Excerpta Medica

The American Journal of Surgery

The American Journal of Surgery 183 (2002) 390–398

Current aspects of mucosal immunology and its influence by nutrition

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Manuscript received December 17, 2001; revised manuscript January 5, 2002

Abstract

Background: A significant body of clinical literature demonstrates that enteral feeding significantly reduces the incidence of pneumonia compared to patients fed parenterally. An immunologic link between the gastrointestinal tract and respiratory tract is postulated via the common mucosal immune hypothesis. This hypothesis states that cells are sensitized within the Peyer's patches of the small intestine and are subsequently distributed to submucosal locations in both intestinal and extra intestinal sites. This system is exquisitely sensitive to route and type of nutrition.

Data Source: This review examines the laboratory data regarding cell numbers, cell phenotypes, cytokine profile, and immunologic function in both intestinal and extra intestinal sites in animals that have been administered either parenteral feeding or various types of enteral feeding. It also establishes links between a specific nutrient, glutamine, the enteric nervous system, by way of neuropeptides, and mucosal immunity.

Conclusion: Progress in understanding relationships between nutrient availability, enteric nervous system stimulation, and nutrient delivery on mucosal immunity offers opportunities to explore immune systems previously not appreciated by clinicians and basic scientists. These opportunities offer new challenges to the physician scientist, basic scientist, and clinician to understand, manipulate, and apply these concepts to the critically ill patient population by favorably influencing immunologic barriers and the inflammatory response.

Keywords: Gut-associated lymphoid tissue; Mucosal immunity; Enteral feeding; Bombesin; Glutamine

In the late 1960s, the introduction of parenteral feeding provided a lifesaving solution to the unchecked progressive lean body mass deterioration that occurred in patients with chronic bowel obstruction, fistulas, loss of mucosal body surfaces, or other clinical problems that precluded an ad libitum oral diet [1]. Intravenous administration of concentrated fluids containing macro- and micronutrients adequate to meet nutrition needs and to avoid progressive starvation-induced malnutrition changed the outcome of patients otherwise destined to die.

In the late 1970s, the first clinical [2] and laboratory [3,4] evidence hinted that the enteral processing of nutrients induced unique effects which affected the metabolic response to septic insults and improved host defenses. In the late 1980s and early 1990s, work focused on gut permeability after injury [5] and translocation [6,7] of intraluminal bacteria to the mesenteric lymph nodes and systemic circulation. Subsequently, two new areas of research have focused upon aspects of gut barrier function related to mucosal immunity and the intestinal priming of neutrophils. The latter two issues appear interrelated and influenced by both the route and type of nutrition. This review summarizes the effect of route and type of nutrition on the body's protection of moist mucosal surfaces. Intriguing interrelationships between the gut and respiratory tract have generated new questions and hypotheses tying nutrition to the gut barrier and the systemic inflammatory response.

The Clinical Issues

At approximately the same time, three studies: one clinical [2] and two laboratory [3,4], generated interest in the gastrointestinal tract as an important modulator of immune defenses. Alexander et al. [2] randomized severely burned children to either a standard enteral diet or protein-supplemented diet. Patients receiving the high-protein diet had a

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0002-9610/02/$ – see front matter
PII: S0002-9610(02)00821-8

higher survival rate and fewer septic complications than patients receiving the standard enteral diet. Little attention was paid to the fact that the high-protein-fed patients received significantly less parenteral feeding than the group receiving the standard enteral diet. This enteral/parenteral issue was directly addressed in studies from Sheldon's laboratory in which malnourished [3] or well-nourished [4] rats were randomized to either enteral or parenteral feeding for two weeks followed by a septic intraperitoneal challenge. Animals receiving a diet by way of the gastrointestinal tract, either by drinking or by gastrostomy infusions of the TPN solution, survived the septic insult at a higher rate, similar to well-nourished animals receiving chow, whereas animals receiving the solution parenterally had significantly higher mortality at a rate comparable to malnourished animals [8].

Moore et al. performed the first controlled clinical trials and randomized patients with moderate to severe intra-abdominal injury (determined by the Abdominal Trauma Index—ATI) to either direct small bowel feeding with a defined formula diet after celiotomy or intravenous fluids alone [9–11]. Parenteral feeding was started on the fifth postoperative day in 26% of patients who had not resumed an oral diet. They noted a significantly lower postoperative intra-abdominal sepsis rate with a trend toward a lower incidence of pneumonia. In a follow-up study [10], patients were randomized to early small bowel feeding with the defined formula diet or to early parenteral feeding and again the enteral group sustained a significantly lower incidence of pneumonia with a trend toward a reduction in intra-abdominal abscess. These results were later reiterated in a meta-analysis of several smaller studies of enteral versus parenteral feeding [12].

In a subsequent study of 98 patients with moderate to very severe intra-abdominal injuries, many of whom had been excluded from the earlier studies due to high severity of injury, Kudsk et al. [13] randomized patients requiring celiotomy to either early small bowel feeding with a defined formula diet or to isonitrogenous, isocaloric parenteral feeding. The enteral-fed group sustained significantly fewer pneumonias, intra-abdominal abscesses, episodes of line sepsis, infections per patient, and infections per infected patient. There was little difference in the less severely injured patients and most of the benefit from early enteral feeding occurred in patients most at risk of developing septic complications. Patients with major abdominal injuries (ATI>24; primarily severe colon, duodenum, liver, or pancreas injuries) or severe blunt injuries (ISS>20), patients receiving more than 25 units of blood, or patients requiring reoperation within 72 hours were the primary beneficiaries of reduced sepsis with early enteral feeding. Subsequently, two studies of specialty enteral diets enriched in omega-3 fatty acids, arginine, nucleotides, and/or glutamine show additional clinical benefits over the standard defined formula diets resulting in a significant reduction in both septic complications [14,15] and the development of the multiple organ dysfunction syndrome (MODS) [14].

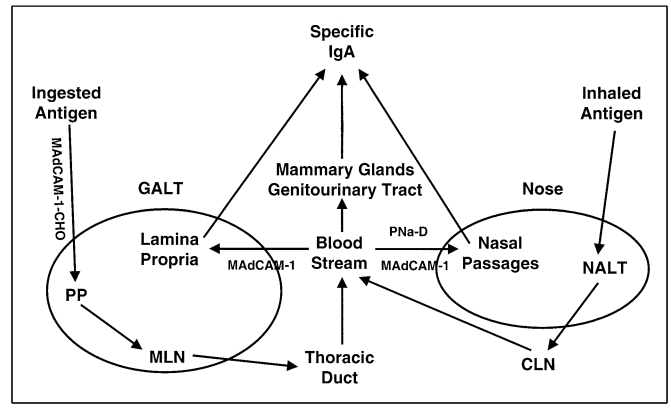

Fig. 1. The common mucosal immune hypothesis: Antigen is taken up by the Peyer's patches (PP) for delivery to antigen-presenting cells. Naïve T and B cells are diverted into the PP through interaction with MAdCAM on the high endothelial venules. After sensitization, cells migrate to the mesenteric lymph nodes (MLN) and to the thoracic duct to be distributed by the bloodstream to various effector sites via MAdCAM-1 including the lamina propria of the gut-associated lymphoid tissue (GALT), the respiratory tract, the mammary glands, and the genitourinary tract. In rodent species, and possibly in humans, the nasal-associated lymphoid tissue (NALT) is analogous to the PP. After sensitization, cells migrate to the cervical lymph nodes (CLN) prior to distribution to mucosal surfaces. Peripheral lymph node addressin (PNa-D) directs cells into the effector sites within the nasal passages. Specific IgA prevents attachment of pathogens to the mucosa by attaching to the specific antigens on the bacteria or viruses.

Respiratory infections frequently complicate the recovery of trauma patients, and two of the studies show that this infection was most dramatically impacted. In our clinical study [15], enteral feeding reduced this complication rate from 31% with TPN to 11%. Most respiratory infections are infections of mucosal surfaces and protected by mucosal immune defenses. Experimentally, this mucosal immune system has been manipulated to define existing links between route and type of nutrition, preservation of gut barrier function, and integrity of respiratory defenses.

Enteral feeding exerts diverse effects in maintenance of intestinal and respiratory integrity. Approximately 50%–60% of the body's total immunity underlies the mucosal surfaces of the upper and lower respiratory tract, small intestine, and colon and accounts for 70%–80% of antibody production (primarily IgA) by the body [16,17]. IgA is synthesized within the lamina propria through interaction of T and B cells, is immediately transported by the overlying epithelial cells onto the mucosal surface using secretory component, and binds to specific antigens on bacteria to prevent their attachment. Without attachment, infection does not occur. Results of experimental and clinical studies have led to speculation of interdependent links between the gastrointestinal tract and extraintestinal mucosal sites. This postulated system is known as the Common Mucosal Immune Hypothesis (Fig. 1).

Immunologic Backdrop: The Common Mucosal Immune Hypothesis

The surface area of the intestinal mucosa is approximately 300 m^2 providing an adequate surface area for the digestion and absorption of nutrients into the body. Because of the huge number of bacteria that lie near the mucosal surfaces, protective barriers have developed allowing a symbiotic relationship between the host and bacteria and at the same time preventing bacterial invasion and sepsis in the host. Multiple extrinsic mechanisms, such as the mucous coat, glycocalyx, resident microflora, peristalsis, proteolytic GI secretions, and other innate humoral factors (lactoferrin, lysosomes, and peroxidases) assist in maintaining the protective barrier, but the strategic immunologic component of the system is secretory IgA [18]. Secretory IgA is the primary immunoglobulin found in all external secretions.

The principal site responsible for sensitization of this immune mass is the Peyer's patches found within the small intestine [19,20]. There is evidence in rodents of a separate system for nasal priming and respiratory protection called the nasal-associated lymphoid tissue (NALT) [21]. Although analogies have been drawn between NALT and Waldeyer's ring in the tonsillary area of humans, no secondary site of sensitization has been clearly delineated in humans. There is also evidence of antigenic processing by epithelial cells, but this process appears to be more important in constantly stimulating the underlying lamina propria cell population than in initial sensitization. In humans, most Peyer's patches are located in the distal small intestine that has microflora similar to the colon presumably through backwash from the colon through the ileocecal valve, but bacterial concentrations are lower in the distal small bowel than in the colon. The intraluminal surface of the Peyer's patches are covered with a follicle-associated epithelium, a one-cell-layer complex composed of columnar epithelial cells, goblet cells, and microvillus (M) cells (Fig. 1) [20,22]. Antigen is transported through the M cells to underlying dendritic and macrophage populations which express major histocompatibility complex Class II molecules and process and present the antigen to CD4+ T cells and naïve B cells destined to become cytokine-producing cells and plasma cells, respectively, in mucosal immunity [23,24].

The adhesion molecule, mucosal addressin cellular adhesion molecule-1 (MAdCAM-1), is located on the high endothelial venule (HEV) of the Peyer's patches and regulates trafficking of naïve T and B cells into and through the mucosal immune system [25,26]. It is a ligand for the adhesion molecules, L-selectin and $\alpha_4\beta_7$, found on naïve lymphocytes [27]. After the T and B cells are sensitized to the processed antigen within the Peyer's patches, their cellular surface changes from an L-selectin++ $\alpha_4\beta_7$+ to L-selectin± $\alpha_4\beta_7$++ profile as they mature and/or proliferate within mesenteric lymph nodes [28]. These cells migrate through the thoracic duct into the circulation and home to lamina propria sites through the interaction between a slightly altered MAdCAM-1 which is more attractive to the $\alpha_4\beta_7$++ molecule. The B cells are transformed under the appropriate T cell cytokine milieu to plasma cells. The T cells produce cytokines which are Th2 type IgA-stimulating (IL-4, IL-5, IL-6, IL-10, and IL-13) or Th1 type IgA-inhibiting cytokines (IFNγ, TNFβ, and IL-2) [29]. It is this interaction of T and B cells and balance of cytokines which controls IgA production for transport and mucosal protection.

Both experimental and clinical data support the concept that cells processed within the gut-associated lymphoid tissue (GALT) can migrate and home to intestinal and extraintestinal sites and induce specific immunity in the external secretions of these sites. In 1974, Montgomery [30] orally and bronchially immunized rabbits to antigens and noted specific IgA antibody against that antigen in the mammary secretions. Subsequently, co-workers harvested cells from the GALT, bronchial-associated lymphoid tissue (BALT), or mesenteric lymph nodes and populated bronchiolar or intestinal lamina propria with these cells in transfer experiments [31]. The functional importance of this observation was noted by Michalek who immunized gnotobiotic rats with killed cells from a cariogenic strain of *Streptococcus mutans* to generate a specific *anti-Streptococcus* IgA [32]. The orally immunized animals sustained significantly fewer carious lesions than unimmunized animals when both groups were infected with the *Streptococcus mutans*. Roux et al. [33] harvested lymphoblasts from mesenteric lymph nodes of sensitized mice and demonstrated that the lymphoblasts homed to the mammary glands of syngeneic recipients in late pregnancy and during lactation and released specific IgA into the milk and colostrum. Lymphocyte transfer studies were performed from orally immunized mice to nonimmunized recipients and specific antiferritin IgA-producing cells were found to home to other mucosal sites [34]. Animals were administered ferritin in plasma cells and studied by immunofluorescence in various tissues. Ferritin-specific plasma cells were found within the intestinal mucosa, lactating mammary gland, respiratory tract, and salivary gland.

These cells can locate in the respiratory tract. In 1987, Weisz-Carrington [35] orally immunized mice with ferritin and studied the lungs and intestine for specific antibody-producing cells by immunofluorescence. There was a 6- to 15-fold increase in IgA antiferritin plasma cells within the bronchial mucosa in sensitized animals. When mesenteric lymph nodes of sensitized animals were transferred to ferritin-naïve animals, there was a fourfold or greater increase in IgA antiferritin plasma cells in the respiratory mucosa after stimulation, this successfully demonstrating that IgA-producing sites from the GALT migrate to the respiratory mucosa after oral immunization.

The relevance to humans was established when three pregnant women ingested 10^9 live *E.-coli* 083 bacteria

within one month of delivery [36]. Large quantities of SIgA antibodies specific for the *E.-coli* were found in the colostrum, saliva, and serum that were previously negative. Antibodies appeared as soon as three days after ingestion of the bacteria. In 1978, Mestecky administered enteric-coated capsules of killed *Streptococcus mutans* to healthy volunteers and found IgA antibody specific to that organism in saliva and tears [37]. Childers et al. [38] gave four volunteers enteric-coated capsules containing antigen to *Streptococcus mutans* in liposomes and noted salivary responses to specific antigens and early plasma IgA responses as well. Czerkinsky et al. [39] administered capsules containing *Streptococcus mutans* to volunteers and noted circulating IgA-producing cells within the blood within seven days which reached a maximal response on days 10–12. Specific SIgA against the bacteria were found within salivary secretions. Interestingly, one IgA-deficient patient exhibited an IgM response. Finding specific IgA-producing cells within the blood stream was consistent with the circulatory system as a means of distributing these GALT sensitized cells.

Recently, human volunteers [40] received oral, rectal, system, or intranasal immunization with cholera toxin B subunit. Systemic immunization resulted in circulating cells which expressed increased L-selectin whereas only a small proportion expressed $\alpha_4\beta_7$. However, intestinal immunization resulted in virtually all IgA antibody-secreting cells expressing $\alpha_4\beta_7$ with a small number expressing the L-selectin molecule consistent with the gut immunization changes on cell profile noted in animal experiments. Nasal immunization resulted in expression of both L-selectin and $\alpha_4\beta_7$. Thus, site of immunization in humans influences expression of adhesion molecules on circulating cells which might explain distribution of cells to specific mucosal-associated tissues.

The importance of enteral stimulation on the GALT system was studied in neonates who expired soon after birth [41]. Infants who received enteral stimulation showed clear evidence of B cells and T cells within the lamina propria; whereas, parenterally fed neonates who expired had an atrophic intestinal lamina propria. Gut and bronchus samples were obtained and related to time of death in a study of infants who died of sudden infant death syndrome between the early postpartum period and 90 months after birth. IgA plasma cells first appeared at 12 days in the gut and later in the bronchi as the system matured. Plasma cells increased rapidly over time as IgA plasma cells predominated by three weeks in the gut and six weeks within the bronchi. Thus, MALT is poorly organized and extremely immature at birth but matures depending upon the presence or absence of enteral feeding. Bacterial colonization and proliferation may play an important role since the GALT and MALT are almost nonexistent in germ-fed mice until animals are colonized with bacteria [42].

Taken in toto, clinical and experimental evidence supports the existence of an interrelated system capable of immunizing cells in nasal passages or intestine of mammals although an upper respiratory site of immunization and antigenic processing has not yet been found in humans. There is strong circumstantial evidence of a communication between various mucosal departments using the circulatory system to generate generalized mucosal protection by local immunization [42]. This concept, the common mucosal immune hypothesis [43], is useful in explaining the immunologic changes induced by nutritional manipulation. Although this area of research is still in its infancy, animal models clearly show that both route and type of nutrition—or the hormonal and neuropeptide response to that nutritional stimulation—affect the vigor and integrity of this specific immunity.

Nutritional Impact in Models of Mucosal Immunity

The stomach and upper small intestine contain low concentrations of bacteria (10^3 organisms/mL or less) which are usually considered transients from the mouth and oropharynx rather than colonization. The bacterial flora in the distal third of the bowel reflects that of the large intestine although at a lower concentration than found within the colon. In the normal fed human, 1×2 cm (or larger) Peyer's patches line the antimesenteric surface of the distal ileum within 30–40 cm of the ileocecal valve, exposing this priming cell mass to a microflora similar to that in the colon [44].

Nutritional Studies of Mucosal Immunity in Mice

Both the route and type of nutrition affect the histology, cytokine levels, IgA levels, and effectiveness of intestinal and respiratory immunity. Histologic changes in GALT occur within three to four days after dietary manipulation of route and type of feeding [45]. Unfortunately, lack of enteral stimulation cannot be studied using a starvation model since a fast for three days results in severe malnutrition and is lethal. However, intestinal effects caused by the presence or absence of enteral feeding can be isolated from generalized malnutrition with starvation by supporting animals with parenteral feeding. This technique permits manipulation and study of nutrition, hormonal or neuropeptide stimulation and specific nutrient administration on mucosal mass and function without lethal malnutrition. For experimental design, groups of animals are fed the TPN solution enterally or parenterally to study the effects of route of nutrition using identical diets. Type of nutrition can then be manipulated by altering the complexity of the enteral diet by using more complex formulas which are isonitrogenous and isocaloric relative to the TPN solution but contain various forms of protein, fat, and carbohydrate. A chow group allows comparisons with the normal fed state.

Impact of Route and Type of Nutrition on Histology and Cellular Characteristics of Gut-Associated Lymphoid Tissue

In 1991, Tanaka [46] found histologic evidence of a reduction in the GALT lymphocyte cell number within intestinal lamina propria of long-term parenterally fed rats. To study this further, our group [45] separated Peyer's patches, lamina propria, and intraepithelial lymphocytes from the small intestine of mice and analyzed them for changes in cell populations using flow cytometry. With just three days of parenteral feeding [47], a marked reduction in cell number developed in all three sites. CD4 and CD8 percentages within the Peyer's patches remained stable, but a significant decrease occurred in the lamina propria CD4: CD8 ratio from 2:1 to 1:1 with parenteral nutrition, suggestive of significant shifts in cell profiles and alterations in cytokine levels (discussed later). Administration of TPN intragastrically as a model of a defined formula diet resulted in similar cellular changes, but infusion of an isonitrogenous, isocaloric complex enteral formula (CED) containing fat, protein, and complex carbohydrates maintained cell populations at levels comparable with chow-fed animals. The changes induced with parenteral feeding rapidly reversed with reinstitution of chow as Peyer's patch and lamina propria cell populations recovered within two to three days of re-feeding [48]. Correction of the lamina propria CD4:CD8 ratio was slightly delayed but recovered by the fourth to fifth day.

Intraluminal intestinal IgA levels dropped rapidly with parenteral feeding, reaching a nadir by the fourth to fifth day in association with the lamina propria T and B cell atrophy [45,47]. Simultaneously, respiratory tract IgA levels also dropped, reaching a nadir by the third day. Both of these markers of specific immunity recovered rapidly with reinstitution of enteral feeding [48]. IgA levels remained normal at both sites with chow or CED feeding.

Cytokine Changes within Gut-Associated Lymphoid Tissue with Parenteral Feeding

Under normal conditions, the Th2 IgA-stimulating cytokines (IL-4, IL-5, IL-6, IL-10, and IL-13) are counterbalanced by the Th1 IgA-inhibiting cytokines (IFNγ, TNFβ, and IL-2) [29]. These cytokine changes also affect maturation of stimulated B cells to plasmacytes. IL-4 stimulates B cells to switch to IgA+ cells within the Peyer's patches whereas IL-10 (as well as IL-5 and IL-6) promote conversion of SIgA+ B cells to mature SIgA-secreting plasma cells in the lamina propria. After five days of parenteral feeding, levels of IFNγ, IL-5, and IL6 remained stable, but levels of IL-4 and IL-10 dropped in small intestine homogenates of parenterally fed animals [49]. Both IL-4 and IL-10 levels correlated with intestinal IgA levels, accounting for approximately 50% of the drop in IgA; the remaining decrease could be accounted for by the 50% reduction in T and B cell populations in the lamina propria induced by parenteral feeding. Because intestinal homogenates contained both mucosa (a source of cytokines) and GALT cells, lamina propria GALT cells were separated and analyzed for IL-4 and IL-10 mRNA [50]. mRNA changes were similar to the gut cytokine levels in homogenates, providing evidence of cytokine changes specifically in GALT tissues.

Functional Effects of Parenteral Feeding on Established Antiviral and Antibacterial Mucosal Immunity

The cellular, cytokine, and IgA alterations noted above are only relevant if direct functional effects result from their changes. In this regard, two animal models have been studied: upper respiratory infections and viral shedding of the APR/8 *Haemophilus* influenza virus [51] and *Pseudomonas aeruginosa* pneumonia [52].

The influenza virus is an infection controlled by IgA-mediated defenses. Temporary protection against viral infection can be provided by administration of influenza-specific IgA to naive animals [53]. When infected, animals generate specific IgA within two weeks, rendering them immune to a subsequent rechallenge as virus is cleared from the airway within hours of the rechallenge. Administration of anti-IgA antibody with the rechallenge temporarily neutralizes this immunity [54], resulting in continued viral shedding from the nasal passages until IgA levels are replenished.

This IgA-mediated protection is affected by route and type of nutrition [51]. After immunizing mice and establishing immunity against the APR/8 virus, animals received parenteral feeding, IG-TPN, an isonitrogenous, isocaloric CED, or chow. In a five-day time period associated with reduced respiratory and intestinal IgA levels, GALT atrophy, and cytokine changes noted previously, 50% of parenterally fed animals lost protection against the virus and had continued viral shedding 40 hours after rechallenge; all animals fed via the gastrointestinal tract cleared the virus. Immunologic memory was not lost in the parenterally fed mice since five days of chow re-feeding returned protection to normal.

Although IG-TPN-fed mice effectively cleared the virus, the quantitative reductions in IgA levels and GALT cell populations to levels slightly above the parenterally fed mice suggested that a defect existed which was not detectable with the APR/8 challenge. This was tested with a more virulent model of *Pseudomonas* pneumonia [52]. A dose response curve was defined in naive animals who were given varying quantities of intratracheal *Pseudomonas aeruginosa*. There was a linear increase in mortality from 0% (with 1.0×10^7 organisms) to 100% (with 1.0×10^8). Mortality to 1.0×10^8 organisms could be reduced to 10%–20%, however, if mice received a previous intranasal im-

munization with *Pseudomonas* antigen in liposomes [52, 55]. The mortality rate dropped as IgA levels increased within the respiratory tract.

After generating immunity against *Pseudomonas*, animals received the four diets as described previously [52]. After five days of feeding, mortality remained 10%–20% in CED- and chow-fed mice, as respiratory immunity remained intact to the *Pseudomonas* challenge. However, parenterally fed animals sustained total loss of the antipseudomonal defense and had a mortality rate identical to unimmunized control animals. IG-TPN animals had a mortality rate significantly better than parenterally fed mice but significantly worse than chow- or CED-fed mice, consistent with partial preservation of mucosal protection following enteral stimulation with the defined formula diet.

Taken in toto, these results provide evidence of a loss in established respiratory mucosal immunity when the GI tract is not stimulated with enteral feeding. Memory remains intact since reinstitution of enteral feeding restores antiviral immunity. The enteral stimulation protects intestinal immunity as well since many prior studies confirmed the bacterial translocation to the mesenteric lymph nodes during parenteral feeding. Explained in the context of our evidence and the data from other laboratories, decreases in IgA levels reduced barrier effectiveness against the bacteria that were then able to adhere to the mucosa, traverse it, and enter the lymphatics for clearance by the mesenteric lymph nodes.

Nutritional Effects Upon MAdCAM-1: The Gateway to Mucosal Immunity

MAdCAM-1 is the primary ligand attracting those naïve B cells which exhibit the L-selectin and $\alpha_4\beta_7$ adhesion molecule profile on their cell surfaces [25,26]. Parenteral feeding depresses MAdCAM-1 expression from normal levels, leading to Peyer's patch atrophy and, presumably, deleterious effects upon established mucosal immunity [56]. This was studied with a dual monoclonal antibody technique in which animals were administered a radiolabeled nonspecific antibody and an antibody (with a second radiolabel) which is specific against a particular adhesion molecule, in this case MAdCAM-1. The ratio of specific antibody binding to nonspecific binding allows quantification of expression on endothelial cells in vivo and overcomes inherent problems with immunohistochemistry, tissue levels of mRNA, or indirect blocking experiments with monoclonal antibodies [57].

MAdCAM-1 expression in Peyer's patches dropped by 60% with four days of parenteral feeding compared with chow feeding [56]. The diet composition affected expression since MAdCAM-1 expression decreased progressively as diet complexity decreased from chow, to CED, and to IG-TPN. Expression was lowest with parenteral feeding. Peyer's patches were also studied after administration of anti-MAdCAM-1 antibody to chow-fed mice for five days to examine the effects on GALT atrophy. Total lymphocyte cell yield in Peyer's patches decreased by 50%–60% with MAdCAM-1 blockade establishing a cause and effect relationship between MAdCAM-1 blockade, GALT cell entry and Peyer's patch atrophy.

Surrogates for Enteral Feeding

Since clinical, technical, and even mechanical limitations often restrict the clinician's ability to enterally feed many patients, surrogates for enteral feeding which maintain the immunologic barrier could provide valuable support in clinical care. Both a specialty nutrient (glutamine) and neuropeptides have been investigated in this regard.

Glutamine

Stress and sepsis up regulates muscle production of glutamine for release into the circulation and delivery to rapidly producing cells and to the immune system. Alverdy and co-workers demonstrated that glutamine supplementation of parenteral nutrition elevated intestinal IgA levels and reduced bacterial translocation [58]. In our models, a 2% glutamine-supplemented parenteral solution maintained relatively normal numbers of T and B cells within the Peyer's patches and lamina propria [59]. IL-4 levels were normalized, but IL-10 levels within gut homogenates remain depressed [60]. Intestinal and respiratory tract IgA levels were lower in glutamine-supplemented animals compared with chow, but levels were increased relative to mice receiving an isonitrogenous, isocaloric unsupplemented parenteral formula. In the functional viral and bacterial models, glutamine supplementation as a mono- or dipeptide improved viral clearance compared with parenterally fed animals, but approximately one-third of animals continued viral shedding, indicating that immunity was not completely normalized [61]. This was confirmed after *Pseudomonas* immunization followed by a *Pseudomonas* challenge [62]. Glutamine-supplementation improved outcome compared with non-supplemented parenteral feeding, but mortality was significantly worse than a chow diet.

Specialty nutrients may hold clinical promise of beneficial effects on mucosal immunity, but to date there is no evidence that immunity can be normalized with a single nutrient. Clinically, one study of bone marrow transplant patients receiving a glutamine supplemented parenteral feeding were reported to have fewer septic episodes and normalization of microbial colonization patterns [63]. However, these data were not confirmed in a larger group of patients undergoing chemotherapy for hematogenous and solid tumor malignancies [64].

Neuropeptides

Each cubic millimeter of intestine is invested with approximately two meters of nervous tissue which constitutes the enteric nervous system (ENS) [65]. The ENS is rich in neuropeptides, such as gastrin-releasing peptide, neurotensin, cholecystokinin and gastrin. Bombesin is found in the skin of frogs and is an analog to gastrin-releasing peptide in humans. Bombesin induces both direct effects on target tissues and also stimulates the release of other neuropeptides, such as cholecystokinin, neurotensin, and gastrin, in a manner similar to gastrin-releasing peptide [66]. Experimentally, bombesin stimulated the production of specific antibodies against *Aeromonas* after bacterial inoculation [67] and reduced bacterial translocation induced by parenteral feeding [68].

In our models, bombesin supplementation of parenteral feedings normalized GALT cell populations in Peyer's patches, the lamina propria, and the intraepithelial space nearly to levels of chow feeding [69]. Administration of this neuropeptide to animals with preexisting TPN-induced GALT atrophy stimulated recovery of GALT cell populations and returned intestinal and respiratory IgA levels to normal without enteral feeding [70]. In the influenza [71] and *Pseudomonas aeruginosa* pneumonia models [70], bombesin supplementation of parenteral feeding maintained normal mucosal defenses at the level of chow-fed animals. This work suggests that it is not the enteral nutrients themselves which determine immunologic awareness and integrity, but the release of neuropeptides in response to feeding which regulates this immunologic function. The effect of neuropeptides on human immunologic integrity has not yet been studied.

Route and Type of Nutrition Affect the Systemic Inflammatory Response

Injury, shock, ischemia/reperfusion, and gastrointestinal tract responses to these insults have been recently related to the development of MODS and respiratory injury [72–74]. Although several links between gut permeability and the inflammatory response have been postulated, recent evidence implicates activation of neutrophils and a neutrophil-mediated endothelial injury with the organ injury. Intestinal lymph has also been studied as an etiologic factor in the inflammatory response that typically targets the lung and liver [75].

The intestine plays an important role in the etiology of damage to these organs. Hypotension results in splanchnic vasoconstriction and intestinal ischemia. During reperfusion, neutrophils (PMNs), primed by the intestinal bed, relocate to other organs. According to the two-hit hypothesis, a second insult, such as tissue injury or hemorrhagic shock, causes the primed cells to react with an augmented response and cause increased endothelial injury within the target organs [72]. PMNs isolated from animals previously injured with ischemia/reperfusion produce much larger amounts of superoxide and other toxic products than unprimed PMNs when stimulated [76]. The gastrointestinal tract is a key factor since PMNs isolated in the mesenteric outflow of the portal vein are primed earlier than cells obtained from the systemic circulation [76].

Recent work relates the gut cytokine changes induced by parenteral feeding to upregulation of PMNs. In addition to effects upon IgA production in GALT cells, IL-4 [77] and IL-10 [78] down regulate the expression of selectins and intracellular adhesion molecule-1 (ICAM-1) on the endothelial surfaces. As IL-4 decreases, expression of ICAM-1 within the small intestine [79] and E-selectin in pulmonary vasculature increases [80]. Simultaneously, a marker of PMN accumulation, myeloperoxidase, significantly increases in the gastrointestinal tract of animals [79]. None of these changes occur in animals fed via the gastrointestinal tract. This accumulation of MPO in the intestine may lead to an augmented inflammatory response to subsequent injury. When parenterally fed animals underwent superior mesenteric artery occlusion and 15 min of gut ischemia, the mortality rate was almost 40% compared with a 5%–10% mortality in chow-, CED-, or IG-TPN-fed mice [81]. IG-TPN-fed animals were compromised to a more severe insult, however, since prolonging the ischemic episode to 30 min increased mortality to approximately 80% in both IG-TPN- and IV-TPN-fed mice but to 10%–20% with CED or chow feedings. Thus, the modicum of protection provided with the defined formula diet given as IG-TPN provides benefit after a short insult but not after prolonged ischemic insult whereas mice receiving a more complex enteral diet are more tolerant to such an insult. Although the absolute number of myeloid cells was not increased in the pulmonary circulation after ischemia and reperfusion, immunofluorescent techniques demonstrated an increase in expression of the activation marker CD18 on the pulmonary myeloid cells of the parenterally fed animals [82].

Summary

In trauma patients at risk of developing subsequent septic complications, enteral feeding significantly lowers sepsis rates and the incidence of multiple organ dysfunction. Route of nutrition exerts more dramatic effects on the gastrointestinal tract than just mucosal atrophy and decreases in absorptive enzymes. Lack of enteral feeding profoundly affects the immunologic status of the specific immunity designed to protect the host from invasion by intraluminal bacteria and toxic products. Changes in cytokines within the lamina propria affect the defense against resident bacteria and influence the vasculature endothelial to response to low-flow states. Although difficult to examine in the human condition, significant circumstantial evidence supports the presence of an immunologic communication between the

gastrointestinal tract and mucosal surfaces throughout the body via a common mucosal immunity. Although this remains a field in its infancy, the ability to manipulate mucosal immunity through nutrition or surrogates of enteral stimulation have potential implications for tipping the immunologic balance in favor of the host.

Acknowledgement

Supported by NIH Grant 5 R01 GM53439.

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