Bacterial translocation: the influence of dietary variables

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Abstract

Transmucosal passage of bacteria in critically ill patients may lead to a significant incidence of systemic sepsis. This has attracted much clinical interest, as it has been shown that malnutrition in itself, impairs various aspects of barrier function. Bacterial translocation is increased in animal models where nutrients are given by the parenteral route, while enteral feeding reverses this. Translocation is also considerably increased in response to a non-lethal endotoxin challenge, if there is pre-existing protein energy malnutrition. Similar results have been obtained where the insult is caused by the inflammatory agent, zymosan. Dietary fibre reduces the deleterious effects of either agent on translocation, although the type of fibre is important. Bulk forming but non-fermentable fibres are more effective than easily fermentable types (for example, pectin). Glutamine was not effective in preventing elemental diet induced bacterial translocation. Thus, although fermentable fibre and glutamine have positive effects on mucosal mass, they do not affect translocation. Enteral nutrition thus seems to be superior to parenteral nutrition in maintaining the functional barrier of the gut. A clearer understanding of the physiology of these effects may lead to use of specifically modified enteral diets in the critically ill patient.

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One of the key functions of the intestine is to prevent luminal bacteria and endotoxins from reaching systemic organs and tissues. Failure of intestinal barrier function resulting in the systemic spread of bacteria from the gut to systemic organs has been termed bacterial translocation. The phenomenon of bacterial translocation has assumed increasing clinical importance, as loss of intestinal barrier function and the subsequent translocation of bacteria or endotoxin, or both have been implicated in the development of systemic infection or multiple organ failure in selected patients.^{1,2} Our initial interest in investigating the role of intestinal barrier failure as a cause of systemic infection was based on the finding that life threatening infections with gut associated bacteria in which no infectious focus could be found commonly occurred in trauma victims, burn and intensive care unit patients, and patients developing multiple organ failure. Therefore, the goals of our

experimental studies have been the investigation of the basic mechanisms by which bacteria contained within the gastrointestinal tract can translocate to cause systemic infections.

Our earlier studies show that although bacterial translocation can be induced in a variety of animal models, one or more of three basic pathophysiological conditions are necessary for bacterial translocation to occur.^{1,2} These are: (1) disruption of the ecological balance of the normal indigenous microflora, resulting in bacterial overgrowth with Gram positive enteric bacilli; (2) impaired host immune defences; and (3) physical loss of the mucosal barrier. These same conditions are commonly seen in the critically ill or injured patient at risk of developing enteric bacteraemia or multiple organ failure. For example, these patients have often experienced considerable blood loss or a hypotensive episode leading to mucosal injury; they are usually immunocompromised and the drug, treatment, or dietary regimens they receive may disrupt the normal ecology of the gut flora, resulting in subsequent overgrowth by certain members of the indigenous microflora or colonisation with exogenous pathogens.

As starvation and protein malnutrition have been reported to impair host immune and antibacterial defences,³ disrupt the normal ecology of the gut microflora⁴ and lead to mucosal atrophy,⁵ there are many reasons to believe that nutritional variables are important modulators of gut barrier function and bacterial translocation. For example, Lowry⁶ showed that human volunteers given a total parenteral nutrition diet have a greater splanchnic and systemic cytokine and metabolic response to parenteral endotoxin than enterally fed volunteers. This study and the results of other human and animal studies⁷⁻¹¹ show that the route by which patients are fed may influence the immunoinflammatory and metabolic response to injury,⁶⁻⁸ affect the incidence of infectious complications,⁹ and modulate clinical outcome.¹⁰⁻¹² One hypothesis to explain the finding that enteral feeding seems clinically superior to parenteral feeding is that parenteral feeding predisposes to an exaggerated cytokine response as a result of the loss of intestinal barrier function.^{6,13,14} As will be discussed, the composition of the diet as well as the route by which it is given have profound effects on intestinal morphology and function as well as on the incidence and susceptibility to systemic infection and the development of hypermetabolism.¹⁻² Thus, because nutritional deficiencies are common in

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Figure 1: (A) Distal ileum from control mouse and (B) mice maintained on a protein deficient diet for seven days, (C) 14 days, or (D) 21 days. Progressive atrophy of villous height and crypt depth is seen although no gaps in the epithelium are seen (original magnification $\times 190$).

severely traumatised or critically ill surgical patients, nutritionally induced changes in intestinal barrier function seem to be of clinical importance.

Experimental results

Most of our work investigating potential relations between nutrition and bacterial translocation has focused on whether the composition or route of administration of specific diets promotes bacterial translocation and, if so, if diet induced bacterial translocation is associated with changes in host immune defences, intestinal morphology, or disruption of the normal intestinal microecology, or all three.

Effect of protein malnutrition on bacterial translocation, intestinal morphology, and host immune defences

Because nutritional deficiencies are comparatively common in patients in hospital and may impair anti-bacterial host defences and disrupt the normal ecological balance of the intestinal microflora, experiments were performed to determine the influence of prolonged protein malnutrition in promoting bacterial translocation.¹⁵ In this study, mice received either normal chow or a solid, specially formulated protein deficient diet from Teklad Laboratories (Madison, Wisconsin). The two diets had an equal energy value with

the Teklad diet containing 20% fat, 67% carbohydrate, and 0.03% protein by weight. They also contained fibre, trace elements, minerals, and vitamins. The animals were killed and their organs cultured for translocated bacteria after 7, 14, or 21 days. Animals fed the Teklad diet for 21 days had lost about 30% of their original body weight. Although the Teklad fed mice had lost weight, had their intestinal mucosal height reduced by greater than 75%, and had the normal ecology of their gut microflora mildly disrupted, bacterial translocation did not occur (Fig 1).

In the second half of the study, mice were challenged with a non-lethal dose of endotoxin (intraperitoneal) after receiving 0, 7, 14, or 21 days of the Teklad diet to discover if endotoxin would act synergistically with protein malnutrition to promote bacterial translocation. The combination of protein malnutrition plus endotoxaemia was associated with a higher incidence of bacterial translocation to the systemic organs than was seen in the normally nourished mice ($p > 0.01$). Additionally, the mice fed the Teklad diet were more susceptible to the lethal effects of endotoxin. The endotoxin induced death rates were directly related to the length of time the mice were fed the Teklad diet and ranged from 7% on day 0 to 66% on day 21 of the Teklad diet ($r^2 = 0.89$; $p < 0.05$).

In a subsequent study,¹⁶ we sought to discover if there was a correlation between the extent of histological damage and the magnitude of bacterial translocation in Teklad diet fed mice challenged with endotoxin induced mucosal injury. We compared the results of normally nourished mice with those of mice fed the Teklad diet for 14 or 21 days. There was no correlation between the gross appearance of the epithelial mucosal barrier and the extent of endotoxin induced bacterial translocation. This finding suggests that the synergistic effects of endotoxin with protein malnutrition on bacterial translocation do not correlate with the histological appearance of the gut mucosal barrier.

To see if our findings were unique to endotoxin, we repeated the series of experiments described above with the inflammatory agent, zymosan.¹⁷ Zymosan was chosen for study because it induces a systemic inflammatory state, which had been implicated in the pathogenesis of adult respiratory distress syndrome and multiple organ failure. The results of this study, in so far as bacterial translocation and death were concerned, were almost identical to that found after endotoxin challenge. That is, the protein malnourished mice were more susceptible to zymosan induced bacterial translocation than the normally nourished mice, and the extent of bacterial translocation and death correlated with the length of time the mice were fed the Teklad diet (Table I). One important difference between the response of the protein malnourished mice to zymosan in contrast with endotoxin was that the intestinal mucosa did not become 'resistant' to zymosan induced mucosal injury after 14 or 21 days of the Teklad

TABLE 1 Protein malnutrition increases susceptibility to lethal effects of endotoxin or zymosan

Group	Death (%)		% Weight loss (mean)	
	Endotoxin	Zymosan	Endotoxin	Zymosan
Normally nourished	7	0	0	0
PM (7 days)	15	0	14	11
PM (14 days)	34	20	23	24
PM (21 days)	66	80	31	36

PM=protein malnourished.

TABLE II Oral and intravenous total parenteral nutrition induced bacterial translocation is reversed with dietary fibre (cellulose)

Group	Incidence of bacterial translocation (%)	Ileal mucosal protein mg/cm ($\times 10^2$)	Caecal log ₁₀ Gram negative enterics (CFU/g)
Chow	0	88 (18)*	6.3 (0.4)*
IV-TPN	60*	31 (19)	8.4 (0.6)
IV-TPN+fibre	0	43 (7)	7.1 (1.0)
Oral TPN	60*	50 (16)	7.7 (0.6)
Oral TPN+fibre	8	47 (6)	8.1 (0.5)

*p<0.01 v all other groups analysis of variance. Data are mean (SEM). IV-TPN=intravenous total parenteral nutrition.

diet, as was seen after endotoxin challenge. In fact, the extent of mucosal injury became slightly greater the longer the mice were protein malnourished before zymosan challenge. The reason for this differential effect of endotoxin v zymosan on the intestinal mucosa is unknown. The two important conclusions of these studies were that Teklad diet induced protein malnutrition did not promote bacterial translocation (though it led to profound mucosal atrophy), but that protein malnourished animals had a higher incidence of systemic spread of translocating bacteria and were more susceptible to the lethal effects of endotoxin or zymosan.

Effect of intravenous or oral total parenteral nutrition on bacterial translocation

The initial study evaluating the route of nutrient administration on bacterial translocation was by Alverdy *et al*,¹³ who found that

Figure 2: Elemental diet (ED) induced bacterial translocation is reversed by cellulose and kaolin (p.0.05) but not citrus pectin or glutamine.

the enteral or parenteral administration of a total parenteral nutrition solution (28% glucose, 4.25% amino acids, and 0% fat) to rats was associated with bacterial translocation. The results of this study were somewhat surprising as our previous work had shown that protein malnutrition did not induce bacterial translocation despite weight loss.¹⁵ Yet, in the Alverdy study, bacterial translocation occurred even though the diet contained adequate amounts of protein and the animals gained weight. One important difference between these two studies was that in our previous work on protein malnutrition, the mice received a pelleted diet containing fibre. In the Alverdy study, however, the rats were fed exclusively through the intravenous route or received a fully absorbable liquid diet. Because dietary fibre is important in maintaining the normal ecological balance of the gut microflora¹⁸ and because the bacterial fermentation end products of fibre are trophic for intestinal epithelial cells,¹⁹ the presence or absence of dietary fibre may have been an important variable in determining the presence or absence of bacterial translocation. Therefore, we tested the hypothesis that elemental intravenous total parenteral nutrition or oral total parenteral nutrition diet induced bacterial translocation could be reduced by dietary fibre.¹⁴

The results of our study, based on Alverdy's model verified that both an intravenous and an orally given total parenteral nutrition solution will induce bacterial translocation in rats and that both intravenous and oral total parenteral nutrition induced bacterial translocation can be prevented by the oral ingestion of a dietary fibre (cellulose) (Table II). Interestingly, although dietary fibre prevented bacterial translocation, it did not reverse the total parenteral nutrition induced decrease (p<0.01 v chow fed controls) in jejunal or ileal mucosal protein content (Table II).

Our next study was carried out to answer two questions²⁰: whether oral total parenteral nutrition solution (28% glucose, 4.25% amino acids, 0% fat) will cause bacterial translocation in mice as well as rats, and, if so, which properties (fermentability, bulk, or both) of dietary fibre are important in preventing oral total parenteral nutrition induced bacterial translocation. The results of this study reported that oral total parenteral nutrition will induce bacterial translocation in rats and that the bulk forming properties of dietary fibre are more important than fermentability in preventing bacterial translocation. This second conclusion is based on the fact that cellulose or kaolin (bulk forming agents) but not citrus pectin (a fully fermentable, non-residue fibre) reduced the incidence of bacterial translocation (Fig 2). Similar results have subsequently been seen in rats, where the incidence of oral total parenteral nutrition induced bacterial translocation was reduced in the kaolin fed but not in the citrus pectin fed animals (unpublished data).

Because none of the diets contained glutamine and because glutamine is one of the

important fuels of enterocytes,²¹ the lack of dietary glutamine may have contributed to impaired intestinal barrier function. This possibility is strengthened by experimental studies showing that glutamine protects the intestinal mucosa from a variety of injurious agents.⁸ The argument is also strengthened by a recent study showing that total parenteral nutrition induced bacterial translocation can be prevented by supplementing the total parenteral nutrition solution with glutamine.²² Thus, we tested whether glutamine (as 30% of amino acids) would prevent oral total parenteral nutrition induced bacterial translocation.²³ As the incidence of bacterial translocation was not different between the glutamine supplemented (88%) and non-supplemented (75%) oral total parenteral nutrition diets, it seems that glutamine does not prevent elemental diet induced bacterial translocation (Fig 2).

The mechanism by which dietary fibre protects against total parenteral nutrition induced bacterial translocation could not be determined from the results of our studies, although the intestinal mucosa of the fibre fed animals looked more normal.¹⁴ Fibre exerts a trophic effect, however, on the intestinal mucosa^{19,24} and is necessary for the maintenance of normal mucosal turnover and intestinal cytokinetics.²⁵ The fact that diets lacking fibre may lead to loss of functional integrity of the epithelium is not completely surprising because the addition of dietary fibre to a low residue oral diet exerts a favourable effect on the preservation of intestinal structure and function.²⁶ Whether fibre exerts this protective effect by directly stimulating the intestinal mucosa or by inducing the release of trophic gut hormones or other factors is not clear. If fibre protected against parenteral or oral elemental diet induced bacterial translocation by stimulating trophic hormones, then inhibition of hormone secretion should negate its protective effects. Conversely, stimulation of the appropriate intestinal hormones, in the absence of fibre, should protect against diet induced bacterial translocation. We tested this hypothesis using sandostatin to inhibit and bombesin to stimulate intestinal hormone secretion.^{27,28} The results of this study reported that bombesin reduced diet induced bacterial translocation from 75 to 20% ($p < 0.05$), while sandostatin abrogated the protective effects of fibre in oral total parenteral nutrition fed rats (unpublished data).

The results of these studies can be summarised as follows: (1) dietary fibre but not glutamine protects against total parenteral nutrition induced mucosal changes and bacterial translocation; (2) cellulose is effective in preventing intravenous or oral total parenteral nutrition induced bacterial translocation, even though it does not prevent total parenteral nutrition induced loss of mucosal mass; (3) the bulk forming properties of fibre are more important than fermentability in preventing bacterial translocation; (4) the protective effects of cellulose fibre seem to be

related to its ability to stimulate trophic gut hormones.

Discussion

One important problem with many of the published studies examining the intestinal mucosal arm of the relation between nutritional modulation and intestinal barrier function is that these studies have not measured barrier function in itself.^{8,22,29-31} Instead, they have measured nutritionally induced changes in selected morphological or biochemical parameters (such as intestinal protein content, villus height or intestinal weight), assuming that a decrease in one or more of these parameters of mucosal atrophy would correlate with impaired intestinal barrier function. This assumption equating mucosal atrophy with impaired barrier function is too simplistic, because our studies clearly show that there is no direct correlation between the development of intestinal atrophy and bacterial translocation.¹⁵⁻¹⁷ Furthermore, although fibre reversed total parenteral nutrition induced bacterial translocation, fibre given orally was not associated with the reversal of total parenteral nutrition induced changes in intestinal morphology, weight, or protein content.¹⁴ Thus, the presence of mucosal atrophy does not mean that intestinal permeability to bacteria or endotoxin, or both will be increased and, conversely, the prevention of mucosal atrophy is not synonymous with the prevention of bacterial translocation.

As presented in the results section, we have reported that bacterial translocation occurs in rats or mice fed a total parenteral nutrition solution containing 4.5% amino acids, 28% glucose, electrolytes, and trace elements (300 kcal/kg/day) intravenously or enterally and that bulk forming fibres but not glutamine prevent this diet induced loss of intestinal barrier failure.^{14,20} Although there seems to be little doubt that glutamine promotes small intestinal growth and limits intestinal atrophy, glutamine's ability to prevent diet induced bacterial translocation is less clear. While Burke, in Alverdy's laboratory, reported that intravenous total parenteral nutrition induced bacterial translocation can be prevented by supplementing the total parenteral nutrition solution with glutamine (2% wt/vol),²² Barber *et al* could not reverse bacterial translocation promoted by an oral defined diet (Criticare HN) merely by glutamine supplementation (2% wt/vol). The glutamine supplemented diet given by Barber *et al* largely prevented loss of intestinal mass³² but did not prevent translocation. The ability of glutamine supplementation to prevent translocation in other models is also variable. Souba *et al* reported that oral glutamine (3% wt/vol) prevented radiation induced bacterial translocation,³³ while Wells *et al* found that glutamine supplementation (4% wt/vol) of an oral diet did not prevent endotoxin induced or antibiotic induced bacterial translocation.³⁴ Thus, although glutamine supports mucosal growth, there is disagreement on whether

glutamine will bolster intestinal barrier function.

In summary, enteral nutrition seems to be superior to parenteral nutrition. This conclusion is based on clinical studies showing that high protein enteral diets improve systemic immunity, reduce the incidence of important infections, and improve survival.^{9 10 12} Further, enteral nutrition bolsters anti-bacterial host defences,¹¹ blunts the hypermetabolic response to injury,⁷ maintains mucosal mass and intestinal barrier function, and limits or prevents disruption of the normal gut microflora.^{2 8} ¹⁴ As more information on the physiology of the intestinal barrier is generated, it is probable that our therapeutic regimens will include the use of specific agents to maintain or bolster intestinal mucosal structure and function. Undoubtedly this therapeutic regimen will include immediate enteral feeding,³⁵ specific nutrients, and mucosal trophic factors, such as fibres, trophic gut hormones,³⁶ and growth factors.³⁷

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