Interactions Mediating Bacterial Translocation in the Immature Intestine1,2

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ABSTRACT Systemic disease caused by transmucosal passage of enterovirulent bacteria and toxins from the gut lumen into the mesenteric lymph nodes (MLN) is reviewed, with particular concern for bacterial interactions in the developing gut of premature newborns. Anaerobic bacteria are rarely observed to translocate to the MLN. Bifidobacterial strains have been tested for their abilities to adhere to enterocyte-like Caco-2 cells in culture. We have investigated the inhibitory effect of adherent human bifidobacterial strains against colonization by a number of diarrheagenic bacteria (Escherichia coli 0157; Salmonella typhimurium) and viruses (murine and rhesus rotavirus), in various in vitro and in vivo models. The phagocytic cell (macrophage) may be a key factor in bacterial translocation (BT). Human breast milk contains abundant bioactive substances (immunologic, nutritional) that provide protective effects through inhibition of bacterial overgrowth and BT. New biotherapeutic therapies that stimulate beneficial anaerobic microflora (Lactobacillus, Bifidobacterium) are promising avenues of research to combat BT in disease treatment. J. Nutr. 130: 432S–436S, 2000.

KEY WORDS: • bacterial translocation • Bifidobacterium • Lactobacillus • phagocyte • secretory immunoglobulin A

The intestinal mucosa appears to function as a defensive barrier, limiting microorganisms present in the intestinal lumen from colonizing enterocytes. Predisposing factors in the pathogenesis of systemic infections such as trauma, immunosuppression and prematurity promote gut permeability in which mucosal barrier function is impaired. Under these conditions, indigenous bacteria, viruses and toxins, normally confined within the gastrointestinal tract, may reach systemic organs and tissues, a process termed bacterial translocation (BT)1 (Katayama et al. 1997). The definition refers more broadly to the transmucosal passage of viable and nonviable microbes and their by-products (endotoxin) across an intact intestinal barrier (Alexander et al. 1990). Among the conditions that have been reported to influence the rate of BT, the factors that have gained the most attention are the immune status of the host, overgrowth of bacterial pathogens and gut permeability of the newborn.

Of the ~500 species of normal intestinal microflora, relatively few have been shown to translocate to the mesenteric lymph nodes (MLN) and other organs with any frequency. Although anaerobic bacteria may outnumber aerobes by 10:1 or 1000:1, anaerobes are extremely challenging to induce to undergo BT experimentally. The mechanism responsible for anaerobes’ low rates of BT is unclear, although their adhesive properties to epithelial cells have been documented (Duffy et al. 1994, Wells et al. 1987). One possibility may be that anaerobes attach to epithelial cells but are either relatively resistant to phagocytosis or more susceptible to intracellular killing by macrophages. Because anaerobes are rarely associated with pathologic conditions and require special processing techniques, the low rates of detection reported in the literature may be underestimations.

Specific anaerobes, classified as lactic acid bacteria (Lactobacillus acidophilus, Bifidobacterium) may play a protective role in BT via immunologic mechanisms promoted by fermentation processes that metabolize varying quantities of lactic, acetic and formic acids; vitamin synthesis; and production of antimicrobial bacteriocins and fatty acids (Salminen et al. 1996). Potential benefits of anaerobic bacterial growth include the following: 1) strengthening of the gut mucosal barrier function; 2) balance of microbial ecology; 3) adherence to intestinal mucosa, impeding invasive pathogens; 4) metabolism of dietary proteins and enzymes by the intestinal microflora; and 5) resilience of the epithelium to gut mucosal permeability.
Bacterial antigens that have been found to translocate most readily are classified as facultative intracellular pathogens (Table 1). Classic examples are Salmonella typhimurium and Listeria monocytogenes. It is speculated that the transit route for BT is similar for facultative intracellular organisms as for pathogens, yet enteropathogens survive phagocytosis and endocytosis.

Enterovirulent gram-negative aerobes bind to the mucous gel at the gut mucosal surface better than nonpathogenic anaerobic bacteria (Lactobacilla, Bifidobacterium) (Beachey 1981, Duffy et al. 1994). Animal models in Sprague-Dawley rats (Alverdy et al. 1992) and Balb/c mice (Duffy et al. 1997) suggest that bacterial adherence by enterovirulent organisms damages the ileal-cecal mucosa preferentially and increases the permeability of the ileum to BT into the MLN.

Protective effect of malnutrition on BT

Deitch et al. (1987) reported that colonization with exogenous Escherichia coli did not occur consistently in normally nourished or protein-malnourished (PM) rats unless they were challenged with endotoxin. Levels of gram-negative enterics in the cecum of endotoxin-treated rats increased significantly over time compared with nonchallenged rats. The effect of PM against bacterial overgrowth–induced BT was also fully reversed by endotoxin challenge. The discovery that protein malnourishment alone does not significantly trigger BT, but that endotoxin exaggerates enteric BT in malnourished rats, implies that ingested antigens play a key role in physiologic and pathologic models of disease.

The potentially protective effect of PM against bacterial overgrowth–induced models of BT highlights the importance of controlling endotoxin-producing bacterial populations in the intestinal lumen, particularly at the mucosal surface. Protein malnutrition was originally thought to play a more significant role in earlier inflammation-induced models of BT (Florey 1933). The divergent findings underline the complexity of the mechanisms of action of BT in humans and other mammalian species.

Intestinal permeability in newborns and BT

Various animal models (rabbits, rats and mice) have been used to postulate mechanisms for transmucosal passage of antigens in premature newborns. The Ussing chamber has been a useful in vitro technique to simulate the occurrence of transmucosal passage across the intestine of newborn pigs in contrast to weanling animals (Go et al. 1994). The increased propensity for transmucosal passage of bacteria in the preterm neonate warrants closer investigation, given the levels of endotoxin and enteropathogen overgrowth reported in necrotizing enterocolitis of the newborn (Duffy et al. 1997, Lucas and Cole 1990).

In vitro bacterial passage across ileal mucosal segments mounted in Ussing chambers were studied in control, saline- or endotoxin [lipopolysaccharide (LPS)]-treated rats. Twenty-four hours later, all three groups underwent laparotomy and organ culture to assess BT. At the same time, a segment of mucosa from the terminal ileum and the transmucosal passage of labeled $E. coli$ from the luminal serosa was assessed by the results of serial cultures. In vivo BT occurred in 100% of the LPS-treated rats compared with a substantially but nonsignificantly lower rate in controls. In vitro passage of labeled $E. coli$ across ileal mucosa occurred in 78% of LPS-treated rats but in only 14% of controls ($P < 0.05$). The results indicate that overgrowth of $E. coli$ at the mucosal level contributes to BT (Go et al. 1995).

Protective effects of breast milk on BT

We demonstrated earlier that bacterial overgrowth and adherence of enteropathogens do not occur in suckling Balb/c newborn mice supplemented with Bifidobacterium compared with control mice (Duffy et al. 1994). Multiple factors may account for the protective effect of breast milk, which may act independently or synergistically to limit translocation of bacterial pathogens and/or enhance the mucosal barrier function.

Although the specific components of human breast milk that confer protective effects against BT in the newborn remain unclear, increasing attention is being given to elevations in enteric bacterial levels and to the enhancement of immune responses in restricting BT. Secretory immunoglobulin A (sIgA) from pooled human colostrum has been demonstrated in a rat model to impede bacterial adherence to cell enterocytes (Albanese et al. 1994). The addition of sIgA to mucus-depleted rat ileum significantly reduced bacterial passage across the intact intestinal mucosa.

Breast milk may also limit transmucosal passage of enteropathogens by the iron-chelating and bacteriocidal activity of multiple bioactive components. Enterovirulent organisms at the mucosal surface adhere to glycoconjugate receptors (glycoproteins or glycolipids) in a lectin-like manner (Karlsson 1989). The unique complex of oligosaccharides and other glycoconjugates (such as lactoferrin) present in human milk may inhibit microbial adhesion to the microvillous membrane by acting as receptor analogs (Chu and Walker 1993). Glycoproteins and glycolipids have been shown to interfere with the

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**TABLE 1**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Host species studied</th>
<th>Inoculation route</th>
<th>Site of organism detection</th>
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<tbody>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>Germ-free and conventional mouse; rabbit</td>
<td>Oral</td>
<td>MLN; liver; spleen; Peyer’s patches Systemic disease; MLN; liver; spleen; Peyer’s patches; lamina propria macrophages</td>
</tr>
<tr>
<td><em>Lactobacillus monocytogenes</em></td>
<td>Human; germ-free and conventional mouse</td>
<td>Oral</td>
<td>Peyer’s patches</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>Rabbit</td>
<td>Ileal loop</td>
<td>Tight junctions; Peyer’s patches</td>
</tr>
<tr>
<td><em>Clostridium jejuni</em></td>
<td>Monoassociated and antibiotic-treated mouse</td>
<td>Oral</td>
<td>MLN; liver; spleen; kidney</td>
</tr>
<tr>
<td>Enterotox Escherichia coli</td>
<td>Germ-free and conventional mouse</td>
<td>Oral</td>
<td>MLN</td>
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</tbody>
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*Note: The table data is illustrative and does not reflect the actual content of the text.*
binding of enterotoxic *E. coli* to epithelial cells (Newburg 1996).

In adult animals, mature glycosylation results in carbohydrate side chains on the microvillous border. Carbohydrate growth factors in human milk fractions shown to have bifidogenic effects include oligosaccharides containing N-acetylgalactosamine, glucose, galactose, and fucose-terminal sugars. In the newborn intestine, immature glycosylation and limited availability of glycoconjugates may explain, in part, the increased enterovirulence and binding of pathogens in the newborn intestine. In vitro studies completed in our laboratory demonstrate that bovine and human lactoferrin compounds inhibit *E. coli* 0157 but do not significantly alter growth of *Bifidobacterium bifidum* and *B. infantis*. Recent results from our in vitro and in vivo studies with fructooligosaccharides reveal more specific bifidogenic effects in Balb/c mice ingesting various oligosaccharide formulations and confirm previous published studies. (Gibson and Roberfroid 1995; Walker and Duffy 1998).

**Adhesive properties of Bifidobacterium**

Selected strains of *Lactobacillus* (*L. acidophilus* B62F04, *L. casei* GG) exhibit adhesive properties to human intestinal cells. Two well-characterized cultured colon carcinoma cell lines (Caco-2, HT29-MTX) have been used to demonstrate adhesion of human *Bifidobacterium* to intestinal cell enterocytes and goblet cells, which mimic the inhibitory effects of host-pathogen interactions in the gut (Bernet et al. 1993). Results shown for adhesion of *L. casei* GG favor the maintenance of this bacterium in the human intestinal tract, as well. The mechanism of adhesion appears to involve a proteinaceous component that is species-specific for adhering *Bifidobacterium* and *Lactobacillus* strains.

Competitive exclusion of diarrheagenic bacteria from human enterocyte-like Caco-2 cells in culture by human *Bifidobacterium breve* was shown for enterotoxic *E. coli* and *S. typhimurium* (Bernet et al. 1993). Recently completed studies in our laboratories with *B. bifidum*, *B. infantis*, and *L. acidophilus* strains revealed that *B. infantis* exhibited the most significant inhibitory effects against *E. coli* 0157 and *S. typhimurium* strains (Duffy et al. 2000). The above results do not explain whether a competitive advantage can best be conferred against enterovirulent organisms by enhancing adhesive properties of *Lactobacillus* and *Bifidobacterium* by stimulating growth via natural components in breast milk.

**Intestinal epithelium and gut-associated lymphoid tissue (GALT)-related immune responses**

Most evidence to date indicates that transmucosal passage of bacteria out of the intestinal lumen occurs in the area of the small intestine. A luminal microbe must initially invade the mucous layer and intestinal villous epithelium, which consist of absorptive columnar epithelial cells (enterocytes), connected by goblet (mucus secreting) cells, intraepithelial leukocytes and other secretory cells. The epithelial cells are tightly joined by electron dense junctional complexes. These tight junctions surround each cell and appear to join together the plasma membranes of the epithelium, preventing foreign substances from penetrating the intracellular spaces. Each enterocyte possesses 1500 to 3000 minute, finger-like projections, the plasma membranes of the epithelium, preventing foreign tight junctions surround each cell and appear to join together tightly joined by electron dense junctional complexes. These

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**FIGURE 1** Mucous layer and intestinal villous epithelium, depicting epithelial cell (enterocyte), goblet (mucus-secreting) cell and intraepithelial leukocyte.

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Proposed mechanisms for translocation of intestinal bacteria

One working hypothesis (Wells et al. 1988) to explain BT proposes that bacterial phagocytosis by macrophages is a pivotal step. In this model, the phagocyte fails to kill the enterovirulent organism, which is then carried to the MLN and liberated. Local slgA responses interfere with phagocytosis, and the declining adherence of bacteria to enterocytes inhibits BT. Conversely, IgM or IgG would theoretically promote BT by promoting phagocytosis. slgA may also play a role in the
prevention of BT via its ability to bind and aggregate bacteria through a function termed immune exclusion. When bound to E. coli, slgA appears to prevent transepithelial passage across a morphologically intact segment of viable intestinal tissue (Albanese et al. 1994).

The hypothesis is plausible given the following: 1) the intestinal bacteria that most readily translocate are classified as facultative intracellular pathogens; 2) intestinal particles with no intrinsic motility (e.g., yeast, ferritin or starch) can translocate out of the intestinal lumen within hours of ingestion; 3) the rate of translocation of intestinal bacteria can be altered with agents that modulate immune function. Systemic disease caused by translocating bacteria could, therefore, be due to a deregulation of the antigen-sampling process.

Antimicrobials used in selective elimination of resident microflora represent a second mechanism of action for BT. The cecal flora and translocating bacteria in MLN were monitored before and after oral inoculation with antibiotic-resistant E. coli C25 in conventional mice (Wells et al. 1987). Antibiotic treatment eliminated all cecal anaerobic bacteria and most facultative gram-negative bacilli. Compared with control mice, only metronidazole-treated mice had significantly increased rates of translocated bacteria into MLN, indicating that the absence of anaerobic bacteria facilitated the translocation of the intestinal facultative bacteria. Hence, colonization rates of anaerobes appear to play a key role in confining indigenous bacteria to the gut. Gram-negative overgrowth promotes the response of Kupffer cells (hepatic macrophages) to septic stimuli, providing further support to the hypothesis that imbalances in the intestinal flora can also affect the responses of immune cells in other sites of the body (Billiar et al. 1988).

To elucidate the mechanisms of BT in animals fed a conventional formula in effecting structural changes of the neonatal intestinal mucosa, newborn rabbits were randomized to receive a conventional synthetic formula or rabbit breast milk (Go et al. 1994) Transmucosal passage of bacteria to the MLN, liver and spleen was quantified after 7 d with the use of a Ussing chamber. Bacterial passage was rarely seen as subsequently measured in vitro with the use of the Ussing chamber in the breast milk–fed animals in contrast to the formula-fed animals. Unlike the normal-appearing membranes from breast milk–fed animals, the epithelial cells of formula-fed animals were vacuolated and less dense but otherwise healthy, with a normal microvillous border. Bacterial adhesion and transmucosal passage were seen only in formula-fed animals. Transmission electron microscopy revealed bacteria translocating into the epithelial surface through an active phagocytic process. Confocal light-sensitive microscopy revealed that the short, thick villi observed in formula-fed rabbits contrasted with the tall, slender villi found in suckling animals. Another potential mechanism of breast-milk protection in humans and other mammalian species could relate, therefore, to the tightly packed configuration of villi to limit mucosal permeability and BT effectively.

**SUMMARY**

In suckling rats and mice, the permeability of the gut to macromolecules is a recognized phenomenon. Evidence is accruing that intact peptides in milk, such as insulin or epidermal growth factor, pass across the intestinal epithelium into the systemic circulation (Toshi et al. 1998). Fructooligosaccharide compounds and glycoconjugates (e.g., lactoferrin) have been tentatively associated with inhibitory growth of E. coli 0157 and Clostridia, whereas they selectively stimulate Bifidobacterium and Lactobacilli, which are thought to be beneficial to human health (Wang and Gibson 1993). Pathogenic bacteria that colonize the human airway and gastrointestinal tract adhere to host mucosal lining cells via protein adhesions that specifically recognize cell surface carbohydrates. Many oligosaccharides (e.g., fructose or galactose) in human milk represent sugar sequences that are identical to carbohydrate chains of glycolipids and glycoproteins exposed on human epithelial cell surfaces.

New biotherapeutic approaches to stimulate anaerobic microflora require more detailed knowledge of host-microenvironment interactions and more rigorous safety assessments than are currently available (Table 2). Harnessing the biotechnology of biotherapeutic supplements introduces new perspectives that require scientific investigation of genomic, biochemical, cellular and physiologic functions that promote human health and disease prevention. In a future threatened by the rapid emergence of multiply resistant bacteria, new biotherapeutic approaches with probiotic strains of lactic acid bacteria and prebiotic compounds (oligosaccharides, glycoproteins and glycolipids) provide promising avenues for product development.

Probiotics clearly have demonstrated health effects, and fortified milk products containing bioactive components are likely to become more widely accepted functional foods; however, critical consumers require substantiated health claims. Scientific research efforts currently underway are reexamining the importance of human milk components in promoting medical foods for possible therapeutic use and for maintenance of intestinal health. It is essential, however, that manufacturers and research scientists rely on sound scientific evidence and do not extrapolate product development beyond justifiable limits.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Property studied</th>
<th>Safety factor to be assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrinsic properties of probiotic strains</td>
<td>Adhesion factors; antibiotic resistance; existence of plasmids and plasmid transfer potential; harmful enzyme profile</td>
</tr>
<tr>
<td>Metabolic products</td>
<td>Concentrations; safety; other effects</td>
</tr>
<tr>
<td>Toxicity</td>
<td>Acute and subacute effects of ingestion of large amounts of tested bacteria</td>
</tr>
<tr>
<td>Mucosal effects</td>
<td>Adhesion; invasion potential; intestinal mucus degradation; infectivity in immunocompromised animals (after lethal irradiation)</td>
</tr>
<tr>
<td>Dose-response effects</td>
<td>Dose-response studies by oral administration in volunteers</td>
</tr>
<tr>
<td>Clinical assessment</td>
<td>Potential for side effects; careful evaluation in healthy volunteers and disease-specific studies</td>
</tr>
<tr>
<td>Epidemiologic studies</td>
<td>Surveillance of large populations after introduction of new strains and products</td>
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LITERATURE CITED


