

THE ROLE OF NUTRITION AND DRUGS IN PREVENTING BACTERIAL TRANSLOCATION AND ASSOCIATED SYSTEMIC INFECTIONS

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SUMMARY

The addition of pharmacologic amounts of arginine or glutamine to balanced diets, as well as substitution of Omega-3 for Omega-6 fatty acids, will independently result in improved resistance to infection and/or enhanced gut barrier function to translocation of intestinal microbes. Combinations of these immunonutrients in diets have further beneficial effects. There are now 7 prospective randomised clinical trials of the use of immunonutrient enriched diets in surgical patients. In the aggregate, these have shown that the use of such diets can reduce wound complications and infection by 50-75% and hospital stay by 20%, also providing considerable economic savings.

However, in individuals who have established sepsis with or without multiple system organ failure (MSOF), aggressive feeding may be harmful. In animal studies, high protein diets (20% of energy) and the same dietary formulas that improve resistance to infection in normal animals will often have adverse effects because they may serve as a substrate for bacterial growth in the intestine, support excessive cytokine synthesis by intestinal cells, and increase the amount of bacterial translocation from the intestine.

The role of specific immunonutrients in disease is complex. Their influence in regulation of immune responses via interactions with chemotherapeutic agents are largely unknown but worthy of intensive research.

INTRODUCTION

Microbial translocation can be defined as the passage of both viable and nonviable microbes and microbial products across the intestinal barrier (Alexander et al., 1990). Translocation occurs through M-cells (which is a normal pathway for processing antigenic material from the intestinal lumen), directly through epithelial cells (which increases after systemic injury)

or through ulcerations in the intestine (which may become an important pathway after cellular injury, such as may occur after the administration of cytotoxic drugs or irradiation). Theoretically, translocation could occur through the tight junctions between cells, but this has never been documented *in vivo*. Most microbial translocation is felt to occur through intact enterocytes. After

injury, such as a thermal burn, this may occur very quickly, i.e., within 5 minutes of injury, peak within a few hours, and persist at an increased level for many days. Once the microbes penetrate through the mucosa, they pass through the enterocyte into the lamina propria where they are phagocytised by macrophages or granulocytes or pass without ingestion through the lymphatics or vascular systems to distant tissues and organs where they may cause disease or affect biological processes.

There are numerous studies that support the concept that microbial translocation occurs in man, but perhaps the clearest demonstration is a single study by *Kraus et al.*, (1969). In this study, a healthy human volunteer ingested approximately 10^{12} *C. albicans* orally. He developed positive blood cultures for *Candida albicans* three hours after ingestion accompanied by a funguria and systemic symptoms of sepsis which lasted for 12 hours. In *ex vivo* experiments using human intestine, we have shown that candidal bodies pass directly through enterocytes, similar to the mechanism identified in animals. *Sedman et al* (1994) took samples of intestinal serosa and mesenteric lymph nodes for a culture from 267 consecutive surgical patients subjected to laparotomy. Growth of organisms in these samples was documented in 25 of 242 evaluable patients (10.3%). Postoperative sepsis occurred in 28% of those demonstrating translocation compared to 11.5% of those without translocation ($p < 0.05$). However, there was no influence of the occurrence of translocation on mortality. More recently, our laboratory has used PCR techniques for detecting the presence of microbial DNA in the blood of surgical patients, and this appears to be a more sensitive method for detecting translocation in humans than previous techniques (*Kane et al.*, 1997). The precise relationship

between the incidence of translocation and the development of secondary complications such as multiple organ failure in humans is currently under study.

Translocation is not a new concept. In 1891, *Fraenkel* (1891) suggested that organisms could pass directly through the entire wall of the intestine to cause peritonitis, and various authors following that time supported this concept. However, it was not until 1928 that *Arnold* (1928) was able to demonstrate that viable bacteria could be recovered from the thoracic duct after translocation occurred through the intact intestinal epithelium. His work was extended by *Fisher* (1931) who demonstrated for the first time that yeast could be demonstrated to translocate from the intestine to distant organs. *Flory* (1933) was the first to demonstrate that translocation of bacteria occurred directly through epithelial cells. The importance of translocation was largely ignored, however, for the next two decades until *Fine* and his colleagues (1952) postulated that translocation of bacteria and endotoxin after haemorrhagic shock was a major cause of morbidity and mortality. More recent interest in the process of translocation has shown that there are several diseases clearly associated with translocation in man (*Alexander and Gennari* 1996). These include pneumatosis intestinalis, nonocclusive intestinal gangrene, necrotising enterocolitis, gamma radiation, cytotoxic drugs, the cytokine release syndrome, Crohn's disease, ulcerative colitis, haemorrhagic shock, severe trauma, thermal injury, severe neutropenia, and colon cancer. Experimental studies have shown that translocation is clearly important in the hypermetabolic response to injury in sepsis (*Gianotti et al.*, 1994), the septic state in the absence of a defined focus, and multisystem organ damage, but these relationships are less clear in man.

Table 1: Partial list of conditions that increase microbial translocation as measured by cultures of tissues or organs

A.	Diminished blood flow or O ₂ delivery
	Hypoxia
	Fever
	Vasoactive agents; e.g., platelet activating factor, zymosan, endotoxin
	Thermal injury
	Hypovolemic shock
B.	Improved host defense
	Neutropenia
	Phagocytic dysfunction; e.g., blood transfusion
	Corticosteroids
C.	Increase in luminal microbes
	Antibiotic therapy
	Elemental diets
	Intravenous hyperalimentation
D.	Epithelial Damage
	Irradiation
	Cytotoxic drugs
	Irritants
	Infection - e.g. CMV
	Mucosal disease; - e.g. Crohn's disease
	Bowel manipulation
	Reperfusion injury

IMPAIRMENT OF GUT BARRIER FUNCTION

Most of the studies that have defined conditions that increase the incidence of microbial translocation have been done in animals. For the most part, these studies have measured viable bacteria in tissues without separating the contributions of the intrinsic barrier function of the mucosa from alterations in host defence. There are three factors which will increase the numbers of viable bacteria in the tissues:

1. the intrinsic barrier function of the mucosa,
2. the numbers and types of microbes

3. the ability of the host defence mechanisms to kill bacteria that have already translocated.

The conditions that increase microbial translocation are measured by bacterial culture of tissues as shown in Table 1. In general, these can be divided into four broad categories, those conditions which decrease blood flow or oxygen delivery to the mucosa, impaired host defence, an increase in the number of microbes within the bowel lumen and direct damage to the epithelium.

ENHANCEMENT OF GUT BARRIER FUNCTION

The ability of various agents to impair the passage of microbes through the

epithelial barrier is shown in Table 2. Various analogues of prostaglandins,

Table 2: Enhancement of gut barrier function by drugs

Prostaglandin E analogues
Growth factors
Epidermal growth factor
GM-CSF (but not G-CSF)
bFGF
Bombesin
IGF-1
Mucous enhancing agents - sucralfate
Vasoactive agents
Heparan sulfate
Enalapril

particularly the E-1 series, have been shown to improve barrier function. A part of this may be related to increased mucous production, as well as an increase in the ability to improve blood flow to the mucosa. Most of the factors which accelerate growth of the epithelium will decrease translocation rates. Notable among these is GM-CSF which both decreases the rate of translocation and improves the killing of bacteria that do translocate. Mucous enhancing agents, such as sucralfate, will also decrease translocation, but there may be other effects as well. In addition, vasoactive agents which increase the circulation, such as heparan sulfate, and enalapril will improve the barrier function. The effect of GM-CSF on gut barrier function and survival provides an example of how drugs may have a dual effect (*Gennari et al., 1994*). Normal mice were given a transfusion of 0.1 ml of allogeneic blood to produce a non-specific immunosuppression. Two days later, they were randomly divided to receive 10 µg/kg GM-CSF or saline as a placebo control daily for three days. All animals were then given a gavage of 10^{10} *E. coli* and a 20% burn was inflicted. They were then followed for survival for 10 days. All mortality occurred within 48 hours. The mice

treated with GM-CSF had a 90% survival compared to a 35% survival in the placebo treated controls ($p < 0.05$). In another study, animals were treated similarly except that they were gavaged with $^{111}\text{Indium}$ labelled *E. coli* and sacrificed 4 hours after burn injury. At the time of sacrifice, mesenteric lymph nodes, liver and spleen were harvested for the determination of radionuclide counts and the persistence of live bacteria. Translocation as measured by the amount of radioactivity reaching the tissues was greater in the control animals than animals treated with GM-CSF in all tissues examined (mesenteric lymph nodes, liver, and spleen), indicating that GM-CSF improved the barrier function of the gut, i.e., inhibited the ability of the organisms to translocate. Cultures of each of these tissues also showed that there were fewer colony forming units in the tissues in animals treated with GM-CSF. Furthermore, calculation of the percentage of translocated bacteria that remained alive showed that killing was enhanced in the tissues by GM-CSF. Thus, this drug has a clear cut dual effect in improving survival from gut origin sepsis: improvement of the gut barrier function and improvement of the ability to kill organisms that do translocate.

Table 3: Effects of nutrients on translocation

	Effect on Barrier function	Effect on Bacterial killing	Effect on Survival
Amino Acids			
Arginine	—	↑	↑
Glutamine	↑	↑	↑
Glycine	—	—	—
Lipids			
Omega-3 PUFA	↑	↑	↑
Omega-6 PUFA	—	—	—
MCT	—	—	—
RNA			
	—	—	—

EFFECTS OF NUTRIENTS ON TRANSLOCATION

During the last decade and a half, there have been several nutrients which have been identified that have pharmacologic effects, particularly on the immune system. These include the amino acids arginine and glutamine, ribonucleic acid, and the polyunsaturated fatty acids (Alexander, 1995). Studies in laboratory animals have shown that these nutrients often have an effect on translocation (Table 3). When either gut barrier function is improved or there is an improvement of bacterial killing, there is usually an enhancement of survival of the animal when subjected to a septic challenge. Arginine improves the ability to kill translocated bacteria and this is related to an effect of nitric oxide (Gianotti et al., 1993). However, arginine does not affect the barrier function. In contrast, glutamine improves both the barrier function of the intestine and the ability to clear bacteria (Gianotti et al., 1997a). Lipids of the Omega-3 fatty acid family slightly improved barrier function but have a greater effect on

bacterial killing (Gianotti et al., 1997b). The Omega-6 fatty acids have usually been used for controls, but high doses of the Omega-6 fatty acids will impair bacterial killing and decrease survival of animals. Combinations of the immunonutrients, when given in excess to animals, have an additive or sometimes synergistic effect (Gennari et al., 1995). In particular, fish oil and arginine, fish oil and glutamine, or arginine and glutamine are superior to individual combinations.

When prednisone is given to animals in high doses (10 mg/kg/day) for three days, there is an augmentation of mortality from gut derived sepsis, largely because it impairs the ability to kill bacteria that translocate from the intestine (Gianotti et al., 1996). This susceptibility can be reversed by diets containing either arginine or glutamine, indicating that the immunonutrients may have clinical applicability in patients who are immunosuppressed for a variety of reasons (Gennari et al., 1997).

Table 4: Controlled clinical trials that show a benefit of immunonutrition

Authors	Subjects	Results
Gottschlich et al., JPEN 1990	Burn patients	75% fewer wound infections 67-78% fewer infections overall 31% shorter hospital stay
Daly et al., Surgery 1992	Surgery for UGI & pancreatic malignancies	70% reduced wound complications 22% reduced hospital stay
Moore et al., J. Trauma 1994	Trauma victims	Fewer intra-abdominal abscesses and MOF (0% vs. 11%)
Bower et al., Crit. Care Med. 1995	Surgical ICU patients	Hospital stay reduced by 27%; 40% in septic patients; reduced acquired infections
Kemen et al., J. Crit. Care Nutr. 1995	Major abdominal surgery	After PO5: 53% reduced wound complications 3.6 day reduced hospital stay
Daly et al., Ann. Surg. 1995	Surgery for UGI and pancreatic malignancies	77% reduced wound complications 23% reduced hospital stay
Kudsk et al., Ann. Surg. 1996	Major abdominal trauma	85% fewer major infections 44% shorter hospital stay 27% reduced hospital charges

CLINICAL STUDIES OF IMMUNONUTRIENTS

The physiologic principles that evolved from early animal studies resulted in the development of therapeutic dietary formulations which contained immunonutrients. The first of these was tested in burn patients (*Gottschlich et al., 1990*) and subsequently, two commercial immunonutrient formulas that were outgrowths of our initial formula, Impact® (Sandoz Nutrition, Minneapolis, MN) and Immune-Aid® (McGaw Inc., Irvine, CA), have been tested in

patients. There are now 7 prospective randomised controlled clinical trials (6 of them double blinded) which have shown a striking benefit of immunonutrition in surgical patients at a high risk of infection (Table 4). In aggregate, these trials have shown that aggressive enteral feeding with an immunonutrient formula will reduce hospital stay by approximately 20%, reduce wound complications and infection rates by 50-70% and significantly reduce the cost of care.

ADVERSE EFFECTS OF NUTRITION IN ESTABLISHED SEPSIS

While the benefit of aggressive enteral feeding is well established in both experimental animals and patients at risk for the development of infection, their

benefit in patients with MOFS is questionable at best and may be harmful. In 1989, we developed a model of prolonged peritonitis to study the effect of

nutrition on outcome (*Alexander et al.*, 1989). This model utilised implantation of a bacteria filled mini-osmotic pump into the peritoneal cavity. This pump delivered a mixture of bacteria into the peritoneal cavity over the course of a week, establishing a severe, but prolonged peritonitis, in which the animals could survive for 14-21 days, thus allowing time for nutritional intervention. By feeding via previously placed gastrostomies, it was possible to show that both restriction of caloric intake (*Alexander et al.*, 1989) and provision of protein deficient diets have beneficial effects on survival (*Peck et al.*, 1989). It has since been possible to demonstrate that the high protein diets were associated with an increase in the numbers of bacteria within the intestinal lumen and impairment of the barrier func-

tion of the intestine to microbial translocation (*Nelson et al.*, 1996). The increased translocation in animals fed a high protein diet was associated with increased expression of message for the inflammatory cytokines in the intestine (IL-6) as well as down regulating cytokines (IL-10, TGF- β). Treatment of the animals with peritonitis with GM-CSF, sucralfate or epidermal growth factor improved survival in animals that had ongoing peritonitis that received a high protein diet, whereas the addition of glutamine or arginine to the diet did not improve survival. Together, these studies provide clear evidence that certain nutrients have pharmacologic effects on the immune system which are generally beneficial but may be harmful, depending upon the underlying disease.

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