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Bacterial translocation during enteral and parenteral nutrition

J. Wesley Alexander*

Transplantation Division, Department of Surgery, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0558, USA

Microbial translocation

Microbial translocation can be defined as the passage of both viable and non-viable microbes and microbial products across the intact intestinal barrier. In human subjects, translocation may be important in the hypermetabolic response to injury, a septic state in the absence of a defined focus, the development of distant infections, and multiple organ failure. The load of microbial products, such as endotoxin, and their role in inciting inflammatory and cytokine responses may be as important or more important than the presence of the organisms themselves.

Translocation is not a new phenomenon; it was vaguely suggested by the writings of Fraenkel (1891). Several more papers in the early 1900s suggested that organisms could penetrate through the intact bowel wall (Alexander & Gennari, 1996), but it was really the work of Arnold and his colleagues in 1928–30 (Arnold, 1928, 1930; Arnold & Brody, 1928) that provided a clear indication for the role of translocation in producing systemic illness in animals. Of interest, he was able to show that alkalinization of the intestinal contents significantly increased the incidence of translocation, and that translocation occurred very early, as soon as 5 min after introduction of bacteria into the lumen of the intestine. Flory (1933) was able to show that translocation occurred through a trans-epithelial process, i.e. through the enterocytes, rather than between them. In the 1950s, Jacob Fine and his colleagues (Fine *et al.* 1952) established compelling relationships between translocation and irreversible haemorrhagic shock.

Numerous diseases have been found to be associated with translocation in man (Alexander & Gennari, 1996). These include pneumatosis intestinalis, non-occlusive intestinal gangrene, necrotizing enterocolitis, radiation injury, response to cytotoxic drugs, the cytokine release syndrome, Crohn's disease, ulcerative colitis, haemorrhagic shock, severe trauma, cerebral injury, leukaemia, neutropenia, and colon cancer.

Translocation from the intestine is most commonly detected by measuring the presence of viable bacteria in the tissues. This can reflect not only the integrity of the intrinsic barrier function of the mucosa but also the numbers and types of microbes in the lumen and the ability of the host to kill the bacteria that do translocate. Translocation may occur by three mechanisms: through M cells, which is a normal processing pathway; through epithelial cells, which increases after systemic injury; through ulcerations, which is an important pathway after cellular injury, such as caused by cytotoxic drugs or radiation (Alexander & Gennari, 1996). Translocation through the tight junctions has not been observed. Factors that increase microbial translocation, as measured by bacterial counts in the tissues, include: diminished blood flow or O_2 delivery, e.g. haemorrhagic shock, burn injury, administration of zymosan or endotoxin, mesenteric occlusion, hypoxia or fever; immunosuppression, e.g. high-dose prednisone or blood transfusions; changes in the intestinal microbial density, e.g. following antibiotic therapy or the administration of an elemental diet or total parenteral nutrition (TPN); direct damage to the epithelium, e.g. irradiation, cytotoxic drugs, irritants, cytomegalovirus infection, mucosal disease, bowel manipulation, bowel obstruction or reperfusion injury (Alexander & Gennari, 1996).

Drugs which will decrease bacterial translocation can be divided into several broad categories: those which will improve the microcirculation of the intestine, e.g. enalopril or prostaglandin E_1 or E_2 analogues; those which increase mucus production, e.g. prostaglandins E_1 or E_2 analogues, sucralfate; those which act as growth factors (basic fibrobast growth factor, granulocyte macrophage colony-stimulating factor, epidermal growth factor, bombesin or insulin-like growth factor-1); those which decrease inflammatory damage, e.g. allopurinol, or anti-interleukin 6. Antibiotics themselves do not seem to influence the mucosal barrier, except via alteration of the numbers and types of microbes in the intestinal lumen (Alexander & Gennari, 1996).

The process of Candidal translocation

We have studied the process of the translocation of *Candida albicans* in the jejunum and ileum of guinea-pigs and rats with thermal injury (Alexander *et al.* 1990). Suspensions of *Candida* were introduced into the intestinal lumen either orally or into an isolated Thiry–Vella loop, and the animals were killed at intervals thereafter to study how the *Candida* crossed the intestinal barrier, using both scanning and transmission electron microscopy as well as light microscopy. Within minutes of introduction, the *Candida* began to become attached to the epithelial membrane. Through an apparent enzymic process, the Candidal bodies eroded the microvilli and began to become embedded in this layer. Fragments of the microvilli could be seen attached to the outer

Abbreviations: TEN, total enteral nutrition; TPN, total parenteral nutrition.

***Corresponding author:** Dr J. Wesley Alexander, fax +1 513 558 3580, email Alexanjw@Healthall.com

membrane of the *Candida*. Once the Candidal body reached the outer membrane, it was quickly incorporated and passed through the cytoplasm of the enterocytes without morphological injury to the enterocyte itself. They were then extruded into the lamina propria where they were engulfed by macrophages or remained free, passing into the lumens of the central lacteals or small venules. Some of the *Candida* were incorporated and engulfed by M cells, whereas others entered through micro-ulcerations at the villus tips. Some formed small micro-abscesses in the submucosal area, whereas others were carried to distant tissues, such as the regional lymph nodes where they were found primarily engulfed within macrophages. They could often be found budding, evidence of invasive growth. Studies with both fluorescent-labelled endotoxin and intact bacteria showed that these translocated in a similar manner, although it was more difficult to study.

Translocation in man

That translocation occurs in man is without controversy. This is perhaps most clearly demonstrated by a study in which a human volunteer was administered orally approximately 10^{12} *Candida albicans* (Krause *et al.* 1969). Positive blood cultures for *Candida* occurred at between 3 and 6 h, and funguria occurred at between 2·75 and 3·25 h. Symptoms lasted for 2–9 h despite vigorous treatment. Sedman *et al.* (1994) studied the prevalence of gut translocation in patients before gastrointestinal surgery. Samples of the intestinal mucosa and mesenteric lymph nodes were taken in 257 consecutive surgical patients. Translocation occurred in 10·3 % of the evaluable patients. Post-operative sepsis was significantly more common in those patients with translocation (28 %) compared with those that had no translocation (11·5 %).

Early enteral feeding reduces translocation

Previous studies in our laboratory had shown that early enteral feeding reduced the hypermetabolic response in burned guinea-pigs (Mochizuki *et al.* 1984). A subsequent study was done to determine whether or not this was related to translocation from the intestinal tract (Gianotti *et al.* 1994). Guinea-pigs were provided with a gastrostomy, and 2 weeks later a 40 % total body surface area thermal burn was inflicted. They were then divided into two groups. One received a complete enteral diet by gastrostomy, whereas the other received an equal volume of Ringer's lactate solution. Both treatments started immediately post-burn and continued for 48 h. *Escherichia coli* (10^{10}, labelled with ^{14}C) were then administered through the gastrostomy tube. Resting metabolic expenditure was determined 4 h after administration of *E. coli*. Radioactivity in the portal blood and ileal mucosa and also plasma cortisol levels, urinary vanalyl mandelic acid and intestinal mucosa weight were determined 5 h after administration of *E. coli*. The mucosal weight was significantly lower in those animals receiving Ringer's lactate solution when compared with those receiving enteral nutrition. There was a strong inverse correlation between the amount of radioactive material within the mucosa and the mucosal weight for individual animals, indicating that as the mucosa atrophied, translocation increased. There was also a strong inverse correlation between mucosal weight and the amount of radioactivity in the portal blood. Not surprisingly, the amount of radioactivity in the mucosa and blood correlated significantly, and in starved animals these tissues had greater amounts of radioactivity than in the fed animals. The amount of radioactivity in the blood and the mucosa correlated significantly with the urinary vanalyl mandelic acid and cortisol, and radioactivity in the blood and tissues also directly correlated with the resting energy expenditure. These studies gave a clear indication that translocation of enteric organisms was an important trigger for the hypermetabolic response following burn injury.

These animal studies are further supported by the findings of a clinical study by Chiarelli (1990). These investigators compared early enteral nutrition with delayed enteral nutrition in burn patients. Twenty patients with burn injuries averaging 38 % were divided into two groups. The early-fed group received nutrition starting an average of 4·4 (SE 0·5) h post-burn, whereas the delayed-fed group received enteral nutrition beginning 57·7 (SE 2·6) h post-burn. There were five positive blood cultures from three patients in the early-fed group compared with thirty-three from seven patients of the delayed-fed group. Early feeding decreased the duration of hospitalization from 89 to 69 d. As in the animal studies, glucagon was normalized in the patients fed early, while it remained elevated in patients for whom feeding was delayed. Early enteral feeding also has been found to be beneficial in a variety of other conditions, such as head injury. Grahm *et al.* (1989) did a randomized study in patients with head injuries in which the experimental group was fed with a naso-jejunal tube within 36 h and the control group was fed by gastric tube when bowel sounds began spontaneously. Infection occurred in fourteen patients in the control group compared with only three patients in the experimental group, and the period spent in the Intensive Care Unit decreased from 10 to 7 d. Studies by Inoue *et al.* (1989) showed that a single bolus of food given either 3 or 12 h post-burn could decrease the incidence of translocation from the intestine. Another study in burned guinea-pigs (Inoue *et al.* 1991) showed that early enteral feeding would reduce the incidence of both translocation and mortality in burned guinea-pigs that were administered *Candida albicans* orally. Thus, in injured subjects, early enteral nutrition will decrease translocation, improve survival, and decrease the hypermetabolic response which occurs as a consequence of translocation.

Total parenteral nutrition *v.* total enteral nutrition and the role of translocation

In a large multicentre study, Busby (1991) evaluated the role of TPN *v.* no TPN in patients before surgery. TPN was given pre- and post-operatively for 10 d. There was no difference in the total complication rate in the two groups of patients, but the 90 d mortality rate was slightly higher in the TPN group (13·4 % *v.* 10·5 %) and infectious complications were significantly higher in the TPN group (14·1 % *v.* 6·4 %; $P = 0{\cdot}01$). Sandstrom *et al.* (1993) randomized 300 patients to receive either TPN or intravenous glucose post-operatively for up to 15 d. There was no difference in outcome when analysed by intent to treat, but the patients

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who received only glucose for 15 d had a higher mortality. These studies indicated that in many surgical patients, TPN not only lacked beneficial effect, but might have harmful effects. Alverdy *et al.* (1988) suggested that one of the harmful effects from TPN might be that it promotes bacterial translocation from the gut. In an animal study, he showed that spontaneous translocation from the intestine exceeded 60 % in animals that received TPN. If the same animals were fed the TPN solution enterally, the incidence of translocation was reduced by half. Control animals receiving regular chow did not show evidence of translocation. This is consistent with numerous subsequent studies which showed that intravenous nutrition increases translocation and also that elemental-type diets are associated with increased translocation, although not as much as diets given by the total parenteral route. Pappo *et al.* (1994) fed rats for 7 d before oral administration of $1{\cdot}5 \times 10^{10}$ *Candida*. Animals receiving a control enteral diet had a low incidence of translocation to the kidneys, but animals receiving TPN with or without antibiotics had significantly higher rates of translocation to blood, lymph nodes and kidneys.

Comparisons of the clinical benefit of total enteral nutrition (TEN) *v.* TPN have been done by several investigators. One of the first of these was by Moore *et al.* (1989). They randomized trauma patients to receive either TEN or TPN beginning 12 h post-injury. The patients receiving TEN had only a 9 % incidence of infections overall compared with a 37 % incidence in the patients with TPN. Perhaps the most extensive study was that of Kudsk *et al.* (1992), also in seriously injured patients. His patients had both a central line and a feeding jejunostomy tube placed at the time of laparotomy for blunt or penetrating abdominal trauma. Patients were then divided randomly to receive either TEN or TPN. The incidence of pneumonia, line sepsis, and intra-abdominal abscess were all significantly less in those receiving TEN, and the benefit was greatest in patients with larger injuries.

These studies are all consistent with the concept that TPN increases translocation compared with TEN, and its associated with a greater incidence of septic complications.

Selected enteral nutrients decrease translocation and improve survival

Arginine

The effect of arginine on survival rates and host defence mechanisms was studied using two clinically-relevant models of infection that included transfusion-induced immunosuppression (Gianotti *et al.* 1993). *Balb/c* mice were fed for 10 d on a defined AIN-76A diet, an AIN-76A diet supplemented with arginine (2 % dietary energy), an AIN-76A diet supplemented with glycine (4 % dietary energy), or standard laboratory chow. In most experiments, the mice were then transfused with allogeneic blood and allowed to feed for an additional 5 d before undergoing either caecal ligation and puncture or oral administration of 10^{10} *E. coli* and a 20 % burn injury. Additional animals receiving the arginine-supplemented diet were treated with the NO inhibitor Nω-nitro-L-arginine before oral administration of *E. coli* and burn injury. The effect of these diets and Nω-nitro-L-arginine on the degree of translocation of ^{14}C-radiolabelled *E. coli* from the intestine and the ability of the host to kill translocated organisms was also investigated. Mice were fed and received the transfusion, oral administration of *E. coli*, and burn injury as described previously. Mesenteric lymph nodes, liver and spleen were harvested 4 h post-burn.

Survival after caecal ligation and puncture was 56 % in the arginine-supplemented group *v.* 28 % in the AIN-76A group and 20 % in the chow group ($P < 0{\cdot}02$). After oral administration of *E. coli* and burn injury, survival was 100 % in the arginine-supplemented group *v.* 50 % in both the glycine-supplemented and chow-fed groups and 35 % in the AIN-76A-fed group ($P < 0{\cdot}01$). In animals receiving the arginine-supplemented diet, treatment with Nω-nitro-L-arginine decreased survival from 95 % to 30·5 % ($P < 0{\cdot}0001$). Greater translocation, as measured by radioactivity, was observed to the mesenteric lymph nodes of the AIN-76A-fed group. However, there was no difference in translocation to the liver and spleen related to dietary group. Quantitative colony counts and the calculated percentage of remaining viable bacteria showed that the ability to kill translocated organisms was significantly enhanced in animals receiving arginine.

Glutamine

Similar studies were done with glutamine using regular rat chow, an AIN-76A diet and an AIN-76A diet supplemented with glycine or glutamine (2 % dietary energy; Gianotti *et al.* 1995). Animals were fed for 2 weeks before a 20 % burn injury and oral administration of ^{14}C-labelled bacteria. A transfusion was given to provide additional mild immunosuppression. As in the experiments with arginine, the intestine was the only source of bacteria to cause infection. All the animals prefed with glutamine survived compared with only 20–40 % of the animals in the control groups. When the spleen, liver and mesenteric lymph nodes were removed and measured for radioactivity, it was found that the animals receiving glutamine had significantly less translocation to these tissues, as well as fewer colony counts, indicating that this amino acid significantly reduced translocation from the intestine and also improved the killing of bacteria.

Another experiment was done in which prednisone (10 mg/kg) was given for 4 d before challenge by oral administration of 10^9 *E. coli* and a 20 % burn injury (Gennari & Alexander, 1997). Controls received diets enriched with glycine. Survival for the groups were as follows: arginine 64 % *v.* glycine 24 % ($P = 0{\cdot}004$); glutamine 55 % *v.* glycine 20 % ($P = 0{\cdot}015$). These studies indicated that single dietary amino acids could improve survival even in immunosuppressed animals.

n-3 Fatty acids

The effect of dietary lipids on bacterial translocation, killing of translocated organisms and host survival was studied in a burned animal model (Gianotti *et al.* 1996). *Balb/c* mice were fed on one of the three experimental AIN-76A diets (containing 15 % dietary energy from fish oil, safflower oil or a 50 : 50 mixture i.e. each oil provided 7·5 % dietary energy), AIN-76A without added lipids, or a non-purified

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Table 1. Effects of selected nutrients on translocation of bacteria

	Effect on barrier function	Effect on bacterial killing	Effect on survival
Arginine	–	↑	↑
Glutamine	↑	↑	↑
RNA*	–	–	–
n-3 Fatty acids	↑	↑	↑
n-6 Fatty acids	–	–	–
Glycine	–	–	–
Dietary fibre (insoluble)	ND	ND	↑
Arginine + glutamine	↑	↑	↑
Fish oil + arginine	↑	↑	↑
Fish oil + glutamine	↑	↑	↑

ND, not determined; ↑, effect was increased; –, no effect.
* Considered a nutrient in that it occurs in natural foodstuffs and provides synthetic building blocks.

stock diet. All animals were transfused on day 10. On day 15, the animals were orally administered 10^{10} *E. coli* labelled with ^{14}C and given a 20 % burn injury. Survival was 84 % in the fish oil-fed group *v.* 36 % in the safflower oil- and 50 : 50 mixture-fed groups, and 25 % and 20 % in the two control groups ($P < 0{\cdot}0001$). The numbers of viable translocating bacteria were reduced in all tested organs in the fish oil-fed groups compared with the other groups.

Combinations of dietary additives

Experiments were done to determine whether combinations of individual nutrients would have an additive effect in improving survival from translocation in a burned animal model (Gennari *et al.* 1995). This model used a 30 % burn injury rather than a 20 % burn injury. Diets containing arginine plus fish oil or fish oil plus glutamine were associated with significantly higher survival than control diets. Interestingly, when medium-chain triacylglycerols were added to the diets with either arginine or glycine, no beneficial effect was obtained. The effects of various enteral nutrients on translocation are summarized in Table 1.

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