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REVIEW ARTICLE

Perspectives on Bifidobacteria as Biotherapeutic Agents in Gastrointestinal Health

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Intestinal microflora carry out a process of fermentation where dietary and endogenous substrates are metabolized within the intestinal tract of the host animal they inhabit (1). Enteric infections may develop when the normal flora are disrupted by antibiotics or medical procedures. Microorganisms mixed in fermented milk products and poultices have been used since antiquity to treat enteric and respiratory infections (2). There is increasing interest in the current literature in promoting the use of live organisms (probiotics) or nutrient compounds (prebiotics) that stimulate beneficial microflora as new biotherapeutic agents in resisting pathogens (3–5).

Stimulation of intestinal microflora that promote the integrity of the gut mucosa and do not overactivate the immune function of gut associated lymphoid tissue (GALT) show promise in a variety of clinical applications (6, 7). Bacterial passage occurs during normal antigen processing in GALT; however, deregulation of phagocyte sampling of bacterial antigens may lead to transmucosal passage of enteropathogens to extraintestinal sites (8, 9). Using bacterial translocation (BT) as a measure of gut barrier function, experimental evidence suggests that animals fed formula have higher incidences of BT compared to those fed breast milk (10, 11).

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Gram-positive anaerobes (lactobacilli; bifidobacteria) stimulated by feeding breast milk play a key role in preventing pathogen colonization and translocation of bacteria into mesenteric lymph nodes (MLNs) after ingestion (12, 13). Alternatively, increased permeability to bacteria at the mucosal level contributes to passage of enteropathogens associated with prematurity and enteric feeding. In addition, decreased colonization resistance associated with increased shedding of epithelial cells, depletion of secretory IgA (SIgA), and intestinal mucus may all play pivotal roles in bacterial passage of potential pathogens (14–16).

ENTERIC FEEDING AND BIFIDOBACTERIA GROWTH

Growth of intestinal microflora stimulated by breast and formula substrates in the gut has been the subject of much debate. Metchnikoff (17) and Tissier (18) originally observed that human milk promotes growth of gram-positive anaerobes, including *Lactobacillium* and *Bifidobacterium* spp. With the availability of rapid assays, DNA hybridization, and fingerprinting techniques, it is well established that human milk contains unique factors (*N*-acetylglucosamine-containing oligosaccharides) that promote growth activity of *Bifidobacterium* variant *pennsylvanicus* (19, 20). Physiologically, bifidobacteria are unique in their ability to ferment glucose and produce acetic and lactic acids in an approximate ratio of 1.5:1, without evolution of gas (21). While the exact mechanisms remain elusive, acetate and other short-chain fatty acids are important substrates for colonocyte nutrient metabolism (22).

Bifidobacteria require ferrous iron, riboflavin, and biotin for growth, and *B. bifidum, B. infantis,* and *B.*

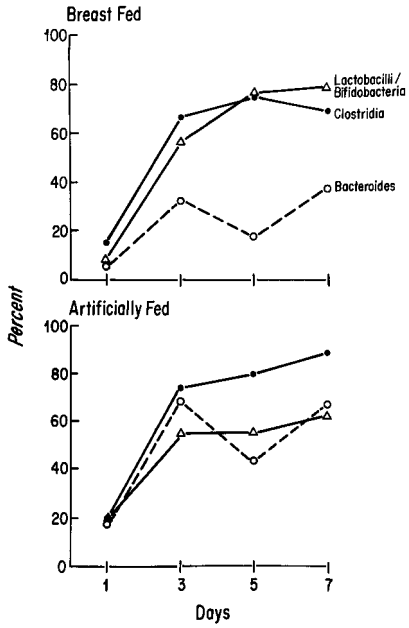

Fig 1. Microflora patterns among feeding groups: —△—, *Lactobacillius/Bifidobacterium*; —●—, *Clostridia*; ---○---, *Bacteroides*.

longum synthesize their own thiamin, folic acid, and vitamin B_{12} (23). Bifidobacteria utilize a wide variety of carbohydrate compounds as a source of carbon. These colonic anaerobes acquire their carbon sources from fiber, ie, complex carbohydrates (mucins, glycoproteins) that are not digested in the small intestine (24).

Investigations in our laboratories over the past two decades have intensively focused on the characteristic shift toward overgrowth of coliforms in artificially (formula) fed infants compared to the distinct proliferation of lactobacilli and bifidobacteria observed in breast-fed infants (Figure 1) (25). Available evidence further suggests that morphological changes in the intestinal mucosa of preterm neonates fed artificial formulas may increase permeability to bacteria (26).

Bacterial adhesion, transmucosal passage, and increased shedding of epithelial cells have been observed in formula-fed animals and preterm neonates (27, 28). To elucidate how breast milk prevents coliform overgrowth and transmucosal bacterial passage, new perspectives in promoting bifidogenic effects require better understanding of physiologic mechanisms of action and of genomic and cellular function of the host microenvironment. Experimental formulas containing synthetic and natural sugars, nucleotide salts, and enzyme hydrolysates of casein have generally failed to sustain beneficial microflora patterns, although reduction in coliform growth has been observed (29, 30).

BIOTHERAPEUTICS AND ENTERIC INFECTION

Biotherapeutic agents most likely act by multiple mechanisms and must be viewed as vehicles aimed at pathogen inhibitory activities and/or host immune stimulation to the intestinal tract. Normally, the intact intestinal epithelium represents a barrier to the movement of pathogenic bacteria from dietary and environmental sources. In healthy subjects this barrier is stable, protecting the host and providing normal intestinal function (31). When dietary antigens or infectious pathogens disturb either the normal microflora or epithelial cells, increased permeability may lead to diarrhea and mucosal inflammation (32).

The intestinal mucosa is an important organ of defense, providing a barrier against as much as 90% of dietary antigens (33). There are specific antigen transport mechanisms in the villus epithelium, particularly in the Peyer's patches, for evoking specific immune responses. Although poorly understood, it is speculated that pericellular leakage of macromolecules in normal infants is not allowed due to intercellular tight junctions maintaining the macromolecular barrier of the gut (34). Maintaining the integrity of the defense barrier is, therefore, critical to preventing inappropriate and uncontrolled antigen transport.

Rotavirus (RV) gastroenteritis, for example, is associated with increased gut permeability resulting from the invasion of the highly differentiated absorptive columnar cells of the small intestinal epithelium where the virus replicates (35). Partial disruption of the intestinal mucosa is followed by a loss of microvilli, atrophy, and a decrease in the villus-to-crypt ratio (36). Epithelial cell interactions appear to affect gut permeability in RV infection, and protracted disease can lead to serious mucosal injury in animals and human hosts.

Colonization resistance is one potential mechanism of action in gut barrier defense made in health claims of biotherapeutic agents over conventional antimicrobial therapies. Earlier investigations in our laboratories found that viral replication and the clinical severity of RV gastroenteritis were significantly reduced in breast-feeding infants who maintained detectable levels of bifidobacteria at the time of virus infection. Closer examination in Balb/c mice challenged with murine rotavirus (MRV) (Figure 2) revealed that adherent properties of bifidobacteria appeared to reduce mucosal atrophy and replication of RV antigen

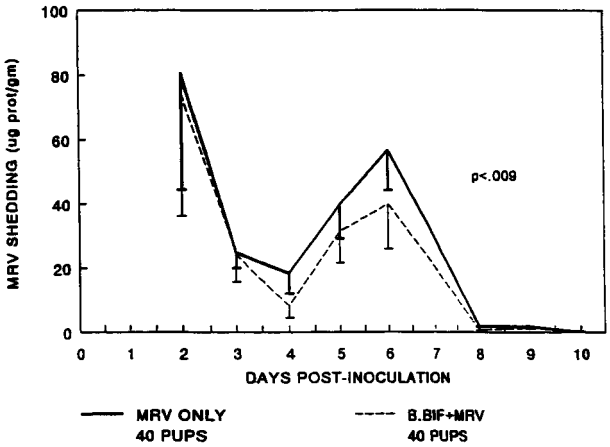

Fig 2. Murine rotavirus (MRV) shedding at various days after inoculation in Balb/C infected mice. MRV = murine rotavirus; B. BIF = *Bifidobacterium bifidum*.

(37). Use of various wild strains of lactic acid bacteria, particularly bifidobacteria, in recent *in vivo* and clinical investigations tentatively indicates that colonization by gram-positive bacilli promotes mucosal immune responses to rotavirus that may be of some importance for protective immunity against reinfection as well (38, 39).

Clostridium difficile depends on monosaccharides for growth and represents a classic example of how competition for nutrients is another mechanism of action by biotherapeutic agents. *C. difficile* proliferates following antibiotic disruption of normal microflora. Infection may result in diarrhea, colitis, toxic megacolon, and in severe cases, death. *Lactobacillus casei* GG has been evaluated in recurrent *C. difficile* colitis, with some promising results. Naaber et al (41) concluded that disturbance of the intestinal microflora was more important in inducing bacterial passage than mucosal inflammatory responses for mild *C. difficile* infection and that treating with lactobacilli and xylitol had some protective effect. Routine use of such biotherapeutic agents as adjuncts to antimicrobial therapy requires further study, however (40).

In two excellent recent reviews (2, 32), biotherapeutic agents (lactobacilli, bifidobacteria, *Saccharomyces bouldari*) were shown to be used successfully in treating recurrent *C. difficile* and antibiotic-resistant infections with few adverse effects. Although promising, the studies reported were small, and several were in nonrandomized patient volunteers. These and other critical reviews (5, 29, 42, 43) present equivocal results. A major difficulty in evaluating health claims is that published studies have rarely presented dose–response efficacy, and pharmacokinetic studies are glaringly lacking as to how these agents should be administered and adverse effects monitored.

BACTERIAL TRANSLOCATION AND GUT PERMEABILITY

The barrier functions are incompletely developed in infancy. Consequently, intestinal permeability can be transiently increased postnatally in premature infants. Predisposing factors that can potentially enhance bacterial translocation in preterm newborns include immunosuppression, thermal injury, hemorrhagic shock, age of subject, and an altered intestinal microbial environment (11, 12, 44). Critically ill neonates with ischemic–reperfusion injury and with sepsis syndrome are highly susceptible to gut failure. The role of the macrophage and immune inflammatory mediators (ie, TNF-α and IL-6 cytokines) on the microcirculation (alterations in mesenteric blood flow) are also important in this process (45).

The selective overgrowth of gram-negative bacilli and enterococci affects their ability to translocate and may help explain why formula-fed and critically ill neonates are at increased risk for necrotizing enterocolitis (NEC). Deitch (33) recently proposed that bacterial translocation occurs if certain enteric bacteria exceed colonization levels of 10^9 bacteria/g stool. Overgrowth of gram-negative enterobacteria may result in passage of enteropathogens to normally sterile mesenteric lymph nodes (MLNs) in large numbers more efficiently than obligate gram-positive anaerobes (bifidobacteria) (38).

Theoretically, coliforms with a very low concentration in the cecum might translocate with the same efficiency as those with high incidence. However, concentration and enterovirulence of the organisms are also critical in pathogenesis (9). Antigenotoxic properties of lactic acid bacteria from *in vivo* investigations in rats may, in part, explain the more rapid bacterial passage of gram-negative aerobes observed (46).

Recent studies provide additional evidence that translocation of bacteria is dependent on metabolic substrates of enteric feeds (Table 1). Neonatal rabbits fed formula showed a significantly greater incidence of bacterial translocation than neonatal rabbits fed breast milk (10). Subsequent experiments showed that levels of bacterial organisms could be reduced by changing the microenvironment and hygienic conditions of the rabbit, but that factors present in rabbit breast milk inhibit bacterial translocation regardless of the environment.

In a more controlled set of experiments (47), coli-

TABLE 1. SELECTED STUDIES SHOWING EFFECTS OF FEEDING MODE ON BACTERIAL TRANSLOCATION (BT)

Investigation	*Feeding mode*	*Study type*	*Model system/design*	*Findings*
Ford et al (10)	Breast-feeding vs pasteurized/nonpasteurized formula	*In vivo*	Neonatal rabbit	Breast-milk inhibited BT; a sterile microenvironment and formula pasteurization reduced BT in formula-fed rabbits
Steffan et al (9)	Breast-feeding vs milk-simulated formula	*In vivo*	Neonatal rat	Artificial feeding enhanced BT to mesenteric lymph node
Duffy et al (52)	Breast-feeding, formula, and NPO	Clinical	Prospective follow-up of preterm infants	NPO and formula-fed infants at increased risk of developing advanced stages of NEC compared to breast-fed infants

form isolates from cecal epithelium and MLNs were fingerprinted and grouped into different biochemical phenotypes in starved rats. Translocation was associated with coliforms that adhered to the cecal epithelium, although not all coliforms adhered to the epithelium during catabolic stress. Related evidence suggests immune exclusion by SIgA may interact with bacterial adhesive properties, preventing enteropathogens from translocating across a morphologically intact segment of viable intestinal tissue (14).

Most pathogenic microorganisms, moreover, require iron to develop maximum virulence. It is not surprising that host animals have developed iron-withholding mechanisms and maintain low levels of iron in the biologic fluids, limiting growth of microorganisms (48). Iron-binding proteins, transferrin (in the circulation) and lactoferrin (in human milk), accomplish this task for ferric iron (49). It is speculated that bifidobacteria may be an extension of the iron-withholding system to bind ferrous iron oxidized under the anaerobic conditions of the large intestine (50).

A hypothesized model (51) of acute necrotizing enterocolitis in infancy gaining increasing attention identifies three components resulting in transmucosal passage of bacteria to MLNs: (1) injury to the intestinal mucosa; (2) overgrowth of gram-negative bacteria; and (3) the availability of an altered metabolic substrate (ie, formula milk in the gut lumen). Our laboratories examined enteric feeding relative to bacterial overgrowth and risk of NEC in a premature cohort (52). Breast-fed infants remained at substantially reduced risk for developing NEC, with the highest risk observed in the nil *per os* (NPO) infants and those infants who were exclusively formula fed.

Bacterial culture results summarized in Table 2 indicate strong concordance ($r = 0.71$, $P < 0.001$) with endotoxin levels in stool filtrates, in sharp contrast with discordance ($r = 0.31$, $P < 0.05$) generally reported between blood culture and endotoxinemia (53). Although much remains to be learned about the exact mechanisms by which bacteria cross the intestinal mucosal barrier, it is clear that adherent properties of bacteria are important in the pathogenesis of infections that originate at mucosal surfaces.

FUTURE DEVELOPMENT OF BIOTHERAPEUTICS

Major interest in the current literature focuses on the role of biotherapeutic agents, particularly raw and bioengineered strains of lactobacilli and bifidobacteria, as probiotic nutritional supplements (3, 54, 55). Most recently, prebiotics have gained attention as nondigestible food components that may help to promote beneficial microflora (56). Carbohydrate growth

TABLE 2. ASSOCIATION BETWEEN STOOL ENDOTOXIN AND BACTERIAL CULTURE RESULTS

	Endotoxin	
	Nondetectable [N *(%)*]	*Elevated* [N *(%)*]
Clostridium spp*		
Low	61 (34.5)	116 (65.5)
High	6 (13.3)	39 (86.7)
Pearson's χ^2 test: $P = 0.0058$		
Klebsiella		
Low	66 (44.6)	82 (55.4)
High	1 (1.4)	73 (98.6)
Pearson's χ^2 test: $P = 0.0000$		
E. coli		
Low	64 (38.8)	101 (61.2)
High	3 (5.3)	54 (94.7)
Pearson's χ^2 test: $P = 0.0000$		
Enterobacters		
Low	61 (35.9)	109 (64.1)
High	6 (11.5)	46 (88.5)
Pearson's χ^2 test: $P = 0.0008$		

* *Clostridium perfringens* and *C. difficile* spp. only.

factors in human milk fractions generally recognized for stimulating growth of bifidobacteria include mixtures of oligosaccharides containing *N*-acetylglucosamine, glucose, galactose, and fucose (57). *trans*-Galacto oligosaccharides have also been studied for cellular receptor binding of glycoconjugates and glycolipids (58).

There are a number of desirable characteristics that should be considered in combining probiotic and prebiotic properties in biotherapeutic products. Optimally, probiotic strains should be of human origin and nonpathogenic in terms of animal results from *in vivo* systems. Additionally, strains need to be easily grown *in vitro* and should be stable, more or less, in predigested form. Desirable characteristics also include demonstrated ability to survive gastric acidity and, preferably, to multiply as part of the endogenous microbiota (4, 26).

Oligosaccharide supplements have shown very direct evidence of bifidogenic stimulation. Many oligosaccharides in human milk represent sugar sequences that are identical to carbohydrate chains of glycolipids and glycoproteins that are exposed on human epithelial cell surfaces (58). Pathogenic bacteria that colonize the human airway and the gastrointestinal tract adhere to host mucosal lining cells via protein adhesions that specifically recognize cell surface carbohydrates. More study needs to be focused on antiadhesive properties of human milk oligosaccharides, including the ability of these compounds to act as competitive inhibitors of bacterial binding to human epithelial cells *in vitro*.

In summary, biotherapeutic agents most likely act by multiple mechanisms. Antibacterial agents (bacteriocins) are produced and secreted by probiotic organisms (including lactobacilli and bifidobacteria), which may have important inhibitory effects on enteropathogens (59). Human breast milk may alter bacterial antagonism for essential nutrients and impede the overgrowth of aerobes by allowing altered receptor binding to epithelial sites (11, 16). Competition for mucosal receptor sites may prevent adhesion and overproliferation of gram-negative aerobes and allow more beneficial organisms to adhere to the surface (8, 10). Also important may be the inhibition of volatile fatty acid production and the reduction of H_2 (12, 15). Finally, human sera carries lower antibody titers to bifidobacteria (1:40) compared with enterococci (1:640) or enterobacteria (1:2560). Stimulation of immune responses that down-regulate dietary antigens to tolerogenic substrates, therefore, is an interesting concept that also merits serious examination (60).

TABLE 3. IMPORTANT STUDIES FOR SAFETY ASSESSMENT OF PROBIOTIC STRAINS*

Type of property studied	*Safety factor to be assessed*
Intrinsic properties of probiotic strains	Adhesion factors, antibiotic resistance, existence of plasmids and plasmid transfer potential, harmful enzyme profile
Metabolic products	Concentrations, safety, and other effects
Toxicity	Acute and subacute effects of ingestion of large amounts of tested bacteria
Mucosal effects	Adhesion, invasion potential, intestinal mucus degradation, infectivity in immunocompromised animals (eg, following lethal irradiation)
Dose–response effects	Dose–response studies by oral administration in volunteers
Clinical assessment	Potential for side effects, careful evaluation in healthy volunteers and disease-specific studies
Epidemiological studies	Surveillance of large populations following introduction of new strains and products

* Adapted from Salminen et al, 1996 (4).

Bifidogenic agents can only be promoted for human consumption if tested in rigorously designed model systems and in large, multicenter clinical investigations. Such "well-being" foods must demonstrate evidence of health-promoting effects (eg, the production of essential amino acids, antitumor activity, bacteriocins, and vitamins) and also should show food-protective activities (ie, food spoilage and poisoning bacterial products are very important to guard against) (56). Important studies for the safety assessment of probiotic lactic acid bacteria, and bifidobacteria specifically, will include the properties listed in Table 3 [adapted from (4)]. While the promise of bioengineered strains and bifidus growth promoters is opening a new generation of "well-being" foods, additional studies demonstrating bioactive properties and safety assessment are warranted.

REFERENCES

1. Cummings JH, Macfarlane GT: A review: the control and consequences of bacterial fermentation in the human colon. J Appl Bacteriol 70:443–459, 1991
2. Elmer GW, Surawicz CM, McFarland LV: Biotherapeutic agents: A neglected modality for the treatment and prevention of selected intestinal and vaginal infections. JAMA 275(11):870–876, 1996
3. Fuller R, Gibson GR: Modification of the intestinal microflora

using probiotics and prebiotics. Scand J Gastroenterol 32(suppl 222):28–31, 1997
4. Salminen S, Isolauri E, Salminen E: Clinical uses of probiotics for stabilizing the gut mucosal barrier: Successful strains and future challenges. Antonie van Leeuwenhoek 70:347–358, 1996
5. Walker WA, Duffy LC: Diet and bacterial colonization: Role of probiotics and prebiotics. J Nutr Biochem 9(12):668–675, 1998
6. Schriffin EJ, Brassart D, Servin AL, Rochat F, Donnet-Hughes A: Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. Am J Clin Nutr 66(2):5155–5205, 1997
7. Gorbach SL: Lactic acid bacteria and human health. Ann Med 22:37–41, 1990
8. Berg RD: Translocation of indigenous bacteria from the intestinal tract. *In* Human Intestinal Microflora in Health and Disease. Orlando, Florida, Academic Press, 1983, pp 333–352
9. Steffan EK, Berg RD, Deitch EA: Comparison of translocation rates of various indigenous bacteria from the gastrointestinal tract to the mesenteric lymph node. J Infect Dis 157:1032–1038, 1988
10. Ford HR, Avanoglu A, Boechat PR, Melgoza D, Lumcheong RS, Boyle P, Garrett M, Rowe MI: The microenvironment influences the pattern of bacterial translocation in formula-fed neonates. J Pediatr Surg 31(4):486–489, 1996
11. Go LL, Ford HR, Watkins SC, Healey PJ, Albanese CT, Donhalek A, Simmons RL, Rowe MI: Quantitative and morphologic analysis of bacterial translocation in neonates. Arch Surg 129(11):1184–1190, 1994
12. Wells CL, Maddaus MA, Simmons RL: Proposed mechanisms for the translocation of intestinal bacteria. Rev Infect Dis 10(5):958–979, 1988
13. Katouli M, Nettbladt CG, Muratov V, LJungQVist O, Bark T, Svenberg T, Mollby R: Selective translocation of coliform bacteria adhering to caecal epithelium of rats during catabolic stress. J Med Microbiol 46:571–578, 1997
14. Albanese CT, Smith SD, Watkins S, Kurkchubasche A, Simmons RL, Rowe MI: Effect of secretory IgA on transepithelial passage of bacteria across the intact ileum *in vitro*. J Am Coll Surg 179(6):679–688, 1994
15. Vandengergh PA: Lactic acid bacteria, their metabolic products, and interference with microbial growth. FEMS Microbiol Rev 12:221–238, 1993
16. Katayawa M, Dazhong X, Specian R, Deitch E: Role of bacterial adherence and the mucous barrier on bacterial translocation. Ann Surg 225(3):317–326, 1997
17. Metchnikoff E: The Prolongation of Life. London, William Heinemann, 1907
18. Tissier H: Repartition des microbes dans l'intestin du nourisson. Ann Inst Pasteur 19:109–115, 1905
19. Bezkorovainy A: Classification of Bifidobacteria. *In* Biochemistry and Physiology of Bifidobacteria. A Bezkorovainy, Robin Miller-Catchpole (eds). Boca Raton, Florida, CRC Press, 1989, pp 1–29
20. McBain AJ, MacFarlane GT: Investigations of bifidobacterial ecology and oligosaccharide metabolism in a three-state compound continuous culture system. Scand J Gastroenterol S222:32–40, 1997
21. Mitsuoka T: Taxonomy and ecology of bifidobacteria. Bifid Microflora 3:11–28, 1984
22. Wang X, Gibson GR: Effects of the *in vitro* fermentation of oligofructose and inulin by bacteria growing in the human large intestine. J Appl Bacteriol 75:373–380, 1993
23. Noda H, Akasaka N, Ohsugi M: Biotin production by bifidobacteria. J Nutr Sci Vitaminol 40(2):181–188, 1994
24. Kunz C, Rudloff S: Biological functions of oligosaccharides in human milk. Acta Paediatr 82(11):903–912, 1993
25. Neter E: Microflora patterns in breast-fed and artificially-fed infants. *In* Textbook of Gastroenterology and Nutrition in Infancy. E Lebanthal (ed). New York, Raven Press, 1994, Chap 22
26. Hentges D: Gut flora and disease resistance. *In* Probiotics: The Scientific Basis. R Fuller (ed). New York, Chapman & Hall, 1992, pp 87–109
27. Wells CL, Maddaus MA, Jechorek RP, Simmons RL: Role of intestinal anaerobic bacteria in colonization resistance. Eur J Clin Microbiol Infect Dis 7(1):107–113, 1988
28. Beach RC, Menzies IS, Clayden GS, Scopes JW: Gastrointestinal permeability changes in the preterm neonate. Arch Dis Child 57:141–145, 1982
29. Tannock GW: Probiotic properties of lactic-acid bacteria: Plenty of scope for fundamental R & D. Trends Biotech 15(7)270–274, 1997
30. Goldin BR, Gorbach SL: Probiotics for humans. *In* Probiotics: The Scientific Basis. R Fuller (ed). London, Chapman & Hall, 1992, pp 355–376
31. Walker WA: The role of the mucosal barrier in toxin/microbial interaction. CIBA Found Symp 112:37–47, 1985
32. McFarland LV, Elmer GW: Biotherapeutic agents: Past, present and future. Microecology Ther 23:46–73, 1995
33. Deitch EA: Bacterial translocation of the gut flora. J Trauma 30(suppl):S184–S189, 1990
34. Shu-Heh, Chu W, Walker WA: Bacterial toxin interaction with the developing intestine. Gastroenterology 104:916–925, 1993
35. Dharakul T, Riepenhoff-Talty M, Albini B, Ogra PL: Distribution of rotavirus antigen in intestinal lymphoid tissues: potential role in development of the mucosal immune response to rotavirus. Clin Exp Immunol 74:14–19, 1998
36. Osborne MP, Haddon SJ, Spencer AJ, Collins J, Starkey WG, Wallis TS, Clarke GJ, Worton KJ, Candy DC, Stephen J: An electron microscopic investigation of time-related changes in the intestine of neonatal mice infected with murine rotavirus. J Pediatr Gastroenterol Nutr 7(2):236–248, 1988
37. Duffy LC, Zielezny MA, Riepenhoff-Talty M, Dryja D, Griffiths E, Ruffin D, Barrett H, Ogra PL: Reduction of virus shedding by *B. bifidum* in experimentally induced MRV infection. Dig Dis Sci 39(11):2334–2340, 1994
38. Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH: Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhea and shedding of rotavirus. Lancet 344:1046–1049, 1994
39. Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, Arvilommi H: Enhancement of the circulating antibody secreting cell respsonse in human diarrhea by a human *Lactobacillus* strain. Pediatr Res 32:141–144, 1992
40. Biller JA, Katz AJ, Flores AF, Buie TM, Gorbach SL: Treatment of recurrent *Clostridium difficile* colitis with *Lactobacillus* GG. J Pediatr Gastroenterol Nutr 21:224–226, 1995
41. Naaber P, Mikelsaar RH, Salminen S, Mikelsaar M: Bacterial translocation, intestinal microflora and morphological changes of intestinal mucosa in experimental models of *Clostridium difficile* infection. J Med Microbiol 47(7):591–598, 1998
42. Lewis SJ, Freedman AR: Review article: The use of biothera-

peutic agents in the prevention and treatment of gastrointestinal disease. Aliment Pharmacol Ther 12(9):807–822, 1998
43. Fuller R: Probiotics in human medicine. Gut 32:439–442, 1991
44. Ewe K, Wanitscke R, Staritz M: Intestinal permeability studies in humans. *In* Pharmacology of Intestinal Permeation II. TZ Csaky (ed). New York, Springer-Verlag, 1984, pp 535–571
45. Wells CL, Maddaus MA, Simmons RL: Role of the macrophage in the translocation of intestinal bacteria. Arch Surg 122:48–53, 1987
46. Pool-Zobel BL, Bertram B, Knoll M, Lambertz R, Neudecker C, Schillinger U, Schmezer P, Holzapfel WH: Antigenotoxic properties of lactic acid bacteria *in vivo* in the gastrointestinal tract of rats. Nutr Cancer 20(3):271–281, 1993
47. Wilson KH, Perini F: Role of competition for nutrients in suppression of *Clostridium difficile* by the colonic microflora. Infect Immunol 56:2610–2614, 1988
48. Weinberg ED: Iron withholding: A defense against infection and neoplasia. Physiol Rev 64(1):65–102, 1984
49. Bellamy W, Takase M, Yamauchi K, Wakabayashi H, Kawase K, Tomita M: Identification of the bacteriocidal domain of lactoferrin. Biochim Biophys Acta 1121(1–2):130–136, 1992
50. Rasic J, Kurmann J: *Bifidobacteria* and their role. Experentia (Suppl) 39:1–295, 1983
51. Santulli TV, Shullinger JN, Heird WC, Gongaware RD, Wigger J, Barlow B, Touloukian RJ, Amoury RA: Acute necrotizing enterocolitis in infancy: A review of 64 cases. Pediatrics 55:376–387, 1975
52. Duffy LC, Zielezny MA, Carrion V, Griffiths E, Dryja D, Hilty M, Cummings J, Morin F: Bacterial toxins and enteral feeding of premature infants at risk for necrotizing enterocolitis. Am J Hum Biol 10:211–219, 1998
53. Duffy LC, Zielezny MA, Carrion V, Griffiths E, Dryja D, Hilty M, Rook C, Morin F: Concordance of bacterial cultures with endotoxin and interleukin-6 in necrotizing enterocolitis. Dig Dis Sci 42(2):359–365, 1997
54. Gibson GR, Beatty EB, Wang X, Cummings JH: Selective stimulation of *Bifidobacteria* in the human colon by oligofructose and inulin. Gastroenterology 108:975–982, 1995
55. Lee YK, Salminen S: The coming of age of probiotics. Trends Food Sci Technol 6:241–245, 1995
56. Gibson GR, Roberfroid MB: Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J Nutr 125(6):1401–1412, 1995
57. Roberfroid MB: Functional effects of food components and the gastrointestinal system: Chicory fructooligosaccharides. Nutr Rev 54(11):538–542, 1996
58. Howard MD, Gordon DT, Garleb KA, Kerley MS: Dietary fructooligosaccharide, xylooligosaccharide and gum arabic have variable effects on cecal and colonic microbiota and epithelial cell proliferation in mice and rats. J Nutr 125(10):2604–2609, 1995
59. De Vuyst L, Vandamme EJ (eds): Bacteriocins of Lactic Acid Bacteria. Glasgow, Blackie Academic and Professional, 1994
60. Schiffrin EJ, Rochat F, Link-Amster H, Aeschlimann JM, Donnet-Hughes A: Immunomodulation of human blood cells following ingestion of lactic acid bacteria. J Dairy Sci 78(3):491–497, 1995