Therapeutic Microbiology: Probiotics and Related Strategies
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3.3. SYNBIOTICS

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24 Synbiotics in Human Medicine

Thirty years have passed since Gilliland and Speck reported that patients with inflammatory bowel disease (IBD) had a significantly different microbiota from that of healthy individuals (Gilliland and Speck, 1977). Finegold and Sutter reported in the following year an altered microbiota in 75% of healthy omnivorous and 35% of vegetarian Americans (Finegold and Sutter, 1978). Similar observations were later made for European populations (Ahrne et al., 1998).

Numerous attempts during the last 30 years to reconstitute or remodel the microbiota in order to prevent or treat diseases were repeatedly made. However, these often produced dissatisfying results. One obvious explanation suggested by recent reviews (Sartor, 2004; Marteau, 2006) is that the majority of clinical studies thus far have been underpowered.

FACTORS INFLUENCING CLINICAL STUDY OUTCOMES

Several factors might contribute to differences in the outcome of interventions with probiotics and prebiotics.

Regenerative Capacity

The spontaneous regenerative capacity of the gastrointestinal tract is much greater in young experimental animals and in animals with induced disease. Regenerative capacity is greater in humans with acute disease than in humans with chronic disease.

Differences in Daily Doses

The daily dose related to body weight or to the gastrointestinal mucosal surface is generally much larger in experimental animals and in pediatric cases. In the majority of studies, the daily dose used in humans has been 1 billion lactic acid bacteria (LAB) once or twice per day, up to 10 billion organisms/day. Larger doses delivered more impressive results. Large-scale doses in liver transplantation (Rayes et al., 2005) and trauma (Spindler-Vesel et al., 2007) with Synbiotic 2000 and Synbiotic 2000 Forte (see below) included 40 and 400 billion LAB per day. In IBD, VSL#3 was administered at a dosage of 1,200 billion LAB per day (Venturi et al., 1999). A total of 80 billion LAB of Synbiotic 2000 per day were administered to patients with chronic liver disease, according

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to recent reviews (Liu et al., 2004; Riordan et al., 2007). Large differences in the relative abilities of various LAB to survive the harsh environment of the upper gastrointestinal tract explain why supplemented LAB may reach the lower gastrointestinal tract in concentrations too low to generate clinical effects (Miettinen et al., 1998). Some of the strains tested (*Lactobacillus plantarum*) demonstrated, however, an increased ability to influence cytokine production and modulate inflammation.

Variability of Probiotic Strains

Probiotic strains vary with respect to efficacy or potency. Studies using LAB have focused on LAB commonly used by the dairy industry and have failed to demonstrate positive effects either in connection with elective surgery (Woodcock et al., 2004) or with intensive care unit (ICU) patients (Jain et al., 2004). In these studies a composition of LABs consisting of *Lactobacillus acidophilus* LA5, *Bifidobacterium lactis* BP12, *Streptococcus thermophilus*, and *Lactobacillus bulgaricus* (TREVIS; Chr. Hansen, Hørsholm, Denmark) was used. Although the treatments in both studies favorably influenced the microbial composition of the upper gastrointestinal tract, probiotics did not influence intestinal permeability, nor were they associated with measurable clinical benefits. Other studies have demonstrated the inability of *Lactobacillus rhamnosus* GG to affect human antibiotic-associated diarrhea caused by *Clostridium difficile* (Thomas et al., 2001) and *Helicobacter pylori* infections (Armuzzi et al., 2001a, 2001b).

LAB and probiotics derived from LAB differ with respect to relative abilities to modulate the innate immune system and to control disease. The strains with the greatest capacity to induce interleukin-12 (IL-12) seem to be the most effective probiotics to up-regulate major histocompatibility complex class II and B7-2 (CD86), indicative of immune cell maturation (Armuzzi et al., 2001b). Striking differences in lactobacillus production of IL-12 and tumor necrosis factor (TNF) were reported, with production ranging in descending order from strong (*Lactobacillus casei*) to somewhat strong *L. plantarum* Lb1 to weak (*L. fermentum* Lb20, *L. johnsonii* La1, and *L. plantarum* 299v) to none (*L. reuteri*). The ability to control various pathogens is also strain specific and seems to be limited to a few strains. For example, when the ability of 50 different LAB to control 23 different pathogenic *C. difficile* strains was tested, only 5 probiotics (LABs) proved effective against all *C. difficile* isolates and 18 strains were antagonistic versus a subset of *C. difficile* strains, but as many as 27 candidate probiotics were totally ineffective (Naaber et al., 2004). The five most effective strains demonstrating potent inhibition against *C. difficile* were *L. paracasei* subsp. paracasei (two strains) and *L. plantarum* (three strains). *L. paracasei* subsp. *paracasei* proved to be the most potent inducer of Th1 cytokines and potently repressed Th2 cytokines when more than 100 LAB organisms were compared with each other (Fujiwara et al., 2004).

Multispecies Communities

A combination of several probiotic bacteria may be necessary as probiotic bacteria function in vivo as multispecies communities. The microbiota consists of approximately 800 or more different bacterial species with at least 40 predominant species. The human microbiota functions like an organ, and different bacterial species interact as a microbial consortium with the host mucosa. The microbiota constitutes a good example of both symbiosis (living together for mutual benefit) and synergy (increased potency that exceeds additive effects). As the knowledge about the mammalian microbiota expands, the scientific community can progress from a probiotic soloist (single-strain) strategy to a "chamber orchestra" of probiotics (multistrain) to a full probiotic or synbiotic "symphony orchestra." Solo or multiprobiotic strategies may be relevant for different health promotion or disease prevention strategies. Such multistrain formulations might benefit from the addition of prebiotics, micronutrients, or other plant-derived products.

The effects of treatment may vary depending on the application of single-strain or multistrain probiotics. Total microbiota replacement may include the transfer of donated microbiota (feces) from one individual to another. Such an approach will most likely never become widely accepted therapy, but dramatic effects have been reported (Borody et al., 2003, 2004). Multistrain probiotics have been reported to reduce antibiotic-associated diarrhea, prevent enteric infections (*Salmonella enterica* serovar Typhimurium), and reduce pathogenic colonization (*Escherichia coli*) (Timmerman et al., 2004). However, limited knowledge and experience have restricted efforts to construct formulas for supplementation of microbiota to more than a handful of LAB. In my experience, multistrain formulations, including more than four or five strains, may at present not provide additional value.

Suboptimal Supply of Nutrients

The supply of substrate/nutrients for growth and function of LAB might not be optimal. The colonic mucosa has a limited ability to derive nourishment from the circulation and depends on specific nutrients, especially short-chain fatty acids (SCFAs) and plant-derived antioxidants produced by microbial enzymes. The microbiota receives its nutrition from sloughed gastrointestinal epithelia, gastrointestinal secretions, and dietary components. A well-functioning microbiota is key to the body's ability to spare nitrogen, and large amounts of nitrogen are absorbed and reused by the body's protein synthesis machinery. The

feeding formulas commonly given to critically ill and other patient groups usually contain relatively small amounts of fiber. Furthermore, processing of foods reduces the ingestion of plant-associated probiotic microbes naturally supplied by eating of raw plants. The degree of milling of grains and mastication strongly influences the proportion of plant food and fiber reaching the colon. Large particles travel more rapidly through the gut. A reduced degree of milling and less mastication will reduce the degree of digestibility by eukaryotic enzymes and increase the amount of food left for fermentation in the colon.

The Influence of Diet

Diet may aggravate systemic inflammation and poses challenges for probiotics/synbiotics. The modern Western diet is based on nutrients received from only a small number of plants, with 80% of the nutrients derived from 17 plants and 50% of the calories from eight grains. Furthermore, foods in the developed world may be extensively processed, which can potentially reduce their nutritional value. Examples of nutrients and antioxidants that do not resist heating and drying are important amino acids such as glutamine, fuel for intestinal epithelial cells, and the "master antioxidant" glutathione. In addition, manipulation of food, especially heating to extreme temperatures, increases the content of unwanted proinflammatory ingredients such as oxidized or *trans*-fatty acids. Heating, ionization, and irradiation of food result in increased production of proinflammatory proteins called Maillard products, otherwise known as advanced glycation or advanced lipoxidation end products. Foods rich in these end products include dairy products such as powdered milk (frequently used in enteral nutrition, infant formulas, and ice cream), cheeses, bakery products (bread crusts, crisp breads, pretzels, and biscotti) and cereals, overheated (especially deep-fried and oven-fried) meat, poultry, and fish, caffeinated drinks including coffee and soda, Chinese soy and balsamic products, and smoked foods in general (Goldberg et al., 2004; Bengmark, 2007). The consumption of such highly processed foods has increased dramatically in recent decades, in parallel to the rise of endemic chronic diseases. The anti-inflammatory effects of beneficial bacteria may counteract dietary influences on immune responses.

CLINICAL EXPERIENCE WITH SUPPLEMENTED PREBIOTICS

Prebiotics in Constipation

Chronic constipation is a common disorder in the developed world. Its etiology remains unclear despite numerous clinical, pathophysiologic, and epidemiologic studies, but a high intake of dairy products and plant fibers may play a significant role in its pathogenesis. A randomized case-control study compared 291 children with idiopathic chronic constipation and 1,602 healthy controls (Kaplan and Hutkins, 2000). Constipation was negatively correlated with a low intake of cellulose and pentose fiber ($P < 0.001$). Fructo-oligosaccharides (FOS) may have potential benefits in constipation due to their soluble dietary fiber-like properties. In a recent study, a total of 56 healthy infants (ages 16 to 46 weeks; mean age, 32 weeks) were randomly assigned to receive either FOS (0.75 g) or a placebo added to one serving of cereal per day for 28 days (Roma et al., 1999). The mean number ($\pm$ standard deviation [SD]) of stools per infant was 1.99 ± 0.62 per day in the FOS-supplemented group compared with 1.58 ± 0.66 in the control group ($P = 0.02$).

Prebiotics To Prevent and Treat Diarrhea

In a large randomized study with acutely ill medical and surgical patients, some individuals requiring enteral nutrition received a supplementation of hydrolyzed guar gum for 5 days and were compared with individuals receiving fiber-free enteral nutrition. The incidence of diarrhea was 9% in the group receiving fiber supplementation, compared with 32% in the group treated with fiber-free nutrition ($P > 0.05$) (Moore et al., 2003). One effect of fiber, especially oligosaccharides, is increased bioavailability and absorption of zinc. In a randomized study including children 3 to 59 months of age in Bangladesh, zinc supplementation was proven to be effective for reducing the incidence and duration of diarrhea (Rushdi et al., 2004). In another study in Bangladesh, unripe banana (250 g/liter; equivalent to two fruits) or pectin (2 g/kg of food) was supplemented to a rice diet given to children suffering from persistent diarrhea (Baqui et al., 2002). The amounts and frequency of stools, the duration of diarrhea, vomiting frequency, and utilization of oral and intravenous rehydration solutions were significantly reduced with supplementation of either unripe banana or pure pectin. Recovery by 3 days was observed in 59% of children in the unripe banana group and in 55% of children in the pectin group, compared with 15% of children in the control group, receiving only rice.

Prebiotics To Support Mineral Absorption

Recent studies suggest that increased intake of plant fibers, fruits, and vegetables is associated with increased bone mineral density in male and female elderly subjects (Tucker et al., 1999; Rabbani et al., 2001). Calcium absorption, bone calcium content, bone mineral density, bone balance, and bone formation/bone absorption index were significantly increased following 3 weeks of

supplementation of a mixture of inulin and FOS (Tucker et al., 2002).

Prebiotics To Control Weight

The effects of dietary fiber on hunger and weight loss were studied approximately 20 years ago. One hundred eight of 135 members completed the trial including 23 controls, 45 individuals consuming ispaghula granulate, and 40 persons ingesting bran sachets (Hylander and Rossner, 1983). Both fiber preparations reduced hunger at all meals. The mean (±SD) weight reductions during the trial were 4.6 ± 2.7 kg for the controls, 4.2 ± 3.2 kg for the ispaghula group, and 4.6 ± 2.3 kg for the bran group ($P > 0.05$ for both groups). Although supply of dietary fiber immediately before meals did reduce the feeling of hunger, this intervention did not provide any additional benefits for weight reduction. A recent crossover study compared the effects on satiety with dietary supplementation of fermentable fiber (pectin, beta-glucan; 27 ± 0.6 g/day) versus similar amounts of nonfermentable fiber (methylcellulose). Daily satiety was significantly greater with nonfermentable (methylcellulose) than with fermentable fibers (beta-glucan, pectin; $P = 0.01$), but no differences were observed in daily energy intake or loss of body weight or body fat (Howarth et al., 2003).

Prebiotics in IBD

Although both patients with IBD and patients with irritable bowel syndrome (IBS) may consume insufficient amounts of dietary fiber, little evidence exists that lack of dietary fiber contributes to the pathogenesis of IBD or IBS. The ability of maintaining remission in patients with ulcerative colitis by a daily supply of *Plantago ovata* seeds (10 g per day; also called psyllium or ispaghula husk) was compared with daily treatment of mesalamine (500 mg/day) and a combination of mesalamine and *P. ovata* seeds (Fernandez-Banares et al., 1999). Twelve months of treatment failed to demonstrate any difference in clinical benefits among the three groups. Germinated barley foodstuff, a by-product from breweries that is rich in hemicellulose and glutamine, was administered to 39 patients with mild-to-moderately active ulcerative colitis (Kanauchi et al., 2001). Daily supply of germinated barley foodstuff (30 g/day) significantly reduced disease activity, increased SCFA concentrations, and increased the numbers of *Bifidobacterium* and *Eubacterium* isolated from stool specimens. Observed effects were probably due to the increased supply of glutamine and other antioxidants such as B complex vitamins rather than the fiber. Glutamine and other antioxidants attenuated proinflammatory cytokines such as TNF and enhanced release of heat shock proteins (HSP-72) (Wischmeyer et al., 2003). A controlled study using oat bran as a fiber source was recently reported, involving 22 patients and 10 controls with quiescent ulcerative colitis. Daily supply with as much as 60 g of oat bran (equivalent to 20 g of dietary fiber) for 3 months resulted in significantly increased quantities of fecal butyrate and diminished abdominal pain. Most patients tolerated elevated quantities of dietary fiber, and signs of disease relapse were not seen in any patients with colitis (Hallert et al., 2003). Butyrate inhibited nuclear factor (NF) κB activation of lamina propria macrophages, reduced the number of neutrophils in crypts and the epithelium, and reduced the density of lamina propria lymphocytes/plasma cells in patients with ulcerative colitis (Luhrs et al., 2002), correlating findings with observed reductions in disease activity. Twenty patients with ileal pouch-anal anastomosis received inulin daily for 2 weeks (24 g/day). Significant reductions in inflammation were observed by endoscopy and histology. In addition to histology, significantly increased fecal concentrations of butyrate, reduced fecal pH, reduced fecal content of secondary bile acids, and diminished growth of *Bacteroides fragilis* were observed with prebiotic inulin supplementation (Welters et al., 2002).

Prebiotics in IBS

Some evidence suggests that various dysmotility disorders including gastroesophageal reflux problems, infantile colic, and constipation manifest food-related features and may be due to intolerance of proteins in cow's milk (Murch, 2000). IBS is a clinical diagnosis based on the occurrence of abdominal distension, abdominal cramps, more frequent stools, and relief of pain on defecation. The prevalence of the syndrome varies between 7 and 22%, making IBS the most common functional gastrointestinal disorder (Bommelaer et al., 2002). A study published in 1990 reported that supplementation of diet with corn (20 g of fiber per day) would significantly improve IBS, ameliorate pain, and increase frequency of stools and may reduce rectosigmoid pressure (Cook et al., 1990). A recent meta-analysis based on 17 studies did conclude that fiber supplementation is generally effective for relief of global symptoms of IBS (relative risk, 1.33; 95% confidence interval [CI], 1.19 to 1.50) (Bijkerk et al., 2004). Patients with constipation-predominant IBS appeared to receive benefit from fiber intake (relative risk, 1.56; 95% CI, 1.21 to 2.02), while fiber seemed to be ineffective for relief of IBS-associated abdominal pain. Clinical improvement was observed only with soluble fiber (psyllium, ispaghula, and calcium polycarbophil) (relative risk, 1.55; 95% CI, 1.35 to 1.78), whereas insoluble fiber (corn and wheat bran) occasionally worsened

clinical outcome. Beneficial effects of supplementation with soluble fiber (guar gum) were also observed in a recent study of 188 adult patients with IBS (Parisi et al., 2002).

Prebiotics To Control Infections

In an effort to prevent nosocomial pneumonia and sepsis, patients with severe multiple trauma were treated with beta 1-3 polyglucose (glucan), a component of cell walls of plants and microbes (de Felippe Junior et al., 1993). Pneumonia occurred in 2 of 21 glucan-treated patients and 11 of 20 control patients ($P < 0.01$). Infectious complications (pneumonia or general sepsis) occurred in 14% of glucan-supplemented patients versus 65% of individuals in the control group ($P < 0.001$). Another study compared the effects of a high-protein formula enriched with fiber, arginine, and antioxidants with those of a standard high-protein formula given as early enteral nutrition to critically ill patients (Caparros et al., 2001). The supplemented group had, in comparison to nonsupplemented controls, a lower incidence of catheter-related sepsis (0.4 episodes/1,000 ICU days) than the control group (5.5 episodes/1,000 ICU days) ($P < 0.001$), but no differences were observed between the groups in incidence of ventilator-associated pneumonia, surgical infections, bacteremia, urinary tract infections, mortality, or long-term survival.

LAB AND VITAMINS/ANTIOXIDANTS

LAB produce important vitamins and antioxidants. One important example is the essential B vitamin folate, which is known to reduce homocysteine quantities and may prevent some chronic diseases. Folate is synthesized by LAB such as *Lactococcus lactis* and *L. plantarum*. Other LAB, however, such as *Lactobacillus gasseri*, are net consumers of folate. A recent publication describes the successful transfer of five genes essential for folate biosynthesis from *L. lactis* to *L. gasseri*, converting *L. gasseri* into a net producer of folate (Wegkamp et al., 2004). Anemia, iron deficiency, and folate deficiency are common among patients with IBD (Pironi et al., 1988; Gasche et al., 2004). Plasma total homocysteine (tHcy) concentrations were examined in a pediatric study of 43 patients and 46 controls and shown to be significantly higher in children with IBD than in control subjects ($P < 0.05$). Furthermore, the level of plasma tHcy correlated well with observed reductions in plasma 5-methyltetrahydrofolate ($P < 0.0005$) (Nakano et al., 2003). A similar study with 108 adult patients with IBD and 74 adult healthy controls yielded significantly lower levels of folate in patients with IBD ($P < 0.05$) (Koutroubakis et al., 2000). The mean concentration of tHcy in serum was significantly higher in patient groups with ulcerative colitis (15.9 ± 10.3 mmol/liter) and patients with Crohn's disease (13.6 ± 6.5 mmol/liter) than in controls (9.6 ± 3.4 mmol/liter) ($P < 0.05$).

COMBINING PREBIOTICS AND PROBIOTICS

Prebiotics and LAB (probiotics) have demonstrated beneficial effects with respect to the function of innate immunity, intestinal barrier function, and increased resistance to disease. The gut mucosa and microbiota are intimately linked in the maintenance of a functional interface between the host and the external environment (Henke and Bassler, 2004; Sansonetti, 2004). The hope is that a combined supply of prebiotics and probiotics (synbiotics) shall have synergistic effects in enhancing immunity and facilitating intestinal barrier function.

The term "defense by diversity" was coined in 1999 (Hill, 1999) and seems applicable to synbiotic treatment. Natural foods may contain both LAB, fiber, and prebiotic components. A recent study concluded that combining fiber has more than additive effects on the functions of microbial ecosystems and host immune responses (Peuranen et al., 2004). A recent review suggests that multispecies probiotics may be superior to single-species probiotics in reducing antibiotic-associated diarrhea, preventing infections (*S. enterica* serovar Typhimurium), and reducing pathogenic colonization (*E. coli*) (Timmerman et al., 2004). The choice of prebiotics and probiotics must be based on scientific evidence, and LAB may have variable effects on immune function and outcome. One consideration is that most LAB have limited abilities to ferment bioactive fibers such as inulin or phlein, variable abilities to adhere to human mucus, low antioxidant capacity, and differences with respect to survival in acid conditions or presence of bile in the gastrointestinal tract. LAB selected for synbiotic studies should be selected for functional activities in the context of a specific combination formulation with prebiotics. Unfortunately, few studies have closely examined potentially synergistic effects of simultaneous administration of synbiotics containing LAB (or other probiotics) and prebiotics.

Synbiotic 2000 consists of a mixture of 10^{10} CFU (or Synbiotic Forte with 10^{11} CFU) of each of four LAB species, including *Pediacoccus pentosaceus*, *Leuconostoc mesenteroides*, *L. paracasei* subsp. *paracasei*, and *L. plantarum*, and 2.5 g of each of the four fermentable fibers or prebiotics including beta-glucan, inulin, pectin, and resistant starch (Medipharm AB, Kågeröd, Sweden, and Des Moines, IA).

Microbiologists Åsa Ljungh and Torkel Wadström at Lund University developed this multicomponent synbiotic formula, which has been extensively used in clinical trials. The choice of LAB for the formulation was finalized after extensive studies of >350 human microbial strains (Kruszewska et al., 2002) and >180 plant microbial strains (Ljungh et al., 2002). Strain selection was based on the ability of LAB to produce bioactive proteins, induce NF-κB signaling, stimulate pro- and anti-inflammatory cytokines, enhance antioxidants, and functionally complement each other. In recent studies both the Synbiotic 2000 Forte and a Probiotic 2000 Forte (no fiber added), containing 10^{11} CFU of each of the four LAB (e.g., 400 billion LAB per dose), have been tested clinically.

Synbiotics and Pancreatitis

Sixty-two patients with severe acute pancreatitis (mean ± SD Apache II scores: Synbiotic 2000-treated, 11.7 ± 1.9; controls, 10.4 ± 1.5) received either two sachets per day of Synbiotic 2000 (80 billion LAB/day) and 20 g of fiber/day or the same amounts of fiber (20 g per day) as in Synbiotic 2000 during the first 14 days after hospital admission (Olah et al., 2007). Notably, 9 of 33 patients (27%) in the Synbiotic 2000-treated group and 15 of 29 patients (52%) in the fiber-only-treated group developed subsequent infections. Consistent with infection data, 8 of 33 (24%) Synbiotic 2000-treated and 14 of 29 (48%) of the fiber-only-treated patients developed systemic inflammatory response syndrome, multiorgan failure, or both ($P < 0.005$) (Olah et al., 2007). Seven pathogenic microorganisms were cultivated in the synbiotic-treated group compared to 17 in the fiber-only group (Table 1).

Synbiotics and Trauma

Two prospective randomized trials involving polytrauma patients, the study using Synbiotic 2000 and the other using Synbiotic 2000 Forte, have been concluded. The first study compared diets including either Synbiotic 2000 (40 billion LAB/day), soluble fiber, a peptide diet, or supplementation with glutamine for patients with acute extensive trauma. Treatment with Synbiotic 2000 resulted in a highly significant reduction in the number of chest infections (4 of 26 or 15% of patients), compared to patients on the peptide diet (11 of 26 or 42% of patients, $P < 0.04$), glutamine diet (11 of 32 or 34% of patients, $P < 0.03$), or fiber-only diet (12 of 29 or 41% of patients, $P < 0.002$) (Spindler-Vesel et al., 2007). The total number of infections was significantly decreased in the Synbiotic 2000 with only 5 of 26 patients developing infections (19%) versus 17 of 29 patients (59%) in the fiber-only group, 13 of 26 patients (50%) on a peptide diet, and 16 of 32 patients (50%) receiving glutamine.

In another study, 65 polytrauma patients were randomized to receive one dose daily of Synbiotic 2000 Forte (400 billion LAB + 10 g of fiber, see above) for 15 days or maltodextrin as a placebo. Significant reductions were observed in patient mortality (5 of 35 versus 9 of 30, $P < 0.02$), severe sepsis (6 of 35 versus 13 of 30, $P < 0.02$), chest infections (19 of 35 versus 24 of 30,

Table 1 Pathogens isolated from acute pancreatitis patients receiving synbiotic treatment or fiber-only treatment[a]

	No. of patients infected	
Microorganism(s) isolated	Synbiotic 2000 group	Fiber-only group
Pseudomonas aeruginosa	1	4
E. faecalis	1	2
Enterobacter spp.	1	1
Streptococcus spp.	2	
S. aureus	1	1
E. faecium	1	
Candida spp.		2
Staphylococcus haemolyticus		1
Serratia spp.		2
Klebsiella spp.		1
E. coli		1
Stenotrophomonas maltophilia		1
Citrobacter freundii		1
Total	7	17

[a]Data from Olah et al., 2007.

Table 2 Pathogens isolated from polytrauma patients receiving synbiotic treatment or fiber-only treatment[a]

Microorganism(s) isolated	No. of patients infected	
	Synbiotic 2000 group	Fiber-only group
Acinetobacter baumannii	21	35
Candida albicans	7	17
Pseudomonas aeruginosa	15	14
Staphylococcus epidermidis	2	10
S. aureus	4	7
Staphylococcus hominis		2
Enterobacter aerogenes		2
Staphylococcus haemolyticus		1
Serratia spp.		2
Klebsiella spp.	5	12
Proteus spp.		1
Total	54	103

[a]Data from Kotzampassi et al., 2006.

$P < 0.03$), central line infections (13 of 32 versus 20 of 30, $P < 0.02$), and ventilation days (average, 15 versus 26 days). Pathogenic microorganisms were cultivated from 54 individuals in the synbiotic-treated group compared with 103 individuals in the fiber-only group (Table 2) (Kotzampassi et al., 2006).

Synbiotics and Surgical Patients

In a randomized controlled study, 45 patients undergoing major surgery for abdominal cancer were divided into three treatment groups including patients receiving enteral nutrition (EN) plus Synbiotic 2000 (LEN), EN plus fiber (FEN) in the same amount (20 g per day), or standard parenteral nutrition (PN). Synbiotic treatment lasted for two preoperative and seven postoperative days. The incidence of postoperative bacterial infections was 47% with PN, 20% with FEN, and 6.7% with LEN ($P < 0.05$) (H. Chunmao, R. Martindale, H. Huang, and S. Bengmark, in preparation). A total of 34 pathogenic microorganisms were cultivated in the synbiotic-treated group compared with 54 pathogens in the fiber-only group (Table 3). Significant improvements were also documented in prealbumin (LEN and FEN), C-reactive protein (LEN and FEN), serum cholesterol (LEN and FEN), peripheral leukocyte count (LEN), serum endotoxin (LEN and FEN), and serum immunoglobulin A (LEN).

In another prospective randomized double-blind trial, 80 patients subjected to pylorus-preserving pancreatoduodenectomy received either Synbiotic 2000 (40 billion LAB twice per day) or fiber only twice daily beginning on the day before surgery and continuing for the first seven postoperative days (Rayes et al., 2007). A highly significant difference in infection rate ($P = 0.005$) was observed, as only 5 of 40 (12.5%) patients in the Synbiotic 2000-treated group suffered infections (four wound infections and one urinary tract infection) versus 16 of 40 (40%) patients in the fiber-only group (six wound infections, five cases of peritonitis, four chest infections, two cases of sepsis, and either urinary tract infection, cholangitis, or empyema). The infecting microorganisms in the synbiotic-treated group were *Klebsiella pneumoniae* (two patients), *Enterobacter cloacae* (two patients), *Proteus mirabilis* (one patient), and *Enterococcus faecalis/faecium* (one patient). In the fiber-only group, pathogens included *E. cloacae* (eight patients), *E. faecalis/faecium* (seven patients), *E. coli* (seven patients), *K. pneumoniae* (two patients), *Staphylococcus aureus* (two patients), and *P. mirabilis* (one patient) (Table 4). Statistically significant differences between the groups were observed in the duration of antibiotic utilization (mean ± SD for the Synbiotic 2000 group, 2 ± 5 days; for the fiber-only group, 10 ± 14 days).

Synbiotics and Liver Disease

Fifty-eight patients with hepatic cirrhosis diagnosed with minimal encephalopathy were randomized into three treatment groups. Group 1 (20 patients) received Synbiotic 2000 (40 billion LAB), group 2 (20 patients) received the same amount of prebiotic/fiber component as in Synbiotic 2000 only, and group 3 (15 patients) received a placebo (nonfermentable, nonabsorbable fiber [crystalline cellulose]) (Liu et al., 2004). A significant increase in intestinal LAB was observed after 1 month of supplementation in the synbiotic-treated group, in contrast to the other two groups. Intestinal pH was significantly reduced in both treatment groups, but pH was not reduced in the placebo-treated group. Significant

Table 3 Pathogens recovered from synbiotic-treated patients versus patients receiving only fiber and undergoing surgery for abdominal cancer[a]

Microorganism(s) isolated	No. of patients infected	
	Synbiotic 2000 group	Fiber-only group
Pseudomonas aeruginosa	17	24
S. aureus	8	11
Staphylococcus epidermidis	1	1
Staphylococcus faecalis		1
E. cloacae	4	
Acinetobacter spp.	2	3
Staphylococcus haemolyticus		1
Serratia spp.		2
Klebsiella spp.		1
P. mirabilis		2
Candida albicans	2	6
Aspergillus spp.		
Bacillus subtilis		1
Klebsiella spp.		1
Total	34	54

[a]Data from Chunmao et al., in preparation.

reductions in fecal counts of *E. coli*, *Staphylococcus* spp., and *Fusobacterium* spp. but not of *Pseudomonas* spp. or *Enterococcus* spp. were reported in the synbiotic-treated group. Significant reductions in ammonia, endotoxin, alanine aminotransferase, and bilirubin levels were observed in the synbiotic-treated group compared to the fiber-only and placebo groups. The improvements in liver function were accompanied by significant improvements in performance of psychometric tests and degree of encephalopathy.

In a follow-up study, 30 patients with hepatic cirrhosis were randomized to receive either Synbiotic 2000 or a placebo (crystalline cellulose) for 7 days (Riordan et al., 2007). Viable fecal counts of *Lactobacillus* species, stage of liver disease (Child-Pugh classification), plasma retention rate of indocyanine green (ICG_{R15}), whole-blood TNF and IL-6 mRNA, serum TNF, soluble TNF receptor I (sTNFRI), soluble TNF receptor II (sTNFRII), and plasma endotoxin levels were evaluated pre- and post-treatment. Synbiotic treatment was associated with significantly increased fecal lactobacilli counts and significant improvements in ICG_{R15} and Child-Pugh classification. No significant changes in any study parameter followed the placebo treatment, but significant elevations in whole-blood TNF and IL-6 mRNA, in addition to concentrations of soluble sTNFRI and sTNFRII in serum, were observed in synbiotic-treated patients. TNF and IL-6 levels correlated significantly with each other, both at baseline and after the synbiotic treatment. Synbiotic-related improvements in ICG_{R15} were significantly associated with changes in IL-6 and unrelated to plasma endotoxin values. Short-term synbiotic treatment may significantly modulate gut microbial function and improve hepatic function in patients with cirrhosis. The observed benefits seemed unrelated to a reduction in endotoxemia but could be mediated, at least in part, by treatment-related induction of IL-6 synthesis by TNF. These results offer hope that synbiotic treatment administered to patients awaiting liver transplantation might prevent septic episodes, improve liver function, and promote patient outcomes.

Table 4 Pathogens isolated from patients treated with synbiotics versus patients receiving only fiber and undergoing pancreatectomy[a]

Microorganism isolated	No. of patients infected	
	Synbiotic 2000 group	Fiber-only group
E. cloacae	2	8
E. faecalis/faecium	1	7
E. coli	0	7
K. pneumoniae	2	2
P. mirabilis	1	1
S. aureus	0	2
Total	6	27

[a]Data from Rayes et al., 2007.

Sixty-six patients were randomized to receive either Synbiotic 2000 or fiber only in connection with human orthotopic liver transplantation. The treatment started

Table 5 Pathogens isolated from patients treated with synbiotics versus patients receiving only fiber and undergoing liver transplantation[a]

Bacterium isolated	No. of patients infected	
	Synbiotic 2000 group	Fiber-only group
E. faecalis	1	11
E. coli	0	3
E. cloacae	0	2
Pseudomonas aeruginosa	0	2
S. aureus	0	1
Total	1	18

[a]Data from Rayes et al., 2005.

on the day before surgery and continued for 14 days after surgery. During the first postoperative month, only one patient in the synbiotic-treated group (3%) showed signs of infection (urinary tract infection) compared to 17 of 33 (51%) patients supplemented with multiple fiber components (Rayes et al., 2005). A single pathogenic microorganism (*E. faecalis*) was cultivated in the synbiotic-treated group compared with 18 pathogens in the fiber-only group (Table 5). The duration of antibiotic utilization was only 0.1 day (± 0.1 day) in synbiotic-treated patients compared with 3.8 days (± 0.9 day) in the fiber-only group.

Synbiotics and IBD

Daily rectal instillations with Synbiotic 2000 reconstituted in saline were administered to 10 patients with distal colitis during 2 weeks. Synbiotic-treated patients demonstrated dramatic improvements in various disease scores, such as episodes of diarrhea (initially 2.4, decreased to 0.8), visible blood in stool (2.2 to 0.8), nightly diarrhea (0.5 to 0), urgency (1.9 to 1.0), and stool consistency (1.1 to 0.8) (Pathmakanthan et al., 2002). Two patients reported significant bloating, but other adverse or side effects were not reported. In a pilot study (Furrie et al., 2005), nine patients with active ulcerative colitis received a synbiotic composed of freeze-dried *Bifidobacterium longum* (4×10^{11} CFU) and a prebiotic FOS/inulin mixture (6 g) daily for 4 weeks. Nine patients received a placebo consisting of powdered maltodextrose (6 g per day). The quantities of intestinal bifidobacteria were increased 42-fold compared to 4.6-fold in the placebo group. The sigmoidoscopy score, based on clinical assessment of disease activity (Baron et al., 1964), decreased by an average of 1.3 units compared to an increase of 0.58 units in the placebo group ($P = 0.06$). The mean histology score was diminished in the synbiotic group and increased in the placebo group. However, the total number of patients was small ($n = 8$), and the results were not statistically significant. The bowel habit index scores decreased by 20.4% in the synbiotic group, and the scores increased by 70.4% in the placebo group. Human beta-defensin 2, 3, and 4, TNF, and IL-1 were reduced after synbiotic treatment but remained unchanged in the placebo group ($P = 0.05$). As stated by Aberra (Aberra, 2005), "slowly, the links of diet to the intestinal environment and the association of the intestinal environment to IBD are becoming evident. The prebiotic and probiotic trials reveal the importance of the intestinal environment as a potent regulator of IBD activity." Seven malnourished patients with short bowel syndrome and refractory enterocolitis were treated for more than 1 year with a synbiotic formulation consisting of *Bifidobacterium breve*, *L. casei*, and galactooligosaccharides. Synbiotic treatment reportedly enhanced the function and composition of the intestinal bacterial microbiota and increased the content of SCFAs in feces (from 27.8 to 65.09 μmol/g of wet feces) (Kanamori et al., 2004).

Synbiotics and IBS

The effects of twice-daily consumption of a probiotic fruit drink, ProViva (Skånemejerier, Malmo, Sweden), containing *L. plantarum* 299v (6×10^7 CFU/drink) or a placebo for 4 weeks were studied in a controlled study including 40 patients (Nobaek et al., 2000). The vast majority (95% of LAB-treated versus 15% of the placebo-treated patients) of individuals in the probiotic consumption group reported general improvement. A total of 20 of 20 patients in the LAB-supplemented group and 11 of 20 patients in the placebo group ($P = 0.0012$) reported resolution of abdominal pain. A similar study, using the same formula, was performed with patients who received the treatment for 4 weeks. A significant enhancement of LAB composition in probiotics-supplemented patients was described. Flatulence was rapidly and significantly reduced in the LAB-treated group, but no difference in bloating was reported between the groups (Sen et al., 2001). The same formula was applied in a crossover trial of 4 weeks of duration involving 12 patients. A significant reduction in breath hydrogen was registered after 2 h of ingestion, without a change in total hydrogen production or any symptomatic improvement (Madden and Hunter, 2002). Several studies have been performed with probiotics including one trial with synbiotics (for further details, see Young and Cash, 2006). Sixty-eight patients with IBS were treated for 12 weeks with a vitamin- and plant fiber-enriched diet containing either live or heat-inactivated LAB including 10^9 each

of *L. acidophilus*, *L. helveticus*, and *Bifidobacterium* spp. (Tsuchiya et al., 2004). Eighty percent and 40% of the patients, respectively, reported significant improvements in pain, bloating, constipation, and bowel habits ($P < 0.01$).

Synbiotics and Short Bowel Syndrome

Seven malnourished patients (aged 2.5 to 24 years) with short bowel syndrome and refractory enterocolitis received a synbiotic formulation consisting of *B. breve* and *L. casei* (approximately 10^9 CFU) and galactooligosaccharides (approximately 3 g) three times daily for a period of 15 to 55 months (Kanamori et al., 2004). Alterations in microbial composition (increased amounts of anaerobic bacteria and suppression of pathogenic microbes) and increased fecal content of SCFAs (from an average of 27.8 to 65.09 μmol/g of wet feces) were described in the synbiotics-treated group. Six of seven patients demonstrated increased body weight from 1.0 to 4.2 kg/year, and prealbumin concentrations were increased in synbiotic-treated patients ($P = 0.05$).

Synbiotics and *H. pylori* Infection

Synbiotics have been applied in the context of *H. pylori* gastritis. A clinical trial was performed in a school from a low socioeconomic area of Santiago, Chile. *H. pylori*-positive children were randomly distributed into four groups. Children received daily antibiotic treatment (lansoprazole, clarithromycin, and amoxicillin) (Ab) for 8 days, "*Saccharomyces boulardii*" (250 mg) plus inulin (5 g) (SbI) daily for 8 weeks, *L. acidophilus* LB (10^9 CFU per day) (LB), or no treatment (Gotteland et al., 2005). A ^{13}C-urea breath test (^{13}C-UBT) was performed before and after the study, and differences in $^{13}CO_2$ quantities were calculated (DDOB). *H. pylori* was eradicated in 66, 12, or 6.5% of the children in the Ab, SbI, or LB groups, respectively, while no spontaneous clearance was observed in children not receiving treatment. A moderate but significant difference in DDOB was detected in children receiving SbI (76.31; 95% CI, 711.84 to 70.79), but not LB (+0.70; 95% CI, 75.84 to +7.24). *H. pylori* infection was eradicated in 12% of synbiotic-treated and 6.5% of probiotic-treated children. Different species of LAB, doses of synbiotics, and combinations of antibiotics and synbiotics may yield a wider spectrum of beneficial effects in different disorders.

Synbiotics and Allergy

A synbiotic combination of *L. casei* subsp. *casei* and dextran prevented cedar-pollen-induced onset of nasal and ocular symptoms, cedar pollen-specific immunoglobulin E responses, and elevation of eosinophil counts (Ogawa et al., 2006). In a recent randomized study, children >2 years of age with atopic dermatitis received either a combination of potato starch and *L. rhamnosus* or potato starch alone three times per day for 3 months. Disease scores were reduced with synbiotic treatment from 39.1 to 20.7 ($P < 0.0001$). No differences were observed after 3 months of treatment ($P = 0.535$) (Passeron et al., 2006).

Synbiotics and Cancer

A synbiotic preparation, consisting of oligofructose-enriched inulin (12 g) (SYN1) and *L. rhamnosus* GG and *B. lactis* Bb12 (BB12) (10^{10} CFU), was recently administered in a 12-week randomized, double-blind, placebo-controlled trial including 37 patients with colon cancer and 43 polypectomized patients (Rafter et al., 2007). The intervention resulted in significant changes in the fecal microbiota, including increases in *Bifidobacterium* spp. and *Lactobacillus* spp. and reductions of *Clostridium perfringens*. The intervention reduced colorectal proliferation and the capacity of fecal water to induce necrosis in colonic cells and improved epithelial barrier function in polypectomized patients. Genotoxicity assays of colonic biopsy samples at the end of the intervention period indicated a decreased exposure to genotoxins in the polypectomized patients. Synbiotic consumption prevented an increased secretion of IL-2 by peripheral blood mononuclear cells in the polypectomized patients and increased the production of gamma interferon in the patients with colon cancer. It was concluded that several colorectal cancer biomarkers may be favorably altered by synbiotic intervention.

LIMITATIONS OF SYNBIOTICS

Two studies with Synbiotic 2000 have resulted in negative outcomes in the context of IBD. After an initial treatment with infliximab, 63 patients were randomized to daily administration of either Synbiotic 2000 or crystalline cellulose as a placebo (Rutgeerts et al., 2004). Median times to relapse for synbiotic or placebo groups were 9.8 and 10.1 months, respectively. In a second study, patients following surgery were supplemented with either Synbiotic 2000 or crystalline cellulose as a placebo. Seven patients in the synbiotic-treated group and two patients in the placebo group completed the scheduled 24-month treatment (Chermesh et al., 2007). No significant differences were observed between the two groups either in endoscopic findings or in rate of clinical relapse. The Rutgeerts disease scores were calculated as 0.6 (SD, ±0.8) in the synbiotic-treated group and 0.8 (SD, ±1) in the placebo group after 3 months of treatment.

SYNBIOTICS AND SEPSIS?

Two large studies have been performed involving ICU groups treated with synbiotics. Synbiotic 2000 (40 billion LAB) was administered to 162 patients. No significant differences were observed in patient mortality or multiorgan dysfunction (C. D. Gomersall, G. M. Joynt, P. Tan, P. Leung, and S. Bengmark, presented at the Australian and New Zealand College of Anaesthetists Annual Scientific Meeting, 2006). In another study, Synbiotic 2000 Forte was administered to 130 patients twice daily during ICU stays (2×400 billion LAB), and the results were compared with those of 129 patients supplemented with a cellulose-based placebo. No statistical differences were demonstrated between the groups with respect to the incidence of ventilator-associated pneumonia, the rate of ventilator-associated pneumonia per 1,000 ventilator days, and hospital mortality (Knight et al., 2004).

CHALLENGING INFORMATION FROM RECENT ANIMAL STUDIES

Prevention of Lung Inflammation and Tissue Destruction

Experimental animals subjected to cecal ligation and puncture and subsequent stress-induced neutrophil infiltration of the lung can be treated prophylactically by oral supplementation of a synbiotic cocktail. Synbiotic 2000 Forte was administered orally during 3 days before the trauma (Tok et al., 2007), and the four LAB species in the cocktail were injected subcutaneously at the time of trauma (Ilkgul et al., 2005). Both treatments effectively prevented neutrophil accumulation and tissue destruction in the lungs. The average neutrophil counts in the lungs of the groups were as follows (average of five fields and SD): mixture of LAB and mixture of bioactive fibers containing inulin, beta-glucan, pectin, and resistant starch (group 1), 9.00 ± 0.44; LAB only (group 2), 8.40 ± 0.42; mixture of bioactive fibers containing inulin, beta-glucan, pectin, and resistant starch (group 3), 31.20 ± 0.98; and placebo (nonfermentable fiber/crystalline cellulose) (group 4), 51.10 ± 0.70) (Tok et al., 2007). The corresponding values of myeloperoxidase were 25.62 ± 2.19 (group 1), 26.75 ± 2.61 (group 2), 56.59 ± 1.73 (group 3), and 145.53 ± 7.53 (group 4). The values for nitric oxide were 17.16 ± 2.03 (group 1), 18.91 ± 2.24 (group 2), 47.71 ± 3.20 (group 3), and 66.22 ± 5.92 (group 4) (Tok et al., 2007). All differences between treatment groups and placebo were statistically significant ($P > 0.05$). The results were similar for animals treated with subcutaneous injections of viable LAB (Ilkgul et al., 2005).

Prevention of Colonic Cancer

Male Sprague-Dawley rats were subjected to a single injection of the genotoxic compound azoxymethane and supplemented for 4 weeks with *L. acidophilus* or *B. lactis* (10^{10} CFU/g) plus or minus resistant starch (10% Hi-Maize) (Le Leu et al., 2005). The administration of resistant starch significantly increased the numbers of bifidobacteria and lactobacilli in the intestine ($P \approx 0.001$) and reduced pH levels and total numbers of coliforms ($P \approx 0.001$). Compared to animals supplemented with resistant starch only, rats fed *B. lactis* and supplemented with resistant starch demonstrated a significantly greater apoptotic deletion of carcinogen-damaged cells, increased cell proliferation, increased crypt column heights ($P \approx 0.001$), and increased levels of SCFAs in the colon.

Influence on Circulatory Neuropeptides

Circulatory levels of neuropeptides like neuropeptide Y (NPY) and peptide YY (PYY) have profound inhibitory effects on gastric acid secretion, gastric emptying, gut motility, and exocrine pancreatic secretions (Yang, 2002). Adult and elderly rats were supplemented with a mixture of *Lactobacillus delbrueckii* GG, *B. lactis* Bb12, and inulin for 3 weeks (Lesniewska et al., 2006). Synbiotic treatment elevated plasma PYY in adult rats, but the same treatment did not affect portal plasma PYY in elderly rats. Furthermore, portal plasma concentrations of NPY were decreased by synbiotics in the elderly, while NPY levels were elevated for adult animals. The results indicate that the results of synbiotic treatment on gastrointestinal function might be dependent on the age of the animal or human individual.

Influence on Plasma Lipid Profiles and Erythrocyte Membrane Properties

A synbiotic formulation containing *L. acidophilus* ATCC 4962, FOS, inulin, and mannitol was administered to hypercholesterolemic pigs on high- and low-fat diets. The aims of this study included assessments of effects of synbiotics on plasma lipid profiles and erythrocyte membrane properties (Liong et al., 2007). The supplementation of synbiotics reduced, irrespective of fat content in the feed, total plasma cholesterol ($P = 0.001$), triacylglycerol ($P = 0.002$), and low-density lipoprotein cholesterol ($P = 0.045$). Synbiotic supplementation also improved membrane fluidity, reduced membrane rigidity, and decreased fluorescence anisotropies in the hemoglobin-free erythrocyte membrane ($P < 0.001$). Reduced deformation of erythrocytes and improved membrane permeability were also observed.

Milk Production in Dairy Cows

Fifty-eight Holstein dairy cows were divided into two groups including one group receiving supplementation with a synbiotic consisting of *L. casei* subsp. *casei* and dextran (Yasuda et al., 2007). Significant improvements were observed in milk yields and in total amounts of fat, protein, and nonfat solids in the group receiving synbiotic supplementation.

CONCLUSIONS AND FUTURE PERSPECTIVES

The intestinal microbiota has unique features in each individual and is largely influenced by host genetics, environment, and lifestyle. Profound changes in our environment and lifestyles and altered exposures to chemical compounds including pharmaceuticals have likely affected the composition of the resident microbiota. Changes in our gut microbiota (microbiome) may be associated with altered environmental factors and trends in chronic diseases. Some studies have suggested that variations in the gut microbiota affect susceptibilities to various chronic diseases including diet-induced insulin resistance and type II diabetes mellitus (Dumas et al., 2006a, 2006b), type I diabetes (Brugman et al., 2006), and obesity (Backhed et al., 2007). Systemic inflammation in a variety of disorders may be difficult to manage with only probiotics or synbiotics. A multitude of treatments including different combinations of probiotics, prebiotics, nutrients, and antioxidants may be important in addition to fundamental changes of lifestyle and dietary habits.

Clearly the research linking our microbial composition and function with health status and susceptibility to disease is in its infancy. Each microbial species has unique functions, and conclusions cannot be generalized for members of the indigenous microbiota. Numerous food ingredients have specific effects on human health. Further scientific investigations are necessary in order to understand the unique interactions between the host-associated microbiota, diet, medical interventions with synbiotics, and aggregate effects on disease susceptibility, treatment, and prevention.

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