT β R) mediates the expression of MAdCAM-1 on PP endothelial cells through interaction with activated circulating lymphocytes. Lymphotoxin α and β expression on activated lymphocytes stimulates LT β R inducing the non-canonical nuclear factor-kappa B (NF- κ B) pathway and involving the p52 and Rel B⁵⁷ to upregulate MAdCAM-1 expression (as well as tissue levels of Th2 cytokines and chemokines). Constitutively different from the MAdCAM-1 molecule expressed in other noninductive sites of GALT, a *modified* MAdCAM-1 expressed on the high endothelial venules contains a carbohydrate residue that exerts a higher affinity binding to the increased L-selectin expression on naïve lymphocytes.⁵⁶ This modification enables trafficking of naïve cells to induction sites. Expression of an unmodified MAdCAM-1 at effector sites such as the LP and IE spaces as well as lymph nodes facilitates tracking and migration of mature, sensitized cells committed to IgA production to these sites. Following attachment of naïve T and B cells to *modified* MAdCAM-1 in PP and lymphoid aggregates, several site-specific chemokines, including CCL19 and CCL21 (T cells) and CXCL13 (B cells), encourage diapedesis of the lymphocytes towards germinal centers.^{58,59}

The follicular-associated epithelium (FAE) is comprised of the intestinal PP and lymphoid aggregates. The FAE promote 2 primary functions within its structure: luminal antigen sampling and antigen processing. As mentioned, M-cells play fundamental roles in antigen

sampling through endocytosis of luminal contents and subsequent presentation to dendritic cells at the basolateral surface. M-cells also preferentially bind and take up sIgA-antigen complexes, which positively reinforce IgA production to specific luminal targets.⁶⁰ Dendritic cells provide the initial antigen processing assuring appropriate presentation to the naïve T and B lymphocytes entering the PP. Researchers believe that dendritic cells display the capacity to directly sample the luminal contents through extended dendrite projections that reach between the epithelial cell layer.⁶¹ However, the role of dendritic cell luminal sampling in vivo remains controversial, since most evidence of this exists from in vitro experiments. Regardless of direct or epithelial mediated sampling of intestinal contents, dendritic cells play an important part in the FAE roles of antigen processing and presentation to lymphocytes. Subsequent interaction between the antigen-presenting dendritic cells and the naïve T and B lymphocytes result in their sensitization. Normally B cells also require T-cell interactions for subsequent assistance in antigen activation; however, GALT B cells are derived from 2 sources, the pleuro-peritoneal (B1) space and bone marrow (B2). B1-derived lymphocytes are capable of T cell-independent activation and this pathway contributes to roughly 50% of the total intestinal sIgA release.^{62,63}

Sensitized lymphocytes leave the FAE and migrate to the mesenteric lymph nodes (MLNs) where they undergo further maturation, activation, and proliferation. Activation alters the lymphocyte's integrin expression in the MLNs with a downregulation of L-selectin expression and increased expression of $\alpha_4\beta_7$ expression.⁵⁶ After they leave the MLNs, mature lymphocytes exit the lymphatic system through the thoracic duct, enter the systemic vascular system to circulate back to mucosal surfaces throughout the body, including the intestinal and respiratory tracts, lactating mammary glands, the nasal passage, and urogenital tracts—collectively referred to as the mucosal-associated lymphoid tissues (MALT).⁶⁴ As mentioned, the trafficking and homing of mature lymphocytes to these surfaces involves interaction between the unmodified form of MAdCAM-1 expressed at MALT effector sites which has decreased affinity to L-selectin expressed on naïve T and B lymphocytes but greater affinity to the $\alpha_4\beta_7$, which increases on the sensitized T and B cells. This mechanism prevents antigen sensitized and active immune cells from trafficking back to induction sites, such as the PP. Other sites such as the MLNs and spleen also express unmodified MAdCAM-1. Within the LP, B lymphocytes complete their maturation into plasma cells capable of producing and releasing IgA. The progression of B lymphocyte maturation into mature plasma cells occurs at each step of the GALT sites; only 2% of total B cells express IgA within the PP, 50% in the MLN cells, 75% leaving the thoracic duct, and nearly 100% within the LP.⁶⁵ The process of sensitization, maturation, and migration begins with PP sampling of luminal antigen that ultimately leads to sIgA release at mucosal surfaces is collectively known as the common mucosal immune hypothesis. In total, this process leads to the production and release of 2–5 total grams of sIgA per day in healthy humans.

B lymphocytes become IgA producing plasma cells within the GALT and MALT, but T cells orchestrate mucosal immune function through the release cytokines, which cooperatively regulate IgA production, release, and transport, as well as direct lymphocyte trafficking through regulation of MAdCAM-1 expression. In all, 3 primary effector lymphoid T subsets exist: proinflammatory Th1 lymphocytes, anti-inflammatory Th2 (Th2) lymphocytes, and a mixed population of Th17 lymphocytes.⁶⁶ In general, the systemic anti-

inflammatory Th2-type cytokines IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, and IL-25 and the Th17 cytokine IL-17 promote IgA-related GALT functions. The systemic proinflammatory Th1-type cytokines IL-2, interferon γ (IFN- γ), and tumor necrosis factor α (TNF- α) attenuate this process.^{67,68} Furthermore, a subset of FoxP3+ T regulatory (Treg) cells appears to exert fundamental supportive roles for GALT function with the release of transforming growth factor beta (TGF- β), since TGF- β receptor knockout mice almost completely lack intestinal IgA levels.⁶⁹ TGF- β also stimulates lymphocyte class switch recombination and IgA+ plasma cell maturation. The Th2 cytokines, IL-4 and IL-6, further promote IgA levels by stimulating maturation of LP plasma cells. IL-4 and IL-10 directly stimulate polymeric Ig receptor (pIgR) expression, which transports IgA across the epithelium into the intestinal lumen after release of IgA from the plasma cell. IL-4 also directs lymphocyte trafficking by promoting MAdCAM-1 expression in GALT induction sites, directing naïve lymphocyte entrance into the GALT system.

Under normal conditions, the intestinal immune system exhibits a predominance of Th2 cytokines with appreciable IgA production and release, secreting between 2 and 5 g per day.^{70,71} The structure of IgA defines and characterizes its function. Plasma cells produce IgA in a dimeric form in a complex of 2 IgA molecules bound together by a peptide called the J-chain. Following release from plasma cells, the dimeric IgA binds to pIgR present at the basolateral epithelial surface for transcytosis of the molecule across the epithelial layer for release into the intestinal lumen as sIgA. pIgR contains 7 domains within its structure, including 1 membrane spanning and 1 cytosolic domain.⁷² Upon release into the luminal compartment, the other 5 domains—termed secretory component (SC)—remain attached to the IgA molecule protecting the J-chain binding site from bacterial proteolysis.

The dimeric form of sIgA provides distinct host advantages because it enables more efficient opsonization of bacteria or bacterial fragment surfaces to one another, inducing clumping, as well as promoting the clearance from the intestine. Similar to the Paneth cell antimicrobial products, sIgA localizes and concentrates within the mucin glycoprotein surface, adding further physical protection to the host epithelium. sIgA provides direct anti-inflammatory activity, attenuating the mucosal immune response to bacterial antigens during sampling by M-cells if sIgA is attached to the antigen.⁷³ A lack of IgA potentiates both bacterial binding to the host and host hyper-immunoreactivity to microbial molecules. Interestingly, SC also exerts direct antimicrobial properties, independent of IgA. SC displays independent activity against both *Escherichia coli* and *Helicobacter pylori*.^{74,75} The mechanism of SC antimicrobial action relates to N-glycan chains in its structure, similar to the action of lactoferrin. The lack of SC makes sIgA susceptible to cleavage into its monomer forms reducing its binding and clumping capability.

In addition to promoting production and release of IgA at mucosal surfaces, Th2 regulatory cytokine levels within the bowel wall affect expression of adhesion molecules within the vascular endothelium. In particular, they inhibit expression of the endothelial receptor intracellular adhesion molecule-1 (ICAM-1), which attracts and slows circulating polymorphonuclear neutrophils (PMNs) allowing their interaction with the endothelium and tissue products such as sPLA₂, which can prime the PMNs to an augmented inflammatory response to injury.^{76,77} PMNs express the surface integrins, P- and E-selectin, which bind

ICAM-1 and enable diapedesis under certain circumstances. Counter to this Th2 suppression of ICAM-1 expression, the Th1 cytokine IFN- γ up-regulates ICAM-1 and p-selectin, encouraging PMN attachment to the endothelium.⁷⁸ Through this mechanism, increased Th1 cytokines occurring as a result of injury, hemorrhagic shock with poor intestinal perfusion, or infection leads to augmented PMN attachment to the endothelium, priming and the capability of increased production of myeloperoxidase, a powerful pro-oxidant used by PMNs to fight infectious pathogens. This Th1 to Th2 cytokine balance critically influences the intestinal response to injury or infection. Normally, the balance remains toward Th2 anti-inflammatory control.

In addition to cytokines, site-specific chemokines regulate movement of immune cells into effector sites. The gradients of specific chemokine concentrations influence endothelial adhesion receptor expression, such as MAdCAM-1 and ICAM-1, facilitate diapedesis and regulate movement of respective cells into and within the tissue. Within PP, CCL19 and CCL21 stimulate T lymphocyte entry, whereas CXCL13 stimulates B lymphocyte entry.^{79,80} CCL25 stimulates mucosal immune cell memory and homing back to mucosal surfaces. CCL28, which has direct novel antimicrobial functions, stimulates antibody production in plasma cells, as does CCL25. In addition to lymphocyte trafficking, the chemokine CCL-20 regulate the movement of dendritic cells in GALT. Together, these chemical signals help fine-tune the movement and regulation of GALT immune cells to appropriately orchestrate the release of sIgA.

The intestinal microbiome

The final layer of intestinal defense is the maintenance of a normal intestinal microbiome. It is estimated that the intestine is home to between 10 and 100 trillion microorganisms, which increase in numbers from the proximal to distal bowel, reaching the highest levels in the colon. Under normal conditions, “commensal” bacteria facilitate metabolism through the degradation of otherwise nondigestible dietary material producing short chain fatty acids providing the host with additional energy sources for tissues such as the colon and de novo vitamin synthesis, while stimulating normal mucosal immune function and serving as a counterbalance against potential bacteria. Diet dramatically influences the composition and function of the gut microbiome.⁸¹ Although bacteria constitute the most prominent intestinal microorganism mass by total numbers, eukaryotes (including microbial fungus and protists), archaea, and viruses (eukaryotic virus and bacteriophage) coexist in the intestine under normal conditions. Research is just beginning to elucidate their respective roles in host homeostasis and metabolism. For example, eukaryotic yeast and archaea correlate with dietary intake and with specific bacterial lineages, suggesting specific symbiotic cooperatives across kingdoms that maximize the host’s ability to digest and utilize dietary intake.⁸² Evidence of this exists when examining germ-free mice, which are uncolonized by intestinal microbes. Germ-free animals must consume 30% more calories to maintain the same growth rate of normal conventionally raised animals.⁸³

Of the 50 known bacterial phyla found in the environment, 10 exist in the mammalian intestine tract. These include *Firmicutes* and *Bacteroidetes*, which comprise roughly 90% of total numbers, and *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, *Verrucomicrobia*, and

Cyanobacteria, which make up the remaining 10%.⁸⁴ Colonization of the intestine by these “commensal” microbial organisms helps exclude and limit pathogens from accessing the mucosal surface through indirect competitive exclusion, direct pathogen-targeted antibiotic production, and stimulation of normal host innate and adaptive immune mechanisms that favor commensals over pathogens. The injudicious use of antibiotics, alterations in diet, and changes in the intestinal milieu—metabolic, hormonal, and otherwise—lead to dramatic shifts and reassembly of microbiome community structure and membership that influences host metabolism and homeostasis.⁸⁴ The methods by which the collective microbial organisms—conceptually considered a functioning “virtual” organ—protect the host remain the least well understood of the GI defensive mechanisms but are a focus of intense current investigation.

The common mucosal immune hypothesis

A critically important—and unique—aspect of the mucosal immune system lies in the ability of the system to process antigens at 1 site, but through widespread distribution of sensitized T and B cells, protect all mucosal surfaces with antigen-specific IgA.⁵³ Animals receiving oral immunization with antigens produce antibodies against those antigens in the intestine, respiratory tract, nasal-associated mucosal tissue (NALT), salivary glands, and genitourinary tract. This also happens in humans. Oral, but not parenteral immunization, results in a sIgA immune response in mucosal secretions.⁵¹ Children immunized with inactivated poliovirus administered orally but not parenterally generate an antiviral sIgA antibody response in the intestine and respiratory tract.⁸⁵ After an intestinal infection with enterotoxigenic *E. coli*, breast milk, saliva, and intestinal fluids contain specific antibodies to the bacteria.⁸⁶ This is not due to transport and excretion of specific IgA produced elsewhere in the body since it occurs with no increase in serum IgA levels. Specific sIgA antibodies to enterotoxigenic *E. coli* in breast milk and saliva are not found in uninfected, lactating Caucasian women but only following exposure to the antigen eliminating any chance of “preprogramming” of the body to the antigen.⁸⁷ Likewise, human colostrums contain high levels of antigen-specific sIgA to pathogenic enteric *Salmonella* only after infection with the organism.⁸⁸ Administration of killed *Streptococcus mutans* orally to healthy volunteers resulted in measurable levels of specific anti *S. mutans* IgA antibodies in colostrum, milk, tears, parotid glands secretions, or saliva after immunization.^{89,90} These levels increase after additional immunizations due to local production since no increases in specific IgA antibodies occur in the serum. Antigen-specific antibody-secreting cells have been found in the circulation after Oral *Salmonella typhi* Ty21a immunization together with the appearance of specific IgA antibody in genital tract.⁹¹ Finally, investigators isolated *E. coli* 083-specific sIgA and the cells that produced this sIgA from the colostrum of women inoculated with live *E. coli* 083 during the last month of pregnancy; no increase in serum levels could be detected.⁹² These immunization studies provide convincing evidence of a common mucosal immune system providing widespread protection after local immunization. Clearly, the evidence supports immunologic communication between the gut and other extraintestinal tissues. These human clinical and epidemiologic findings help to explain the results in human and animal studies of nutrition, injury and extraintestinal infectious complications.

The clinical rationale for enteral nutrition use in the critically ill and injured

The first clinical trials began to appear within a decade of the demonstration that ENT improved the survival of animals after septic peritonitis, compared with those parenteral feeding. In 1986, Moore and Jones¹⁸ at the University of Colorado published a randomized trial of trauma patients with moderate degrees of injury (with an ATI between 15 and 45) to jejunal feeding with a chemically defined diet or to intravenous fluids containing only dextrose as an energy source. Patients were to be advanced to a goal rate of feeding within 72 hours, which limited the severity of injury. Patients excluded from this trial included those with (1) severe abdominal injuries and an ATI > 45, (2) a major pelvic fracture, (3) requiring early reoperation, or (4) a large number of transfusions since patients with this magnitude of injury rarely tolerate a rapid advancement of tube feeding rate to meet the nutrient needs. Consistent with the animal work, patients fed enterally sustained significantly fewer infections (intraabdominal abscesses in this trial) than patients provided IV dextrose only, with a trend toward a reduction in respiratory infections. Critics of the trial suggested that development of malnutrition in the un-fed group increased their infectious risk. This produced a second study,¹⁹ by the same group, randomizing patients to either early PN or to jejunostomy feedings with the same chemically defined low fiber diet. The same exclusion criteria were used to recruit patients as the first trial. This study demonstrated a significant reduction in infectious complications with a lowered rate of intraabdominal abscesses and significantly less pneumonia than the PN fed group. In the early 1990s, the University of Tennessee-Memphis group randomized patients who had undergone celiotomy for trauma to either enteral or parenteral feeding using isocaloric, isonitrogenous formulas.²⁰ Patients with an ATI ≤ 15 were included with no exclusion by ATI, blood loss, need for transfusions, severity of intraabdominal operations, severe pelvic fractures, or planned early reoperation. All patients received a feeding jejunostomy with subsequent randomization to either jejunostomy feedings or PN. In this trial, enterally fed patients developed both significantly less pneumonia and fewer intraabdominal abscesses than patients randomized to PN, with an 11.8% incidence of pneumonia in the enteral group and a 31% incidence in the PN group. The intraabdominal abscess rate increased from 3.9% with ENT to 17.8% in the PN group, a 460% increase. Magnitude of injury influenced the differences in infection rate. Although no significant difference occurred in patients with lesser injuries (i.e.; those with less than a 15%–20% chance of developing septic complications), patients with a 25% or greater chance of developing septic complications increased their pneumonia rate from 18% with ENT to 56% in the PN group, a 300% increase. In this same high-risk segment of trauma patients, intraabdominal abscesses increased from 9% in the enterally fed group to 33% in the PN group, a 366% increase. When stratified by the ATI and ISS scoring systems, patients with an ISS ≤ 20 or an ATI ≤ 24 demonstrated no significant differences in infections between enteral and PN fed individuals. These populations sustained the lowest rates of infection. However, if a patient sustained an ISS ≥ 20 , an ATI ≥ 24 , or a combination of an ATI ≥ 24 and an ISS ≥ 20 , the risk of infection with PN increased by 6.3 times, 7.3 times, and 11.3 times, respectively, compared with the enteral group. In addition, patients fed parenterally sustained more infections per patient and infected patients sustained significantly more infections when they became infected.

In 1992, Moore et al²¹ combined data in a metaanalysis from 8 prospective randomized trials that recruited both general surgery and trauma patients to compare the usefulness and efficacy of PN in reducing septic complications and organ dysfunction. After confirming that the data were homogenous across the various study sites that cooperated in this trial, an intent to treat analysis confirmed that patients fed enterally sustained significantly fewer septic complications with the primary effect occurring in trauma patients.

Subsequent trials of general surgery patients confirm the conclusion of the VA cooperative study that perioperative nutrition is much more effective in malnourished patients. Heslin et al⁹³ randomized patients undergoing resections of the esophagus, stomach, pancreas, and duodenum for malignancy. Patients were randomized to either early jejunal feeding or to IV fluids alone. They noted no significant differences in length of stay, infectious complications, or major complications between the 2 groups. This contrasted with 2 studies^{94,95} performed by Daly and colleagues who randomized patients undergoing resection of malignancies of the same foregut organs to a specialty diet or a non-isocaloric non-isonitrogenous diet. This study showed a significant reduction in complications from 36.4%–12% and a reduction in length of stay from 12–16 days in the group receiving the specialty diet. Since the investigators recruited patients undergoing similar types of operation, it was unclear why nutrition affected the recovery in the Daly trials but not the Heslin or Brennan trial. The preexisting nutritional status explains at least part of this discrepancy. The first trial recruited well-nourished patients with minimal weight loss and normal serum albumin levels, a population expected to have a relatively low rate of nutrition-related complications and minimal response to nutrition support. In the Daly trials, more than 30% of the recruited patients lost more than 10% of their body weight prior to operation and had markedly depressed albumin levels. Just as in the VA Cooperative study,⁴ the identification and recruitment of an at-risk population is needed to test the effectiveness of any particular therapy administered to treat that risk. In review of the literature, Kondrup et al⁹⁶ examined how the results of clinical nutrition trials were affected by both severity of injury and degree of malnutrition. Their study ranked the patients included into each trial by the severity of disease and degree of malnutrition and, using a validated numeric score, ranked the risk of the patient populations as absent, mild, moderate, or severe. Trials that recruited patients with low severity of stress and low preexisting malnutrition were unlikely to show benefit with nutrition support. However, trials that recruited at-risk patients proved to be much more likely to show positive clinical results benefits with nutrition intervention (Fig 3). The trauma studies reviewed previously demonstrated this same concept: benefits of nutrition occurred predominantly in the more severely injured trauma patients with no differences in patients unlikely to develop septic complications.

The trials defined above were subsequently followed by many studies of enteral or delayed ENT in hospitalized postoperative trauma, head injured, burn, or intensive care unit (ICU) patients. These trials were analyzed by a systematic review using metaanalysis, the majority of which support the experimental data demonstrating that there are benefits gained when nutrition is provided via the GI tract early after surgery or injury which are not gained when those nutrients are administered intravenously.^{97–100}

However, the mechanisms by which ENT reduces septic complications is especially intriguing. Certainly, any relevant explanation should be measurable and testable under both experimental and clinical conditions. Over the past 3 decades, investigators have postulated explanations for why patients benefit from enteral nutrition or do poorly with PN including uncontrolled hyperglycemia, bacterial translocation, GI tract atrophy, augmented cytokine responses in response to sepsis, alterations in mucosal immunity, or changes in the microbiome. The concept of bacterial translocation as an infectious etiology is primarily of historic interest since clinical work failed to confirm the postulates underpinning these observations.²² Although many of these issues are currently under active experimental scrutiny, experimental evidence of the involvement of the mucosal immunity—in both clinical and experimental work—proposes a very cogent link between the route of nutrition, individual nutrients, and the intestinal and respiratory mucosal defenses with intriguing confirmation of results under both experimental and clinical conditions.

The normal clinical balance between bacteria and the host

Humans and bacteria (as well as intestinal fungus, yeast, and archaea) coevolved over time. They provide advantages to each other through their interactions. An estimated 500–1000 species of bacteria exist within the human gut^{101–103} in numbers which exceed the number of human cells in the body 10-fold and contain 150-fold greater genetic diversity than humans. Generally, a mutually beneficial relationship exists between the human intestine and the bacteria: the intestine provides nutrients to the bacteria while bacteria aid in digestion of food, absorption of nutrients, and de novo production of vitamins. Under normal conditions, oral feeding occurs at regular intervals, providing continuous physical and chemical stimulatory signals throughout the GI tract. In response to enteral nutrients and dietary bulk during feeding and digestion, the ENS releases gastrin-releasing peptide (GRP) and enteroendocrine cells release hormones, including gastrin and other enteroendocrine molecules, which stimulate intestinal motility as well as the production and release of gastric acid, digestive enzymes, bile, and bicarbonate. Eating increases blood flow to the intestine carrying oxygen to the tissue and transporting nutrients absorbed from the gut to the liver and systemic circulation. Importantly, eating also provides a healthy environment for the gut luminal bacteria while stimulating the multiple physical, chemical, and immunologic components, which protect our bodies from pathogens and toxins present in the gut lumen.

Illness interrupts this process and critical illness disrupts the bacteria: host balance. Whether GI dysfunction occurs due to medical illnesses or following trauma, abdominal or extraintestinal surgery, intestinal obstruction, or inflammation, patients cannot eat. Hypoperfusion due to hypotension, lack of enteral nutrition, acidosis, endogenous and exogenous opioids, antibiotic pressure, and a host of other physiologic and medical therapies negatively alter the mucosal barrier. If these conditions are prolonged and especially if associated with hypermetabolism, patients develop progressive malnutrition from starvation weakening their immunity and ability to heal.

The key to the symbiotic relationship between bacteria and the human host is an intact mucosal immune barrier complex, which prevents attachment and invasion of the bacteria. The physical barriers include the mucosa with its tight junctions, a thick mucus layer that

concentrates antibacterial peptides and Igs, and a layer of mucosal immune cells that reside in the submucosal LP. The immune defenses consist of both acquired immunity and innate immune systems. MALT and, more specifically, GALT, generate specific acquired Igs against specific intraluminal bacterial antigens in the form of IgA. IgA produced in the LP is transported by the mucosal cells into the lumen where the IgA adheres to antigens on the bacteria, inhibits expression of bacterial virulence factors by the bacteria, and prevents bacterial attachment to the mucosa.¹⁰⁴ Preventing attachment prevents mucosal invasion and infection. The innate immune system, a teleological ancient defense system (compared with the acquired immunity), consists of antimicrobial, bactericidal peptides. These peptides—such as defensins, lysozymes, and sPLA₂—attack the bacterial cell walls. Both the IgA (acquired immunity) and the antibacterial peptides (innate immunity) are concentrated within the mucus layer to provide an effective defense in health. Within the lumen, 2 major bacterial phyla, *Firmicutes* and *Bacteroidetes*, and 5 other minor bacterial phyla compose the intestinal gut flora in healthy adult humans.¹⁰⁵

In the 1960s, the widespread clinical adoption of PN allowed the direct administration of nutrients into the vascular system, bypassing the GI tract altogether¹⁰⁶ enabling clinicians to save many patients who would otherwise die from malnutrition. PN solves 1 problem: providing the body with adequate amounts of macronutrients and micronutrients to meet metabolic and healing needs. However, significant clinical and experimental work convincingly demonstrates that immunologic benefits occur when nutrients are delivered via the GI tract fail to occur when similar nutrients are provided intravenously. PN, however, provides a powerful experimental tool to study the impact of decreased enteral stimulation (decreased enteral stimulation [DES], ie, gut starvation) on the normal mucosal immune system parameters described previously while simultaneously preventing the confounding problem of severe malnutrition. Most changes in mucosal immunity occur over 3 days in our murine model. However, 3 days of starvation is fatal in this animal.

DES and adaptive (acquired) immunity

One of the most important immune mechanisms in the intestine—and respiratory tract—is the release of sIgA into the luminal compartment, providing the host with the ability to target specific antigens when needed, such as bacterial pathogens and virus. Considering the responsiveness of the GALT to luminal antigens, it is not surprising that the route and type of dietary alimentation produces dramatic effects upon GALT histology and function.

Normally, circulating naïve T and B cells expressing the integrins L-selectin and $\alpha_4\beta_7$ interact with modified MAdCAM-1 expressed on the high endothelial venules of the PP, which tether the cells and cause them to roll along the endothelium.⁵³ After tethering, chemokine receptor activation produces a firm arrest and subsequent diapedesis of cells into the patches under the influence of the chemokines CXCL13, CCL19, CCL21, and others. Within the PP, the T and B cells are sensitized to antigens processed by the dendritic cells, migrate to the lymph nodes, and subsequently are released into the thoracic duct and vascular system for delivery to effector sites throughout the body. They interact with an unmodified MAdCAM-1 in these sites and migrate into the small intestine under the influence of CCL25 and CCL28 in the small intestine and CCL28 and CXCL12 in the

respiratory tract. Within effector sites, the B cells mature into plasma cells that produce IgA in response to production of IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, and IL-25 and the Th17 cytokine IL-17 by Th2 T cells. IgA is released, binds to pIgR on the basolateral membrane of the epithelial surface for transcytosis to the lumen for release. The intraluminal IgA retains a fragment of the pIgR to become sIgA. sIgA concentrates within the mucin layer to bind to the IgA-specific antigens on bacteria and prevent their attachment to the mucosa. If there is no attachment, there is no infection. PN and DES negatively affect each of these steps (Fig 4).

Experimentally, the most noticeable effects of 5 days of IV feeding were the noted atrophy of the GI tract, in particular, the PP and the thickness of the small intestine.^{107,108} Other investigators noted decreases in intestinal mucosal wet weight, villus length, and levels of protein in DNA in animals fed PN compared to those fed enterally. Early studies using rats noted that the mucosal thickness decreased by 50%, with most of the changes occurring in the proximal half of the intestine. However, both mice and humans do not experience mucosal atrophy to this degree—perhaps 10% at most. However, investigations of mucosal immunity demonstrate significant changes occurring in PP, the IE spaces, and the LP.¹⁰⁹

DES and PP

M-cells on PP initially sample luminal antigen; decreased antigen uptake occurs but otherwise no differences in cellular structure have been observed.¹¹⁰ It remains unclear if PN causes impaired M-cell function or simply decreases available luminal antigen for uptake. However, the total lymphocyte numbers dramatically drop during PN over time. In studies of the PP, LP, and IE spaces, decreases in total lymphocyte yields decrease within 24 hours reaching their nadir by 3 days¹⁰⁸ (Fig 5A). Within PP, there are no changes in the T and B lymphocyte ratio of 1:2 compared with controls.¹⁰⁷ The distinct T-cell subpopulation ratios remain unchanged following PN and the CD4 to CD8 remains 4:1 although the absolute numbers of cells drop. Within the CD4⁺ T cells, PN leads to no changes in the relative proportion of CD4⁺ CD44⁺ (memory effector cells), CD4⁺ L-selectin⁺ (Naïve cells), CD4⁺ CD25⁺ $\alpha_4\beta_7$ ⁺ (activated cells), or CD4⁺ CD25⁺ Foxp3⁺ (Treg cells). Likewise, the relative percentage of PP B cells, including IgD⁺ (Naïve), CD44⁺ (memory), IgM⁺ L-selectin⁺ (Naïve), or IgM⁺ $\alpha_4\beta_7$ ⁺ (activated cells)¹¹¹ remains the same after PN feeding. Since the decreased cellularity only influences total numbers, these changes accumulatively suggest attenuated global lymphocyte entry into PP, an observation that led to detailed study of the mechanisms responsible for cell entry into the PP.

Overall, 4 molecules appear to be related to migration of mucosal T and B cells into the PP. T and B cells express 2 of these integrins—L-selectin and $\alpha_4\beta_7$ —whereas the high-endothelial venules in PP express 2 adhesion molecules—ICAM-1 and modified MAdCAM-1. However, only 3 of these molecules appear essential to cell entry. ICAM-1 blockade with a monoclonal antibody had no effect on PP yield while blockade of any of the remaining 3 reduced PP cell numbers to those of PN fed mice despite chow feeding. Subsequent studies of MAdCAM-1 expression over time with PN confirmed stepwise decreases in MAdCAM-1 expression, which occurred within 4 hours of starting PN with a nadir at 48 hours. This time course was consistent with temporal decreases in the PP (as well

as LP and intraepithelial compartment) lymphocyte recovery. Refeeding PN animals with chow began to reverse MAdCAM-1 expression within 4 hours, returning to normal by 12 hours and repopulating the PP with lymphocytes within 2 days (Fig 5).¹¹²

MAdCAM-1 itself is the end product of stimulation of LT β R expressed within the stroma of PP (as well as the LP).¹¹⁴ The unifying concept that ties ENT with regulation of MAdCAM-1 expression (as well as the Th2 cytokine IL-4) is a heterodimer of lymphotoxin α and β (LT α 1 β 2) expressed on activated T and B cells that stimulate LT β R. This interaction releases the signal for IL-4 and MAdCAM-1 production and expression through an activation of NF κ B.⁵⁷ This relationship with PN was confirmed through a series of experiments. First, PN significantly decreased LT β R expression in the intestine and PP compared to mice fed chow or a complex enteral diet (CED) administered via a gastrostomy tube.¹⁰⁸ PN mice simultaneously administered an agonistic anti LT β R monoclonal antibody experienced significant increases in PP lymphocyte counts and intestinal IgA levels.¹⁰⁸ This work confirmed that PN affected LT β R expression interfering with this critical GALT signal for MAdCAM-1 expression by inhibiting lymphocyte entry into PP for subsequent distribution to intestinal and extraintestinal mucosal immune sites. Finally, confirmation that this interaction regulates transcription of MAdCAM-1 was documented through the blockade of LT β R using an chimeric Ig fusion protein.¹¹⁵ This LT β R blockade significantly decreased PP MAdCAM-1 mRNA and PP lymphocyte counts to PN levels.

A final series of investigations verified the role of the NF κ B pathway in the regulation of MAdCAM-1, LT β R, and various cytokines. A total of 2 NF κ B pathways exist. Inflammatory cytokines or stress stimulates the canonical or classical NF κ B pathway. These inflammatory stimuli induce a breakdown of IkBA causing an accumulation of the P65 dimer within the nucleus to regulate anti-apoptotic and immune regulatory genes but not proteins. The noncanonical or alternative NF κ B pathways are activated by receptors important in lymphoid formation and lymphocyte development. The noncanonical pathway includes processing of P100-P52 and nuclear accumulation of P52/RelB dimers, which control production of MAdCAM-1, cytokines, and chemokines important in T and B cell entry into PP. PN reduced PP levels of all NF κ B proteins compared to animals fed enterally. In addition, LT β R blockade with an anti-LT β R fusion protein in chow fed animals produced the same results, reducing the protein important in the NF κ B noncanonical pathway without affecting the inflammatory pathway. Blockade resulted in significant reductions in the noncanonical downstream products including MAdCAM-1, CCL19, CCL20, CCL25, IL-4, and IL-10, which dropped to levels of PN fed mice. Finally, stimulation of LTBR in PN mice with agonist antibodies increased the noncanonical pathway molecule P52 and RelB increasing MAdCAM-1 and IL-10. No significant effects were noted on CCL19, CCL20, or CCL25 in the PP.

In summary, maintenance of adhesion molecules and chemokines critical to cell T and B entry into the mucosal immune system are dependent on enteral stimulation. Although PN prevents the development of progressive protein calorie malnutrition, PN appears to have no effect upon maintenance of normal mucosal immune mechanisms in PP.

DES and cell migration

Following the exit from the PP, lymphocytes travel to the MLN to undergo further maturation. Examination of the MLN compartment revealed no differences in total cellularity following PN compared with enteral fed animals and it is unclear whether the MLN contribute to PN-associated GALT impairment.¹⁰⁷

DES and the LP

The impairment of naïve T and B cell entry into the PP produces significant downstream effects on the mucosal immune system. The effect upon the LP is the most dramatic affecting all T and B cell populations, cytokine levels, IgA production, and even the transport protein, pIgR, which is expressed on the basolateral surface of the mucosal cells. Just as in the PP, gradual but rapid decreases in total lymphocyte populations within the LP reach a nadir by approximately 3 days (Fig. 5). The CD4/CD8 ratio drops from the normal 2:1 in animals receiving an enteral diet to 1:1 with PN.

The cell phenotype changes within the LP are substantially greater than the relatively minor changes seen in the PP. First, PN induces significant reductions in the phenotypic markers of cell activation (CD25⁺), memory (CD44⁺), and homing. The result is a reduction in the percentage of activated T cells (CD4⁺CD25⁺) expressing the homing molecule $\alpha_4\beta_7$ (LPAM-1), which directs cells into the LP. No effect upon the CD62 marker (L-selectin) occurs since it is primarily responsible for migration into PP. However, $\alpha_4\beta_7$ is expressed on activated lymphocytes and is necessary to direct migration of activated cell into effector sites. In addition, significant reductions also occur in markers of naïve B cells and Th cells, as well as CD4 and CD8⁺ populations. Of particular note, the Treg cell population (CD4⁺CD25⁺Foxp3⁺) also decreases. The Treg population plays a critical role in antigen-specific stimulatory signals. The Treg cells produce the cytokines TGF β and IL-10 that provide the stimulatory signal for IgA⁺ B-cell IgA production while simultaneously suppressing the important IgA-inhibiting cytokine, IFN γ , within the LP. This cell population also maintains immunologic cell tolerance through its suppression of autoreactive T-cells. Finally, B-cell subpopulations are diminished within LP with drops in IgD⁺, IgD-LPAM⁺, and CD44⁺ memory cells. IgD⁺ cells represent mature B-cells, which can present antigen and stimulate other cells.¹¹⁶ The IgD⁺ cell population concentrates in the mucosal surface and respond to T-cells for activation. The IgD⁺ B-cells lose IgD expression upon switching to IgA producing cells. The reduction in CD44⁺ expression reflects a reduction in B-cells committed to production of antigen-specific IgA. Similar to the PP, refeeding causes repopulation of the LP cellularity within 2 days (Fig 5).

These global changes in cell populations—particularly the T-cell populations—alter cytokine production and overall mucosal immune function. In both the intestinal and extraintestinal LP sites, IgA production depends upon the balance between regulatory and counter-regulatory signals produced by T cell. The Th2 type IgA-stimulating cytokines include IL-4, IL-5, IL-6, IL-10, and IL-13. Although some of these cytokines are also responsible for B-cell maturation into IgA producing plasma cells, all are important in IgA production by those plasma cells. IgA-stimulating cytokines are counterbalanced by Th1-type IgA-inhibiting cytokines including IFN γ . As PN with DES reduced the CD4/CD8 ratio,

levels of IL-4 and IL-10 present in gut homogenates drop simultaneous with levels of IL-4 and IL-10 mRNA obtained from isolated GALT LP cells. IFN γ levels, however, remain unaffected, shifting the balance away from IgA production to IgA inhibition. Overall, the changes result in reduced production and release of IgA from the plasma cells so that less IgA is available for transport to the lumen via pIgR, which binds the dimeric IgA molecules produced by plasma cells. pIgR is a transmembrane protein found on the basolateral of the epithelial cells; it forms a pIgR-IgA complex resulting in endocytosis and transcellular transport across the mucosa. The IgA is released into the lumen combined with a small residual fragment of pIgR (known as SC) still attached, which stabilizes the molecule. Expression of pIgR drops with PN. With a reduction both in the pIgR available for transport and the reduction in IgA production, the result is lower levels of IgA within the intestinal lumen and impairment of this important mechanism of specific, acquired immunity.

Similar changes occur in the respiratory tract. Just as the cells are released from the PP to migrate to the small intestine, cells migrate to the pulmonary tissue as well. Although PN and DES do not affect on respiratory tract pIgR levels, PN and DES decrease the number of T and B cells within the lung resulting in lower levels of airway sIgA, which create functional defects and measureable loss of IgA-mediated antiviral and antibacterial respiratory protection. These impairments were confirmed through a series of experiments. In the first experiments, mice were immunized with the A/PR8-H1N1 virus intra-nasally. This immunization generates a protective IgA-mediated defense against subsequent infection by the virus in the mice.¹¹⁷ Following the initial immunizations, mice continue to shed the virus from their nasal passages for 10–13 days as mucosal immunity responds to the challenge and IgA levels increase to functional levels. After immunization, animals eliminate a subsequent dose of the virus from the nasal passages in hours. To test the effect of PN and DES on this established antiviral defense, immunized mice received either chow, another form of ENT in the form of the CED administered by gastrostomy, or PN for 5 days. Each animal was inoculated with a second dose of the virus and subsequently cultured for viral shedding. Although all animals fed via the GI tract cleared the virus from the respiratory tract quickly, PN mice continued to shed the virus for days demonstrating loss of established antiviral immunity (Fig 6). The increased viral shedding response was associated with lower sIgA release in the intestinal compartment (Fig 6).¹¹⁷ When additional immunized PN animals were fed chow for 2 days, antiviral immunity returned to normal, establishing that PN does not affect immunologic memory but only function. The changes are rapidly reversible with enteral stimulation.

In a second experiment, animals immunized against *Pseudomonas aeruginosa* were randomized to receive either PN or enteral nutrition.¹¹³ The dose of *P. aeruginosa* administered in this experiment normally kills 90% of unimmunized animals, but prior intranasal immunization with the *Ps* antigens delivered in liposomes reduces mortality to 10% in healthy mice. After generating an effective immunization with the antigen/liposome mixture, animals received either chow, intragastric feeding with a CED, or PN. Although mortality remained low in animals that fed enterally reflecting an intact antibacterial defense, 90% of PN mice died with most deaths occurring within 24 hours. A control group of unimmunized mice also sustained at 90% mortality rate (Fig 7). These experiments

confirmed that functional loss of IgA-mediated immunity occurs following PN and DES. However, evidence points to an intact immunologic memory.

In addition to IgA-mediated function, PN-DES also impairs the ability of acquired immunity to respond to new antigens reducing the ability to generate antibody-forming cells to an immunologic challenge. Mice are capable of generating immune response to both swallowed antigens via PP and inhaled antigens via NALT. Whether humans have a functional NALT remains unproved (Waldeyer ring has been suspected); processing of antigens via cervical lymph nodes in mice allows investigation of the generation of specific immunity in mice using elispot analysis of cells as they migrate from the site of inoculation through cervical lymph nodes back to the upper respiratory passages.¹¹⁸ After immunization of enterally fed and PN mice with the A/PR8-H1N1 virus, PN resulted in significantly fewer lymphocytes in the cervical lymph nodes and nasal passages over the first 2 weeks. IgG-specific, IgA-specific, and total antibody-forming cells increased in enterally fed mice over the course of the experiment while no changes occurred during PN feeding (Fig 8). At day 8 after immunization, most of the PN mice continued to shed the virus whereas only 2 of 15 enterally fed mice shed virus (Fig 8). Antiviral IgA was significantly lower in PN mice immunized 3 weeks prior to diet administration compared to enterally fed mice. These observations have implications for critically ill and critically ill patients that may need to respond to a new microbiome when hospitalized.

DES and the intraepithelial lymphocytes

PN with a DES also affects lymphocytes located within the IE space (the IE lymphocytes—IEL). These cells constitute the initial lymphocytic defense against antigens within the lumen and appear to regulate the immune responses to enteral antigens. Their phenotype differs from the underlying LP lymphocyte populations.¹¹⁹ Approximately, 75%–80% of the cells are cytotoxic CD8⁺ cells and 30%–60% of the cells are Γ ⁺-TCR⁺. Approximately 20% of the cells are CD8[−] with half of them being CD4[−] and the other half being CD4⁺. The populations arise from both thymic-dependent and thymic-independent sources and are 1 of the first lymphocyte populations to interact with intestinal lumen antigens and are capable of releasing IFN γ , TNF α , IL-2, and IL-4. PN affects both the phenotype and the function of the IELs. Parenteral feeding alters these cell populations.¹²⁰ Phenotypically, CD4⁺, CD44⁺, CD8 AB⁺ cells are reduced as IL-4, IL-6, IFN γ , TGF- β ₁, and TNF α increased simultaneously with a drop in IL-2 and IL-10. These events occur simultaneous with a decrease in villi height, increased epithelial cell layer permeability, and elevated bacterial translocation. It is presumed that these changes are integral with the loss of epithelial tight junctions, which occurs simultaneously with reduction and production of antimicrobial peptides by the Paneth cells, mucin by the goblet cells, and IgA by the underlying LP cells.^{119,120}

Decrease enteral stimulation and innate immunity

Innate immunity consists of both non-immunologic physical and chemical defenses that provide the initial defense against invasion by bacteria. Mechanical barriers such as cell-cell tight junctions, the thickness of the mucosa, secretions, dynamic peristalsis, and commensal microbial flora constitute a non-immunologic physical wall. Stem cells of the intestinal track

produce mucus secreting goblet cells, hormone secreting enteroendocrine cells, and Paneth cells in addition to absorptive enterocytes, the most abundant type of cell lining the intestine. The tight-junction proteins including occludin, zonulin, and claudin bind the epithelial cells together. These tight-junction proteins appear to be decreased following PN.¹²¹ Tight-junction protein changes also occur in inflammatory bowel disease and have been implicated in the loss of mucosal barrier function.¹²² Although laboratory work has associated PN with increased barrier dysfunction,¹²¹ increased permeability and bacterial translocation, these phenomena do not correlate with the development of extraintestinal infections during PN and additional mechanisms have been implicated.

PN also alters the functional capability of both goblet cells and Paneth cells. Although the number of goblet cells remained stable in our model, PN in some neonatal models resulted in significant decreases in tissue protein and luminal levels of MUC2 and trefoil factor 3, as well as tissue levels of RELM β and RELM β mRNA. Simultaneously, PN results in decreased levels of 2 cytokines known to stimulate goblet cell products, IL-4 and IL-13. The lower levels of luminal MUC2 and other goblet cell products during PN thins the protective physical layer that normally aids in host epithelial defense; this impaired mucosal defense reduces the barrier against luminal bacteria demonstrated by experiments to be described subsequently. DES impairs the intestinal chemical barrier due to intestinal Paneth cell dysfunction. Paneth cells manufacture and release a host of antimicrobial peptides, a form of natural antibiotics. Under normal conditions, Paneth cell antimicrobial peptides including lysozyme, sPLA₂, angiogenin4, RegIII γ , and α -defensins, which localize and are concentrated in the mucin layer adding a chemical layer of protection to the physical viscoelastic mucin barrier. Each antimicrobial molecule exerts a slightly different mode of antibacterial action, providing an array of defenses against luminal microorganisms. Lysozyme degrades peptidoglycans in bacterial cell walls, sPLA₂ digests phospholipids in bacterial membranes, and α -defensins form pores in bacterial membranes that induce membrane disruption in bacterial cell walls. PN interferes with each process of Paneth cell function.

In a series of experiments, PN significantly reduced both tissue levels and luminal levels of sPLA₂ and lysozyme as well as the transcription of sPLA₂ and lysozyme mRNA. Impaired transcription of cryptdin-4 also occurred with PN although luminal and tissue levels of this α -defensin remained unaffected. Interestingly, only the luminal levels of RegIII- γ increased following PN although transcription and tissue levels of RegIII- γ dropped with PN. The results became understandable when considering the mechanisms, which control RegIII- γ in context. Increased release of RegIII- γ occurs through activation of toll-like receptors by bacterial products. RegIII- γ normally maintains a 50 μ m zone of physical separation between the mucosal surface and bacteria. It appeared that the conditions of a reduced mucin layer and reduced antimicrobial peptides resulted in a compensatory response by the intestinal mucosa to maintain this zone, which increased the luminal levels of RegIII- γ . Functionally, the changes in mucin and antimicrobial peptide defenses increased ileal tissue susceptibility to invasion by *E. coli* (Fig 9).

The decrease in production and release of antimicrobial products by Paneth cells appears to be due, at least in part, to the decreased tissue levels of IL-4 and IL-13. Paneth cells express

an IL-13 receptor that binds these 2 cytokines. This was confirmed in a related experiment in which IL-25—which stimulates IL-4 and IL-13 levels in the serum and tissues—was administered to PN fed mice. IL-25 administration completely eliminated the PN-associated changes in Paneth cell antimicrobial expression.¹²⁴ Paneth cells also released antimicrobial peptides into the intestinal lumen in response to IL-13¹²⁵ implicating cytokine regulation in both the production and release of microbial-modulatory compounds.

The overall innate immunity effect associated with the decreases in the mucin layer and Paneth cell antimicrobial peptides is a loss in resistance of the mucosal barrier to bacteria. In a series of experiments, comparisons of intestinal secretions obtained from intestinal tissues from PN and enterally fed mice were analyzed for bactericidal activity. Paneth cell granules from enterally fed contained significantly more sPLA₂ than PN and released significantly more sPLA₂ into the media with stimulation. Although other antimicrobial peptides were released into the media as well, most of the bactericidal activity appeared to be due to sPLA₂ since simultaneous administration of an sPLA₂ inhibitor suppressed the bactericidal activity by 50%–70%. When mucosa from enteral and PN fed mice were incubated with an enteroinvasive *E. coli*, mucosal resistance to the bacteria was dramatically reduced following PN. Therefore, PN significantly impairs the innate immune response through this inhibition of Paneth cell and goblet cell function.

Inflammation and route of nutrition

There is both clinical and experimental evidence that the route of nutrition affects the inflammatory response to injury. These effects occur on a systemic basis but also are organ specific particularly within the lung, liver, and intestine. The systemic effects provide reasonable insight into interactions between nutrition support and the development of multiple organ system failure.

The systemic response and route of nutrition

The route of nutrition affects the activation of systemic inflammation. PMNs control bacterial invasion via non-specific immune inflammatory responses; this inflammation also produces collateral damage to tissues. The vascular endothelium is integral to PMN activation and priming. Normal conditions inhibit expression of ICAM-1 as well as P- and E-selectins on the vascular endothelial cell. Conditions that increase their expression allow an interaction between these integrins and ligands on the circulating PMNs. The P- and E-selectins initiate rolling and adhesion of the PMNs to the vascular endothelium while firm attachment occurs between the ICAM-1 molecule and the PMN.^{126,127}

Interaction between the PMNs, vascular endothelium, and metabolic products released following injury results in PMNs priming. Priming allows an augmented inflammatory response by the primed cells. Initiating inflammatory events such as sepsis, tissue injury, and hemorrhagic shock increase expression of the endothelial adhesion molecules to start the process. In their early work, Moore and colleagues coined the term “first hit” to distinguish the importance of this initiating event in overall inflammation.¹²⁸ This first hit renders the primed cells capable of generating an augmented inflammatory response if a secondary insult occurs (the second hit). As patients and animals are resuscitated from the

“first hit,” the PMNs do not stay within the intestinal vascular endothelium but are released, circulate and distribute themselves to organs throughout the body in their primed state. The mechanism of priming has been well defined following ischemic injuries.¹²⁹ Hypoxic tissue breaks adenosine triphosphate into the metabolic products of adenosine diphosphate, adenosine monophosphate, adenosine, and hypoxanthine. Xanthine oxidase is produced after conversion of xanthine dehydrogenase through uncontrolled intracellular calcium accumulation within the hypoxic cells. During resuscitation, reperfusion, and oxygenation of the cells, xanthine oxidase converts hypoxanthine to uric acid resulting in creation of reactive oxygen metabolites.¹³⁰ These metabolites activate cytosolic phospholipase-A₂, which is found in high concentrations in the gut mucosa. Activated cytosolic phospholipase-A₂ releases lysophospholipids and fatty acids into the cytosol to increase eicosanoid production. This process primes the PMNs, preparing them to generate an augmented response to subsequent injury. The role of the gut has been well established in this process. Experimentally, only PMNs harvested from the portal vein after ischemia reperfusion demonstrated significant evidence of activation and priming whereas cells recovered from the superior mesenteric artery did not.

Parental nutrition plays an important role in this process as well. As previously noted, PN significantly decreases the CD4/CD8 ratio from the normal 2:1 in enterally fed animals to 1:1 with PN. This change results in significant decreases in the levels of 2 Th2 cytokines, IL-4 and IL-10, which drop simultaneously with intestinal IgA levels. The Th1-IgA-inhibiting cytokine, IFN- γ , remains unchanged. Those LP cytokine changes influence the blood vessels, which traverse these tissues since these 3 cytokines control ICAM-1 expression by the vascular endothelium. In addition to their role in stimulating IgA production by plasma cells, normal levels of IL-4 and IL-10 suppress the expression of ICAM-1 on the endothelium.¹³¹ In these roles, IFN- γ performs the opposite function by inhibiting IgA and stimulating expression of ICAM-1 expression. With a normal ratio of Th2 to Th1 cytokines in the LP, ICAM-1 expression remains suppressed; however, as the ratios change with PN, ICAM-1 levels increase. This results in PMN attachment and interaction with the endothelium. Myeloperoxidase, a marker of PMN accumulation, increases dramatically within the intestinal vascular endothelium during PN. After priming, PMNs are distributed to extraintestinal sites in their primed state. Reinstitution of ENT reduces the expression of the adhesion molecules quite quickly but as long as PN is administered, the process continues. Evidence that this PMN-ICAM-intestinal epithelial interaction actually primed the PMNs was confirmed in a series of experiments studying hepatic and pulmonary permeability changes and cell activation marker following a “second hit” with gut ischemia reperfusion. Mice are sensitive to episodes of gut ischemia; most will survive if the insult is limited to only 15–30 minutes. After 15 minutes of ischemia, survival of animals fed via the GI tract with either chow or a CED administered through a gastrostomy tube is approximately 90%–95% at 72 hours.¹²⁷ PN, however, reduces survival to 50%. The expression of 2 PMN activation markers, CD11a and CD11b, were measured in blood samples following exposure to gut ischemia or reperfusion and samples of lung tissue were stained for CD18, a marker of myeloid activation using immunohistochemistry. Prior to the ischemia or reperfusion event, PMNs obtained from PN or enterally fed mice expressed identical levels of CD11A and CD11b. However, within 3 hours of reperfusion, CD11b

expression increased significantly only after PN feeding. The lungs demonstrated significantly increased levels of CD18+ myelocytes after exposure to gut ischemia reperfusion only in the PN fed group.¹³² Over the course of the experiment, increases in permeability of the liver and lung to albumin occurred only in the PN fed group as well. The results of these experiments—the increases in (1) CD11b in circulating PMNs, (2) CD18+ myeloid cells, (3) mortality, and (4) hepatic and pulmonary permeability—following gut ischemia and reperfusion only with PN feeding confirmed our hypothesis that DES induces a first hit rendering the animals susceptible to subsequent injury.

Organ-specific inflammatory responses of the acquired immune system after PN

Establishing a link between clinical and experimental results

A host of factors affects IgA levels within the respiratory and GI tracts including the Th1-Th2 cytokine balance, availability of pIgR, and the number and phenotypes of T and B cells within the LP. PN and DES exert a detriment effect on each aspect of mucosal immunity. But recent work provides significant evidence that PN affects the organ-specific response of mucosal immunity to injury by altering the release of IgA as well as the production and release of inflammatory cytokines such as TNF α and IL1- β . The results of these studies testify to the similarities between patients and our murine model and the clinical relevance of the experimental observations.

Route of nutrition and the pulmonary response to injury

The impetus for these experiments was based upon the clinical respiratory responses noted to occur following severe blunt trauma. Airway changes in response to injury provide a unique window into alterations in mucosal immunity following trauma. This is possible through bronchoalveolar sampling of upper and lower airway epithelial lining fluid (ELF) for analysis of changes in cytokine and IgA levels over time. Aspiration of saline lavages from distal bronchioles using fiberoptic bronchoscopy and simultaneous measurement of blood urea nitrogen allows calculation of the volume of ELF recovered by the equation: $\text{volume ELF (mL)} = (\text{mg urea in BAL})/(\text{plasma urea concentration in mg/mL})$.^{133,134} This technique can be applied to determine IgA and cytokine concentration changes in ELF and well as changes in ELF itself. Samples were obtained for analysis from 12 trauma patients sustaining severe blunt injury who were expected to require intubation for a minimum of 5 days in the ICU.¹³⁵ The trauma samples were compared to samples obtained immediately after intubation from a healthy control population of patients undergoing elective operations such as inguinal or ventral hernia repair or IVC filter removal. Compared with the healthy control patients, the absolute volume of ELF increased significantly within 30 hours of admission following trauma. Simultaneously, concentration of IgA within the ELF significantly increased following trauma resulting in elevated levels of total IgA following trauma.

Surprised by these results, experiments to confirm these events were undertaken in mice using a limited injury model consisting of neck and celiotomy incisions, which were immediately closed. At 0, 4, 8, and 24 hours, bronchoalveolar lavage samples were obtained

from the group of mice for analysis.¹³⁵ Just as occurred in the trauma victims, levels of respiratory IgA levels spiked at 8 hours (Fig 10). Because of the limited degree of injury, the IgA response to injury returned to normal by 24 hours. The IgA increase was due to active processing and transport of IgA by mucosal immunity since analysis confirmed that the Ig was sIgA containing the remnants of SC. Therefore, the levels were not due to pulmonary permeability changes with leakage of serum IgA into the airway but because of active production, release, and transport of the molecule across the epithelium by pIgR. Subsequent work indicated a very similar and critical role of cytokines in this response.¹³⁶ TNF α , IL1- β , and IL-6 levels in the bronchoalveolar lavage specimens increased in a bimodal manner when studied 3 and 8 hours following the experimental injury. The only change in serum cytokines occurred from an IL-6 increase by 5 hours; serum TNF α and IL1- β remained unchanged throughout the experiment. This established the cytokine inflammatory response to be a (local) pulmonary rather than a systemic response. However, systemic administration of a cocktail consisting of TNF α , IL1- β , and IL-6 was capable of generating an airway although administration of individual cytokines—even at higher levels—produced no airway IgA response.

In further studies of the clinical and experimental data, cytokine levels in the BAL specimens following injury were compared in the human and murine samples.¹³⁷ Injured patients had significantly increased concentrations of TNF α , IL1- β , and IL-6 in both BAL and serum samples, with BAL cytokine concentrations significantly higher than serum levels. Similarly, mice increased their BAL fluid concentrations of the same 3 cytokines without changes in the serum TNF α or IL1- β or IL-6. These results imply a local pulmonary response rather than a systemic response to injury in both mice and men although some contribution for the serum could not be excluded in injured patients.

Finally, factors that eliminated the airway IgA response were studied in several experiments. Most notably, pretreatment of mice prior to injury with either 5 days of PN or administration of an anti-TNF α antibody completely obliterated the respiratory sIgA increase to injury; a similar blunting of the response occurred with anti-IL1- β monoclonal antibody blockade.¹³⁸ These results substantiated the hypothesis that PN with DES significantly affects mucosal immune integrity and impairs the ability of mucosal immunity to protect a patient following injury. Overall, although PN appears to augment systemic inflammation, a global mucosal immune deficit impairs the mucosal defenses.

Route of nutrition and the intestinal response to injury

The GI tract also generates an IgA mucosal immune response following injury.¹³⁹ Within 2 hours of limited injury created by celiotomy and neck incisions, enterally fed mice experience significant increases in intestinal IgA levels, which continue to increase over the first 8 hours (Fig. 10). Counter to the lung response, this IgA increase occurs without any increase in either TNF α or IL1- β levels within the serum, intestinal fluid, or the small intestinal tissue. Serum and tissue IL-6 levels, however, increased by 5 hours and remained elevated at 8 hours. IL-6 also increased in the intestinal fluid but this response was brief, returning to normal within 2 hours of injury. Intraperitoneal injection of the same recombinant TNF- α , IL-1 β , and IL-6 cocktail used in the pulmonary studies described

previously did increase intestinal IgA levels to that same degree as injury, administration of TNF- α , IL-1 β blocking antibodies failed to blunt the injury response as long as animals received ENT. PN feeding, however, completely eliminated the small bowel IgA response to injury.

Because of these experiments, it was concluded that the normal mucosal immune system plays an important role in defending mucosal borders throughout the body both in healthy and injured. In injury, local proinflammatory releases of TNF α and IL1- β have appeared to play a critical role in the pulmonary response but a less important role in the intestinal response to injury. At both sites, however, parental nutrition adversely affects the ability of the mucosal immune system to respond altering both the inflammatory responses and the defenses against infectious challenge posed during stress. In addition, PN aggravates the systemic inflammatory response to the mechanism of PMN priming.

No clinical correlates comparing the inflammatory intestinal response in human to these experimental models exist currently. However, comparisons between human GALT phenotypic alterations with PN show remarkable similarities between the experimental work and human changes. Fukatsu and colleagues similarly documented the effects of preoperative dietary intake on GALT and infectious morbidity in patients undergoing right hemicolectomies for colon carcinoma.¹⁰⁹ They examined terminal ileum specimens from 62 patients who received either preoperative PN or preoperative oral feeding using immunohistochemistry. They quantified the numbers of T cells, IgA producing cells, as well as mature and immature dendritic cells within the LP and IE spaces. Consistent with the murine data, PN significantly reduced the total number of T lymphocytes in both the LP and the IE spaces. Likewise, PN decreased IgA producing cells and mature dendritic cells within the LP although total dendritic cells numbers remained unchanged. The PN patients sustained significantly higher rates of total infectious complications including a greater incidence of surgical site infections, pneumonia, infectious colitis, as well as infected central venous catheters. This clinical work in general surgical patients mirrored the early studies performed in trauma patients and provides another link between mucosal immunity and infectious morbidity.

DES influences the intestinal microbiome

Intestinal microbes have important roles in host homeostasis including short chain fatty acid production from complex dietary carbohydrates, de novo vitamin synthesis, stimulation of epithelial proliferation, and stimulation of intestinal immune responses. The microbiome is highly responsive to dietary changes.^{81,141} Therefore, it may not be surprising that the lack of dietary nutrients during PN dramatically alters the intestinal microbiome community structure. At the phylum level, PN leads to a decreased percentage of the level of Firmicutes found in the ileal luminal microbial community, but elevated phylum levels of *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*.¹⁴² Analysis of mucosal-associated bacteria in the ileal mucosa also confirmed decreased levels of *Firmicutes*, but greater levels of *Bacteroidetes*, *Proteobacteria*, and *Verrucomicrobia*.¹⁴³

The changing microbial populations must adjust to a starved state under which only secreted host molecules, such as glycosylated mucin glycoproteins, are available for energy

utilization. Consistently, the expansion of the rare mucosal-associated mucin-degrading bacteria *A. muciniphila* of the phylum *Verrucomicrobia* has been described under PN.¹⁴² Likewise, the phylum *Firmicutes* prefer dietary carbohydrates as energy sources, expand preferentially under high carbohydrate diets, and are subsequently decreased under PN alimentation without ENT. With decreased *Firmicutes*, a reciprocal expansion of *Proteobacteria* occurs, a phylum of bacteria that are generally more starvation resistant and able to utilize a greater variety of metabolic sources. Several known pathogens, such as *Escherichia*, *Salmonella*, *Vibrio*, *Helicobacter*, and *Yersinia* are of the phylum *Proteobacteria*, and under certain circumstances may adhere to and attack host tissues in an attempt to survive conditions of GI nutrient scarcity.

In addition to dramatic dietary-dependent shifts in the microbial populations, the loss of sIgA and antimicrobial production by the epithelium decreases the ability of the host to respond to specific opportunistic pathogens that might thrive in the absence of competitive exclusion. Furthermore, the loss of ‘commensal’ populations decrease the beneficial immunostimulation that certain bacteria exert upon the host. For example, *Bacteroides fragilis* stimulates proliferation of Treg lymphocytes and supports luminal sIgA in the intestine.¹⁴⁴ Additionally, the loss of normal bacterial diversity is associated with the expansion of fungal pathogens, such as *Candida albicans*, that are normally “commensal” diploid yeast in the intestine but can become virulent and attack the host under altered morphological states.¹⁴⁵ Experimental administration of PN along with intestinal challenge of *C. albicans* leads to increased fungal translocation and systemic candidemia compared with enterally fed controls.¹⁴⁶ Indeed, *Candida species* remain a major infectious pathogen on catheter surfaces and in the ICU,¹⁴⁷ which may be in part derived from the intestine when microbial dysbiosis potentiates fungal opportunism.

The practical effects of dietary induced changes in microbiome have been demonstrated by Alverdy and colleagues who demonstrated the development of pathogenic responses by bacteria during various stresses and by dietary manipulation.¹⁴⁸ This group showed that in a friendly, nonhostile environment, sets of virulence genes—important for survival in time of stress—remain downregulated in bacteria. However, the addition of a number of stressors results in an up-regulation of these genes. Stressors such as hypotension, opioids hypoxia, ischemia, gut starvation, or reductions in gut motility from opioids upregulate genes responsible for the expression of endotoxin-A and pa-1.^{149,150} These changes increase the ability of the bacteria to attach to mucosal cells and render them more toxic. When these activated bacteria were induced prior to injury, mortality rates of the injured animals increased.¹⁵¹

From a standpoint of ENT, these changes in bacterial virulence occur in the setting of reduced IgA, depressed innate immune factors, and a proinflammatory response of the GI tract that distributes primed PMNs throughout the lung, liver and other tissues. Alverdy and colleagues introduced this concept of quorum sensing where by bacteria respond to the surrounding environment and changes in auto-inducer, which regulates specific target genes within the bacteria.^{148,152} This concept, together with their work in the use of vasoactive drugs, opiates, and PN and gut starvation in the face of dietary related mucosal defense defects constitute a concept that is clinically relevant.

Surrogates for ENT

Although ENT appears to be the preferred method for nutrition support in hospitalized patients, a significant number of hospitalized patients cannot be fed any ENT or fail to tolerate quantities adequate to meet their caloric and protein needs. Although PN has been lifesaving in many of these patients, the PN-induced vulnerabilities contribute to infectious morbidity. The availability of a supplement to PN, which stimulates mechanisms capable of maintaining mucosal defenses, could provide a clinical advantage. A total of 2 areas of research deserve attention. The first is neuropeptides released by the ENS and the second is glutamine, a metabolic nutrient important to the support of the intestinal mucosa, T lymphocytes, and all rapidly proliferating cells.

The enteric nervous system

The GI tract is innervated with fibers from both the autonomic nervous system and the ENS. The sympathetic and parasympathetic fibers of the autonomic nervous system reach the small intestine via the dorsal root ganglia and the spinal cord; in the mucosal immune system, their role appears to be important in the release of antimicrobial peptides by the Paneth cells.¹⁵³ The ENS innervates the intestine much more extensively. The ENS consists of 10^8 sensory neurons, interneurons, and motor neurons that affect peristalsis, blood flow, and secretion of water and electrolytes.¹⁵⁴ The cell bodies of the ENS are located within the submucosal Meissner plexus and in the myenteric Auerbach plexus between the longitudinal and circular layers of the tunica muscularis. These cells release acetylcholine and neuropeptides, but not catecholamines. The various neuropeptides released by the ENS include calcitonin gene-related peptide, vasoactive intestinal peptide, somatostatin, GRP, and substance P. These neuropeptides influence proliferation, function, and differentiation of immune cells as well as cytokine release. An analog of the GRP neuropeptide is bombesin (BBS), which interacts with neuropeptidergic receptors on immune cells. GRP and BBS contain an identical 7-amino acid carboxyl terminus, which accounts for their similar effects. The enteric nerve fibers infiltrate the GI tract with approximately 2 m of nerve per each cubic cm of GI tissue and most of the fibers reside within 13 mm of the mucosa to exert their effect.^{155,156} There are also neuropeptidergic receptors on peripheral nerve endings suggesting that there is a bidirectional communication between the ENS and the immune cells.

BBS administration reverses many of the PN-induced changes that occur within the LP, PP, and cytokines. Although BBS administration fails to affect MAdCAM-1 expression,¹³² simultaneous administration of BBS with PN results in a significant increase in the T and B cell numbers within the PP, LP, IE space, and even the salivary glands.^{157,158} Simultaneously, pIgR expression increases with BBS and both intestinal¹⁵⁷ and respiratory tract IgA¹⁵⁹ levels return to the levels of enterally fed animals (Fig 11). In studies of phenotypic markers of activation (CD25), memory (CD44), homing (L-selectin and A4B7), Treg cells, and B cells, the addition of BBS to PN reversed the depression in the CD4-CD8 ratio occurring within the LP increasing the CD4⁺, CD25⁺, $\alpha_4\beta_7^+$ cells within the LP.¹¹¹ Of particular note, the CD4⁺ CD25⁺ Foxp3⁺ population of cells in the LP returned to normal. These Treg cells provide antigen-specific stimulatory signals via IL-10 and TGF β to drive

IgA production by the IgA⁺ B cells while simultaneously inhibiting the Th1-type IgA-inhibiting cytokine, IFN- γ .⁶⁹ The Treg population appears important in maintaining immunological self-tolerance through inhibition of autoreactive T cells. In addition, BBS increased the B cell populations by increasing the phenotype IgD⁻ $\alpha_4\beta_7$ + subpopulation within the LP. These changes reflect an increase in activated B cells within this effector site since these cells are capable of promoting IgA production. BBS also normalizes the CD44⁺ expression within the B cells. The CD44 molecule facilitates lymphocyte interaction with the epithelium and functions as a marker of memory cells.¹⁶⁰

Functionally, BBS normalizes established antiviral and antibacterial defenses. As discussed previously, PN impairs IgA-mediated antiviral defense against the A/PR8-H1N1 virus. PN does not destroy immunologic memory since refeeding with chow returns immunity to normal. When PN was administered simultaneously with BBS, antiviral immunity remained normal such that re-challenge with the virus resulted in rapid clearance and sterilization of the upper respiratory tract. The effects of BBS upon IgA-mediated antibacterial defenses, which are also lost during PN are even more clinically relevant. Overall, 90% of immunized chow fed animals survives an intratracheal injection of *Pseudomonas* bacteria compared to unimmunized animals, which survive 10% of the time. Although ENT preserves this immunity, PN treatment results in 90% mortality, the same as unimmunized animals. However, when BBS is administered with PN over the 5 days of feeding, immunity remains intact with BBS animals surviving at the same rate as enterally fed animals. Other neuropeptides including cholecystokinin and neurotensin also exert phenotypic changes upon the GALT but none appear as effective as BBS.¹⁶¹ This is likely due to the effect of GRP, which stimulates downstream release of multiple neuropeptides by the ENS.

The beneficial effects of these neuropeptides are not relegated only to changes in acquired immunity; the neuropeptides directly affect innate immunity as well.¹²³ BBS administration to PN fed mice affects both goblet cells and Paneth cells. Within Paneth cells, BBS increased levels of both mRNA transcription and protein levels of sPLA₂ and lysozyme within the Paneth cell granules compared to PN feeding but intraluminal levels of lysozyme and sPLA₂ remained unchanged¹²³ (Fig 11). Release of these antimicrobial peptides from Paneth cells appears dependent upon autonomic stimulation with acetylcholine. Both the ENS and the autonomic nervous system work together providing antimicrobial peptide production and release. Reg3 γ levels (which increase during PN feeding) remain at the level of enterally fed animals suggesting that BBS treatment maintains a barrier of adequate thickness to prevent interaction between bacteria and the epithelial surface since Reg3 γ is released in response to a loss of this thickness. Interestingly, BBS treatment preserved the bactericidal activity of the intestinal secretions compared to PN alone and reduced in vitro mucosal invasion by pathogenic *E. coli* in vitro to levels of ENT. Thus, this work established the close interaction between the ENS and both acquired and innate immune mucosal defenses.

Glutamine

During stress and following injury, both protein synthesis and catabolism increase dramatically due to changes in cytokine and hormonal milieu as well as immobilization. The

metabolic response to injury drives glucose production and hyperglycemia, which quickly depletes hepatic glucose stores. Since significant amounts of glucose cannot be manufactured from metabolism of fat (only the glycerol backbone of triglycerides could be used for glucose production), continued glucose production depends upon amino acid metabolism.

Within the skeletal muscle, the branch chain amino acids (BCAA) are utilized for an energy substrate while the nonBCAA are released into the extracellular fluid.^{163–165} As the BCAA are metabolized, waste nitrogen is dealt with in 2 ways. First, glucose is converted to pyruvate, which receives a nitrogen moiety converting it to alanine. Alanine is released from the cell, transported to the liver, and the nitrogen is converted to urea whereas pyruvate molecules are recombined into a glucose molecule. The secondary transport mechanism for waste nitrogen from the cell is through the formation of glutamine. As the BCAA are incorporated to in the Krebs cycle, transamination of a single nitrogen molecule onto α -ketoglutarate forms glutamate while transamination of a second nitrogen results in the production of glutamine. Glutamine is released from the cell and serves as a specific fuel for enterocytes, T lymphocytes, and is metabolically required for all proliferation.^{166–171}

The kidney converts a small amount to glucose. Glutamine is converted by splanchnic tissue and the immune cells into ornithine, citrulline, and alanine, which are cleared by the liver for entry into appropriate metabolic cycles. Cytosolic glutamine levels drop during severe stress becoming conditionally essential; supplemental glutamine is not usually necessary in times of health. Glutamine and alanine account for 70% of the amino acids released by skeletal muscle although they only constitute approximately 7% of normal muscle protein. Glutamine also regulates glucose metabolism by improving insulin sensitivity.¹⁷² Besides these metabolic pathways, glutamine is a precursor necessary for the production of the antioxidant glutathione, which is found in high concentrations within the intestinal mucosa.^{173,174} Reductions of glutathione result in mucosal atrophy, malabsorption, as well as diarrhea.^{175,176}

Another function of glutamine is the expression of heat shock protein, which requires adequate glutamine levels.¹⁷⁷ In models of ischemia reperfusion, glutamine administration improves survival of the ischemic tissues and overall survival in the animal models.^{178,179} Unfortunately, free glutamine is not a common component of PN due to instability in solution especially during heat sterilization that accelerates conversion of the glutamine molecule to pyroglutamate, which is toxic. Therefore, shelf life is very limited. Although more expensive in PN solutions, glutamine remains stable as a dipeptide, bound either to another glutamine molecule or another amino acids such as alanine. Freshly made glutamine solutions using filter sterilization can also be used, but this usually is not practical for most hospital pharmacies. A number of clinical trials studied the use of parenteral glutamine in critically ill patients. Although a recent study¹⁸⁰ demonstrated no significant benefit with the administration of free glutamine, 1 metaanalysis of combined enteral and parenteral studies noted a significant reduction in mortality and improvements in length of stay and infectious complications in 1564 patients.¹⁸¹

Experimentally, glutamine reversed many of the PN-induced changes in both acquired and innate immune parameters. A 2% glutamine supplemented PN significantly improves the number of T and B cells within PP and LP to normal levels and significantly improved IL-4 levels in gut homogenous levels.¹⁶⁶ IL-10 cytokine levels, however, were not affected.¹⁶⁷ Compared to PN fed mice, intestinal and respiratory tract IgA levels significantly improved although they remained lower than enterally fed mice receiving chow. Glycyl-L-glutamine produced similar effects on the small intestinal immunity and improved respiratory tract IgA.¹⁶⁸ From a functional standpoint, glutamine supplementation with either free glutamine or a dipeptide maintained IgA-mediated antiviral defense against the A/PR8-H1N1 virus although approximately 30% of animals continued viral shedding, indicating that antiviral immunity improved relative to PN but not to completely normal levels. Similar results occurred after immunization against intratracheal *Pseudomonas* where glutamine supplementation improved survival relative to PN but mortality still exceeded mice fed enterally.¹⁸² Just as occurred with BBS, preservation of mucosal immunity was not due to increased MAdCAM-1 expression. This suggests a direct effect on the T and B cells themselves rather than changes in entry of cells into the PP, possibly due to reduction in apoptosis since glutamine modulates gene regulation related to apoptosis in signal transduction.¹⁸³

Glutamine, however, improves survival of PN fed mice after gut ischemia/reperfusion.¹⁸⁴ Animals randomized to ENT with chow, PN, or 2% glutamine supplemented PN were exposed to 15 minutes of gut ischemia and reperfusion. PN significantly reduced the survival compared to chow animals at 72 hours, but glutamine supplementation significantly improved survival to chow levels. These were postulated to occur through 1 of 2 mechanisms. Firstly, glutamine normalizes the IgA-stimulating cytokine, IL-4, and reduces small intestinal ICAM-1 expression and accumulation of PMNs within the small intestine. This presumably reduces priming of neutrophils, which are distributed throughout the body.¹⁸⁵ Secondly, since glutathione preserves the antioxidant glutathione, glutamine might have maintained mucosal glutathione levels, providing a scavenger for the reactive oxygen species created after ischemia reperfusion. Glutathione levels reduce cell membrane lipid peroxidation and increase nitric oxide.⁶⁰ Gut permeability increases as levels of TNF α , IL-1, and IL-6 increase, so glutamine may have blunted the release of proinflammatory cytokines as well. This is a reasonable assumption since glutamine also improves innate mucosal immunity including the defensins, lysozyme, and the mucin layer.¹⁸⁶ Compared to PN alone, glutamine supplementation increases the MUC2 and lysozymes while significantly increasing expression of IL-4, IL-10, and IL-13. Treatment with glutamine significantly improves resistance to mucosal cell invasion by *E. coli*, returning gut barrier resistance against the bacteria to normal.

In summary, evidence is accumulating that methods exist to stimulate or maintain mucosal immunity when ENT is impossible or impractical. The evidence highly supports that glutamine metabolism and the ENS products play integral roles in the maintenance of both acquired and innate immunity of the intestine. Although the neuropeptide work has not been translated into clinical results, some experimental and clinical evidence suggests that glutamine supplementation may be clinically beneficial.

Summary

There are immunologic benefits gained when nutrients are delivered via the gut that do not occur when nutrients are delivered parenterally. Clinically, these differences are noted when the 2 routes are studied in appropriate patient populations who are at risk of nutrition-related complications. The critical difference appears to be the lack of enteral stimulation and gut starvation upon the GI immune system during PN. Immunological changes are evident throughout the multiple layers of mucosal defenses employed to protect the host from intestinal microorganisms and environmental toxins. The changes to these layers range from mucosal atrophy and increased epithelial permeability to the loss of physical and chemical secretions that protect the epithelial surface and modulate the intestinal microbiome community. Breakdown in these intestinal layers of defense renders the host susceptible to subsequent infectious and inflammatory complications.

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Fig. 1.

(A) Overall major infectious and noninfectious complications in TPN and control fed groups. Overall infectious complications were significantly higher in the TPN group with no significant reduction in major noninfectious complications. (B) Major infectious and noninfectious complications following nutritional status stratification. The increase in infectious complications in TPN patients occurred in the mild and borderline malnourished groups, with no increase in the severely malnourished patients. However, major noninfectious complications were lower in severely malnourished patients fed TPN although this failed to reach statistical significance due to the population size. TPN, total parenteral nutrition. (Adapted with permission from The Veterans Affairs Total Parenteral Nutrition Cooperative Study Group.⁸)

Fig. 2. Depiction of the common mucosal immune system. Naïve T and B lymphocytes enter the Peyer's patches through interaction with MacCAM-1 on the high endothelial venules and enter the structure to become sensitized to antigen, which is sampled from the intestinal lumen and presented to antigen-presenting cells by specialized microfold cells. Following sensitization, lymphocytes migrate to the mesenteric lymph nodes (MLN) to undergo maturation and proliferation before exiting the thoracic duct and migrating back to mucosal surfaces throughout the body to support IgA production. IgA is transcytosed by the epithelial cell layer on to the mucosal surface and functions as a key immunological strategy for maintenance of commensalism and prevention of pathogenic opportunism. (Color version of figure is available online.)

Fig. 3.

Variation in nutritional risk score and clinical outcome. The studies were categorized according to total score and for each category, the number of studies with no effect (No) or with a positive effect (Pos) on clinical outcome are shown. The analysis demonstrate positive outcomes in studies of nutrition support which recruit patients with greater degrees of malnutrition. (Adapted with permission from Elsevier and Kondrup et al.⁹⁶)

Fig. 4.

(A) Total cellularity within the mesenteric lymph nodes (MLN), Peyer's patches (PP), and lamina propria (LP) following TPN and chow feeding in mice. In contrast to the MLN, the PP and LP exhibit significantly decreased cellularity following TPN compared with chow (B) Circulating IgA and intestinal sIgA levels within the lumen following TPN and chow in mice demonstrate the intestinal lumen has significantly lower levels of IgA following TPN. Comparably, serum IgA levels are not significantly affected. * $P < 0.05$ vs TPN. TPN, total parenteral nutrition. (Adapted with permission from Li et al.¹⁰⁷)

Fig. 5. (A and B) Decreased cellularity of both T and B lymphocytes occurs in the Peyer's patches (PP) and lamina propria (LP) sites during PN, reaching a nadir by 3 days, where PP exhibits the greatest loss of cell populations compared with baseline. (C and D) Strikingly, refeeding chow following 5 days of PN rapidly restores normal T and B lymphocyte cellularity within the LP and PP space following 48 hours of refeeding. (A and B—Adapted with permission from King et al.¹¹³; C and D—Adapted with permission from Janu et al.¹¹²)

Fig. 6. (A) Despite previous vaccination in all animals, TPN delays clearance of viral shedding compared with animals fed enteral diets of complex enteral diet (CED) and Chow. (B) The delayed clearance of virus was associated with impaired IgA levels at mucosal surfaces, where TPN exhibited a 30%–40% reduction in respiratory IgA levels compared with Chow fed animals. TPN, total parenteral nutrition. (Adapted with permission from Kudsk et al.¹¹⁷)

Fig. 7. Route of nutrition influences 24-hour mortality following *Pseudomonas aeruginosa* where TPN animals survive like non-immunized animals despite previous vaccination. In contrast, enteral feeding of CED or Chow exhibit low mortality rates following vaccination. TPN, total parenteral nutrition. (Adapted with permission from King et al.¹¹³)

Fig. 8.

(A) Total antibody-forming cells (collective IgM+, IgG+, and IgA+ cell numbers) in the nasal passage of TPN, complex enteral diet (CED), or chow fed animals on days 6, 9, and 13 post-immunization for H1N1 virus. CED and chow fed animals significantly elevated total antibody-forming cells in the nasal passage by day 13 compared with TPN. (B) Percentage of animals shedding virus 8 days after immunization is influenced by the route of feeding, where TPN exhibits significantly more shedding than CED and Chow fed animals. TPN, total parenteral nutrition. (Adapted with permission from Johnson et al.¹¹⁸) * $P < 0.05$ vs TPN.

Fig. 9.

(A) Bactericidal activity of intestinal mucosal secretions following 5 days of TPN or chow feeding, where chow was significantly more bactericidal than TPN. TPN, total parenteral nutrition. (B) Relative expression of small intestinal antimicrobial peptides following 5 days of TPN or chow feeding, where TPN tissues express significantly less antimicrobial transcript. (Adapted with permission from Busch et al.¹²³) * $P < 0.05$ vs TPN.

Fig. 10.

(A) Respiratory and (B) small intestinal sIgA response following injury is influenced by the route of nutrition. TPN animals fail to elevate sIgA levels in either the lung or intestine following injury, compared with chow fed controls that significantly increase airway and intestinal sIgA by 8 hours post-injury. TPN, total parenteral nutrition. (A—Adapted with permission from Jonker et al.¹³⁹; B—Adapted with permission from Sano et al.¹⁴⁰) * $P < 0.05$ vs TPN alone. # $P < 0.05$ vs chow alone.

Fig. 11.

(A) The addition of bombesin (BBS) to total parenteral nutrition (TPN) (stripes) normalizes the level of respiratory and intestinal sIgA to levels observed in chow (black) fed animals, compared with TPN alone (white). (B) The addition of BBS to TPN also increases the relative expression of intestinal mucosal antimicrobial peptides compared with TPN alone, to levels at or greater than observed in chow fed animals. TPN, total parenteral nutrition. (A—Adapted with permission from Zarza et al.¹⁶²; B—Adapted with permission from Busch et al.¹²³) * $P < 0.05$ vs TPN.