Gastroenterol Clin N Am ■ (2005) ■–■

GASTROENTEROLOGY CLINICS
OF NORTH AMERICA

Bioecologic Control of the Gastrointestinal Tract: The Role of Flora and Supplemented Probiotics and Synbiotics

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EPIDEMIC OF CHRONIC DISEASES

Although in certain parts of the world acute infectious diseases still dominate as causes of death, especially in children, globally chronic diseases constitute the leading causes of morbidity and mortality today. The World Health Organization estimates that 46% of global disease burden and 59% of global mortality are due to chronic disease; 35 million individuals die each year from chronic diseases, and this statistic has been increasing steadily. The increase, which seems to have its beginning at the time of the Industrial Revolution (eg, the mid-1850s), was relatively slow during the first 100 years, but during recent decades the increase in morbidity and mortality has obtained epidemic proportions. Circumstantial evidence supports an association of chronic disease with the transition from natural unprocessed foods to processed and often calorie-condensed foods. The correlation between increases in chronic diseases and reduction in intake of plant fibers and plant antioxidants with increase in consumption of refined sugars is obvious; the individual consumption of sugar has increased from about 1 lb/person/y in 1850 to about 100 lb/person/y in 2000. Today the fastest increase in chronic disease seems to be in the Third World. These diseases are imported to Third-World countries with an enormous surplus of cheap agricultural products, including grains, especially wheat, and dairy products, especially butter. Little consideration is given to the fact that a large proportion of individuals in these parts of the world are gluten intolerant or to the detrimental effect of such imports on local production of health-promoting fresh fruits and vegetables.

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0889-8553/05/$ – see front matter
doi:10.1016/j.gtc.2005.05.002

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FOOD CONNECTION

The modern Western lifestyle is characterized not only by significant alterations in food consumption. Stress; lack of physical exercise; use of alcohol, tobacco, and pharmaceuticals, and increasing exposure to environmental chemicals also seem to contribute to the burden of chronic disease in Western society. Foods that are consumed–refined and calorie-condensed food products–contain large amounts of saturated and trans fatty acids, sugar and starch, and bioactive peptides such as gluten and are low in omega-3 polyunsaturated fatty acids, plant antioxidants, and health-promoting plant fibers and bacteria. Common to most of the above-mentioned food ingredients is that they affect the function of the innate immune system, the inflammatory response, and the individual's resistance to disease [1]. Plant fibers, antioxidants, and, to some extent, polyunsaturated fatty acids enforce the resistance to disease, whereas saturated and trans fatty acids, sugar and starch, peptides such as gluten, and many chemicals and pharmaceuticals, including antibiotics, suppress the resistance to disease. Consequently, most patients with chronic disease have increased acute and chronic phase response, increased inflammation/superinflammation, and metabolic syndrome [1]. Saturated fat and trans fatty acids induce significant alterations in the immune response [2]; inhibit the macrophage functions [3]; stimulate the Th2 response relative to the Th1 response; and increase the risk of getting chronic diseases, such as diabetes, certain cancers, and rheumatoid arthritis [3]. Antibiotics result in suppression of the various macrophage functions, including chemiluminescence response, chemotactic motility, bactericidal and cytostatic ability, and lymphocyte proliferation [4,5].

The links between chronic diseases of the gastrointestinal tract, especially inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), and chronic phase response and metabolic syndrome have not been studied to the extent they should. Elevated levels of proinflammatory cytokines, increased coagulability, and signs of epithelial and endothelial dysfunction, changes that significantly relate to disease activity, are observed repeatedly in IBD [6,7], especially in ulcerative colitis (UC). A few studies also report insulin resistance in IBD patients [8]. Its role is not clear, but it is reported that insulin sensitivity improves when disease goes into remission.

The intolerance to various foods and their role as risk factors in various digestive tract chronic diseases have not been studied to any larger extent. When the British gastroenterologist Hunter studied food intolerance in IBS in the 1980s, he reported grains, particularly wheat (60% of patients) and corn (34% of patients); dairy products, particularly cow's milk (44% of patients) and cheese (39% of patients); and coffee (33% of patients) were the most common foods to induce intolerance [9]. Although less well studied, the pattern seems to be similar in other digestive tract chronic diseases. Cow's milk is strongly associated with high blood levels of growth factors, such as insulin-like growth factor-1, and with certain chronic diseases, such as breast cancer and prostate cancer [10,11]. Gluten-containing grains and dairy

products contain morphine-like molecules, which destabilize cellular membranes in the body and induce dysfunction of the immune cells, especially in individuals with the specific genetic marker HLA-B8, which is said to exist in 30% of Western populations [12]. These individuals all seem to suffer "a leaky gut," even if only a small fraction of them develop full-blown celiac disease. The ability of human gut to digest small peptides is limited and depends much on the presence and composition of flora. Only a few lactic acid bacteria (LAB) have the ability to disintegrate these peptides. In some individuals, but not all, the liver possesses individual enzymes that eliminate all peptides before entering the general circulation. In this connection, it has been shown that long-term fermentation of dough totally eliminates gluten to a degree that the bread produced with long-term fermentation is tolerated by individuals with pronounced gluten intolerance [13]. This observation gives a new dimension to the Paleolithic diet. Early humans did not consume grains or cow's milk, and most of the food they ate was vigorously fermented, as it was commonly stored in the soil for days, weeks, and months. Early humans consumed considerably less salt, fat, and sugar, but consumed about twice as much minerals, 10 times more plant fibers, greater than 20 times more antioxidants, greater than 50 times more omega-3 fatty acids, and billions of times more live bacteria [14].

MICROBE CONNECTION

Studies in animals, including germ-free animals, and in humans show a series of benefits from living in symbiosis with microbes. Life would be difficult to sustain without access to plants and microbes. The human body, similar to that of all other animals, is "self-cleaning." It is for that purpose equipped with a "layer" of microbes at all surfaces to the exterior world: large intestine, 1 to 2 kg; skin, 200 g; oral cavity, lung, and vagina, each 20 g; nose, 10 g; and eye, 1 g [15]. The human body contains 10 to 20 times more prokaryotic cells (10^{14}) than eukaryotic cells (10^{13}). The flora is suggested to contain 30 times more genes as the rest of the body–greater than 2 million prokaryotic genes compared with 65,000 karyotic genes [16]. The beneficial/probiotic bacteria seem to tolerate poorly the Western lifestyle with its stress and poor eating habits. The intestinal mucosa is said to contain more nerve endings than any other tissue in the body, and when the individual is in stress, release of norepinephrine into the intestinal lumen reduces the good bacteria and changes the phenotype of the potentially pathogenic microorganisms, which become considerably more virulent and sometimes life-threatening [17]. Studies in animals with induced disease, such as pancreatitis, show that the preventive bacteria disappear after 4 to 6 hours, and overgrowth of pathogens and microbial translocation occurs about 6 hours after induction of disease [18,19]. Studies in critically ill humans indicate that the entire LAB flora is lost in patients after a short stay in an intensive care unit [20]. Astronauts have been shown on return to earth to have an absent or much reduced LAB flora and clear signs of overgrowth with pathogens [21].

Westerners in general have a much reduced flora, in diversity of microbial strains and number of bacteria, compared with people who live under more rural conditions; Swedish children show a much reduced flora compared with Estonian [22] and Parkistani [23] children. A United States study from 1983 showed that *Lactobacillus plantarum,* a dominating LAB among plant eaters, is found in only approximately 25% of omnivorous Americans and in about two thirds of vegetarian Americans [24], and nothing supports an assumption that this has changed to the better. A more recent Scandinavian study suggested that even in healthy individuals the most common colonic LAB are present in only about 50% or less: *Lactobacillus plantarum* in 52%, *Lactobacillus rhamnosus* in 26%, and *Lactobacillus paracasei* subsp *paracasei* 17% [25]. [Q3]

Only a minority of the intestinal flora is cultivable, perhaps 30%. There is a "large dark hole" about which little is known. It is possible that specific, yet unknown microbes exist, which in the future might be recognized as responsible for "unexplained" diseases such as chronic diseases of the gut, rheumatic arthritis, and autism. The increasing understanding of inflammation and function of acute phase proteins has shown that many chronic diseases in the past labeled as degenerative are actually inflammatory. More recent availability of molecular biology techniques and the understanding of the function of nanomolecules have made it possible to associate some diseases with specific microbial infections–not only chronic gastritis with *Helicobacter pylori* and arteriosclerosis with *Campylobacter* and *Helicobacter* infections, but also a range of other diseases [26].

In the 1990s, the authors' group showed a significant reduction in numbers of bacteria in patients with IBD [27]. A more recent study using modern molecular biology techniques showed a significant reduction in diversity of microbial strains in IBD patients: in UC with approximately 30% and in Crohn's disease (CD) with 50% [28]. It also has been shown that especially patients with active UC have lost the ability of holding back the potentially pathogenic flora from the intimate mucosal surface [29], and it was shown that patients with CD have a high prevalence of adherent-invasive *Escherichia coli* on the intestinal mucosa; adherent-invasive *E coli* strains were found in 22% of patients with chronic lesions of CD versus 6% of controls and 36% of patients in the so-called neoterminal ileum (eg, the last 10 cm of ileum before an ileocolic anastomosis) [30].

ANTIOXIDANT CONNECTION

Increased levels of pro-oxidants, such as homocysteine, and low levels of vitamins and key antioxidants, such as folic acid and glutathione, often are observed in patients with all types of chronic disease. It has been suggested that IBD results from an imbalance between pro-oxidant and antioxidant mechanisms [31,32]. Glutathione, a tripeptide and key intracellular antioxidant, has generated special interest [33]. Low levels of glutathione especially have been reported from studies of colonic biopsy specimens from patients with CD [34]. Vitamins and antioxidants often are given as supplements to patients with

chronic gastrointestinal disease, but, to the authors' knowledge, no controlled study with systematic supply of antioxidants is reported in the literature. Several such studies have been performed in experimental animals, however. Superantioxidants (eg, antioxidants supposedly >10 times stronger in effect than vitamins such as vitamin C and E), including resveratrol (rich in red wine and peanuts), quercetin (apple, onion, tea), epigallocatechin gallate (green tee), [Q4] lycopene (tomato), and curcumin (turmeric), all have the potential alone or in combination to provide health benefits. Special attention has been given more recently to curcumin, an ingredient in the spice turmeric obtained from the rhizomes of the plant *Curcuma longa Linn,* which contains 1% to 15% of the active substance. Similar to all other plant-borne antioxidants, curcumin most likely is released and absorbed after microbial fermentation in the large intestine. In experimental studies, curcumin has shown strong anti-inflammatory effects and capacity to prevent various chronic diseases, including neurodegenerative diseases such as Alzheimer's disease and various cancers. Significant prevention of digestive tract disease development has been shown in animals with liver injuries induced by carbon tetrachloride [35] and alcohol [36] and in intestinal mucosa and colitis development when induced by TNBS [37,38]. Therapeutic effects, which by no means are inferior to what has been [Q5] observed with other therapeutic modalities tried in experimental studies, including that of probiotics, also have been shown.

FIBER CONNECTION

There is little to support a pathogenic connection between digestive tract chronic diseases and reduced intake of plant fibers per se, even if chronic diseases in general are associated with unsatisfactory consumption of fresh fruits and vegetables, NASH and cryptogenic cirrhosis suggested as possible [Q6] exceptions. Efforts to supplement various plant fibers to patients with digestive tract chronic diseases most often have been unsuccessful. Some more recent studies have led to results, however, that might change clinicians' attitudes. The ability to maintain remission in UC patients through daily supplementation with 10 g of *Plantago ovata* seeds (also called psyllium or Ispaghula husk) alone or in combination with 500 mg of mesalamine was compared with daily [Q7] treatment with 500 mg of mesalamine only [39]. Twelve months of treatment showed no statistical support in favor of any of the groups, a result that seemingly favors the use of psyllium because of its cheaper price and lack of side effects. Daily supply of 30 g of germinated barley foodstuff, a by-product from breweries, rich in hemicellulose and in glutamine, was tried in 39 patients with mild-to-moderate active UC [40] and shown to increase significantly numbers of LAB in stool, especially of *Bifidobacterium* and *Eubacterium*; reduce the disease activity; and increase concentration of short-chain fatty acids, a key energy source for colonic mucosa, essential for mucosal growth. Short-chain fatty acids, especially butyrate, are shown significantly to inhibit nuclear factor-κB activation of lamina propria macrophages, reduce the number of neutrophils in crypts and surface epithelia, and reduce the density of lamina

propria lymphocytes/plasma cells in patients with UC [41]. By-products from breweries are rich in various important nutrients, including large amounts of vitamins such as various B vitamins, and the effects observed also can be linked with several such ingredients or a combination of ingredients. A controlled study with oat bran as the fiber source was conducted in 22 supplemented and 10 control patients with quiescent UC. Daily supplementation during 3 months of 60 g of oat bran (equivalent to 20 g of dietary fiber) resulted in a significant increase in fecal butyrate (average 36%) and significant reduction of abdominal pain. All UC patients tolerated this large dose of fiber well, and signs of relapse were seen in none of the colitis patients [42]. Another controlled study reported significant reduction in inflammation of the mucosa of the ileal reservoir on endoscopy and histology after 3 weeks of daily supplementation of 24 g of inulin to patients with ileal pouch–anal anastomosis [43]. More recent studies also suggest that supplementation of nondigestible oligosaccharides per se enhances bacterial colonization resistance against *Clostridium difficile,* at least in vitro [44]. Nonabsorbable disaccharides, such as lactulose, and plant fibers, such as psyllium, also have proved effective to decrease blood ammonia and reduce encephalopathy in patients with liver cirrhosis [45]. Daily supplementation of about 5 g/d of pectin or about two unripe bananas (rich in pectin and cellulose) was proven extremely effective as supplement to rehydration against persistent diarrhea in children in Bangladesh. Recovery on the third day was seen in 59% in the green banana group and 55% in the pectin group compared with 15% in a rice-only group [46]. Studies also suggest significant reduction in encephalopathy and improvement of liver function in patients with chronic liver disease when given a daily supplement of a combination of 10 g of four fibers (β-betaglucan, inulin, pectin, resistant starch) [47].

PREBIOTICS, PROBIOTICS, AND SYNBIOTICS

Prebiotics

Prebiotics are substrates to be fermented by flora (eg, nondigestible food ingredients, mainly plant fibers, that undigested reach the colon; food ingredients often referred to as colonic foods). Prebiotics have important functions in the body. They are essential to maintain mucosal growth and functions, to maintain water and electrolyte balance, to provide energy and nutrients for the host and for the flora, to enforce the body's resistance against invading pathogens, and to stimulate growth. Prebiotics are known to stimulate an increase in numbers and diversity of intestinal flora; relieve constipation and diarrhea; reduce serum triglycerides, serum cholesterol, and very-low-density lipoproteins; reduce the glycemic response to eating; improve water and electrolyte balance; and increase bioavailability and absorption of minerals such as calcium, magnesium, iron, and zinc.

Probiotics

Probiotics are live microorganisms supplied from the outside of the body, most commonly to the digestive tract. Flora and supplied probiotics have the ability

to reduce or eliminate potentially pathogenic microorganisms from the body; reduce or eliminate the content of various toxins, mutagens, and carcinogens; promote apoptosis and disappearance of premalignant cells; release numerous nutrients, antioxidants, growth factors, coagulation factors, and other factors; modulate the innate and adaptive immune defense mechanisms; and stimulate gastrointestinal motility. Flora and supplied probiotics also are known to release numerous plant antioxidants; to synthesize some important vitamins, such as vitamin K, folic acid, niacin, thiamine, riboflavin, and B-complex vitamins [48]; to support mineral absorption [49]; and to reduce serum cholesterol [50]. A condition for clinical effect of supplied probiotics is that the supplied microbes remain viable during gastric and intestinal transit and that more than 10^7 colony-forming units/mL of viable bacteria reach the intestine [51]. Most probiotics available, such as those provided by dairy products or those sold in health stores, do not meet that condition. The ability to survive the acidity of the stomach and bile acid content of the small intestine seems to be limited to a few microorganisms. When a probiotic fruit drink (PRO VIVA; Probi, Lund, Sweden) containing no more than 10^7 of *L plantarum* 299V was tried in a controlled study of 129 patients undergoing abdominal surgery, no differences in bacterial translocation, gastric colonization with enteric organisms, or septic morbidity could be observed [52]; additionally, there were no differences in concentrations of plasma cells, IgA-positive cells, or IgM-positive cells in the lamina propria [53]. Great differences in ability of some LAB to survive and to influence cytokine production after passage through the stomach and small intestine were shown in a study in ileostomy patients [53]. Four different LAB species were compared: *L plantarum, L paracasei, L rhamnosus,* and *Bifidobacterium animalis*. Of originally orally administered 10^8 cells/mL of each LAB, after the passage through the stomach and small intestine only between 10^7 (*L plantarum*) and 10^2 (*L rhamnosus*) bacterial cells remained. Most of the strains tested showed after passage through the small intestine a significantly reduced or weak (especially *L rhamnosus*) ability to influence cytokine production (eg, the state of inflammation). If the study had included yogurt bacteria, even smaller survival and biologic effects could have been expected. The absence of clinical efficacy of yogurt bacteria when used in postoperative and severely sick patients has been documented in two controlled studies. A standard commercial product (TREVIS; Ch Hansen, Denmaark) containing *Lactobacillus acidophilus* LA5, [Q8] *Bifidobacterium lactis* BP12, *Streptococcus thermophilus,* and *Lactobacillus bulgaricus* was mixed with 7.5 g of oligofructose and given to 45 critically ill patients [54] and 45 controls and to 72 elective abdominal surgery patients and 65 controls [55]. No clinical benefits were reported from either of the studies. The study in intensive care unit patients reported significant reductions in number of potentially pathogenic organisms in the stomach of the treated patients, but no influence on intestinal permeability or clinical benefits. The perioperative study reported no differences in bacterial translocation, gastric colonization, systemic inflammation, or septic complications. See also a commentary to the ICU study by Bengmark [56].

Only a few LAB can ferment semiresistant prebiotics, such as the oligofructans inulin and phleins. When the ability of 712 different LAB to ferment oligofructans was studied, only 16 of 712 were able to ferment the phleins, and only 8 of 712 were able to ferment the inulin-type fiber. Only four LAB species fermented these fibers: *L plantarum* (several strains), *L paracasei* subsp *paracasei, Lactobacillus brevis,* and *Pediococcus pentosaceus* [57]. Also the ability to control various pathogens is strain specific and often limited to a few strains. When the ability of 50 different LAB to control 23 different pathogenic *C difficile* organisms was tested, only 5 proved effective against all, 8 were antagonistic to some, and 27 were totally ineffective [58]. The five most effective strains were *L paracasei* subsp *paracasei* (two strains) and *L plantarum* (three strains). Information such as this is important for the choice of probiotics for clinical use.

[Q9] [Q10]

Synbiotics

Synbiotics is the word coined for the combined treatment with specific bioactive LAB and specific prebiotics with ability to stimulate the growth of certain LAB and to provide definite health benefits by synergistic action. A condition for such effects is that the LAB used have a documented ability to metabolize simultaneously supplemented prebiotics, which is often not the case, especially when it comes to different oligosaccharides [57].

YOGURT AS CARRIER OF PROBIOTICS AND SYNBIOTICS

Cow's milk is not an ideal carrier of probiotics, especially for specific clinical use. In addition to its proposed role as risk factor for chronic diseases [9,10,59–62], concerns with cow's milk include the following:

1. Cow's milk is a poor carrier of bioactive fiber-fermenting probiotics because, in sharp contrast to breast milk, it contains no fibers or fiber-like molecules (only elephant milk contains as much as human milk). The complex fucosylated oligosaccharides in human milk, with structural similarities to immunomodulating cell surface glycoconjugates, protect breast-fed infants against infection and inflammation. These oligosaccharides most likely also serve as prebiotics, provide key nutrients to breast-fed infants, and stimulate growth of the nonpathogenic health-supporting gut microflora [63].
2. Cow's milk is known to release inflammatory mediators; induce inflammation; induce leakage of molecules, such as albumin and hyaluronan; increase intestinal permeability; and cause translocation and leaky gut [64–69].
3. Cow's milk is known to be rich in free polyunsaturated fatty acids. It was shown that presence of polyunsaturated fatty acids, even in lower concentrations than provided in fermented dairy products such as yogurt, cause *Lactobacillus* to lose their ability to adhere to mucous membranes and to grow, supporting that dairy products are not ideal as carriers of probiotics [70].

Supplementing with yogurt bacteria or similar bacteria yields small or no clinical benefits [54,55,71].

PROBIOTICS AND SYNBIOTICS IN CLINICAL STUDIES

The hope for identification of a "magic bug" capable of controlling all types of inflammation in all stages of disease seemingly has remained an illusion. The promising effects observed in experimental studies often have not been possible to repeat in patients, especially in patients with chronic diseases. Chronic diseases that develop spontaneously are more therapy-resistant than similar diseases when induced in animals. Induced diseases rarely remain chronic, and more dramatic effects of probiotics generally are obtained in acute conditions in animals and humans. Another possible explanation may be that animals usually receive much larger doses of probiotics in relation to mucosal surface and to body weight. No systematic dose-response studies have been published yet. The most successful results so far have been obtained with the use of cocktails of LAB, with or without simultaneous supplementation of prebiotics. These cocktails commonly are supplied in significantly larger doses, however, than is the case with the use of single-strain probiotics or single-strain/single-fiber synbiotics, which treatments most often are provided with daily doses of 1 to 10 billion LAB/d. The current trend is toward more complex compositions and toward use of much larger doses of LAB. Studies with the multistrain probiotic VSL#3 use doses between 1800 and 3600 billion LAB/d, and studies with the multistrain/multifiber Synbiotic 2000 use doses between 40 billion and 1200 billion LAB.

COMMERCIALLY AVAILABLE PREBIOTICS AND PROBIOTICS

Although some trials in the past have used LAB from health stores, dairy products, or a plethora of LAB available on the "spot market," most of the trials have been done with fewer than 10 different formulations:

Single-strain probiotics

Saccharomyces boulardi (Laboratories Biocodex, Montrouge, France) is commonly given in doses of 2 capsules containing 250 mg morning and evening, equivalent to approximately 10 billion live organisms/d. The nonpathogenic *E coli* serotype O6:K5:H1 (Mutaflor; Ardeypharm GmbH, Herdecke, Germany) is referred to as Nissle 1917 after early observations done by Nissle during the World War I. It is commonly given in doses of less than 10 billion LAB/d. *Lactobacillus GG* (LGG) (Valio, Helsinki, Finland) commonly is given in doses of 1 to 5 billion LAB/d. *L acidophilus* LA1 (LA1) (Nestle, Vevey, Switzerland) is commonly given in doses of less than 5 billion LAB/d (sometimes <1 billion LAB/d).

Multistrain probiotics

The probiotic cocktail VSL#3 (Sigma-Tau, Pomezia, Italy, and VSL Pharmaceuticals, Fort Lauderdale, Florida) is the only multistrain probiotic tried so far. It consists of four *Lactobacillus* strains (*L acidophilus, L casei, L delbruecki* subsp *bulgaricus,* and *L plantarum*), three *Bifidobacterium* strains (*B longum, B infantis, B breve*), and *S salivarius* ssp *thermophilus* (5×10^{11} cells/g). VSL#3 has been tried in several studies and yielded results that have attracted the interest of gastroenterologists and patients around the world. VSL#3

contains 300 billion live bacteria per gram. It is commonly given in high doses, usually 1800 billion LAB and more recently up to 3600 billion LAB/d.

Single-strain/single-fiber synbiotics

The composition of *L plantarum* 299 or 299V plus oat fiber (PRO VIVA; AB Probi, Lund, Sweden) was constructed after extensive studies of human *Lactobacillus* strains [72]. The composition contains 10 g of oat fiber and 10^9 (1 billion) LAB of *L plantarum*. A common dose is 1 or 2 billion LAB/d; occasionally 5 billion LAB/d has been tried. Most of the experience with this synbiotic stems from studies in critical care and in connection with extensive surgery.

Multistrain/multifiber synbiotics

Synbiotic 2000 (Medipharm, Kågeröd Sweden, and Des Moines, Iowa) consists of a mixture of four LAB, one from each of the four main genera of lactobacillus: 10^{10} of *P pentosaceus* 5-33:3, 10^{10} of *Leuconostoc mesenteroides* 32-77:1, 10^{10} of *L paracasei* subsp *paracasei* 19, and 10^{10} of *L plantarum* 2362 (eg, 40 billion LAB per dose, plus a mixture of four well-studied bioactive plant fibers: 2.5 g of β-glucan, 2.5 g of inulin, 2.5 g of pectin, and 2.5 g of resistant starch, total 10 g plant fibers. The composition was determined after extensive studies of more than 350 humans and more than 180 plant strains by Lund university microbiologists Ljungh and Wadström and their group [73,74]. They chose the LAB to be used in the composition based on the ability of the various LAB to produce bioactive proteins, transcribe nuclear factor-κB, produce proinflammatory and anti-inflammatory cytokines, produce antioxidants, and complement each other functionally. The four LAB individually function differently, but show synergistic effects when supplemented together. Newer supplements Synbiotic 2000 FORTE and Probiotic 2000 FORTE (no fiber added) are based on 10^{11} of each of the four LAB (eg, 400 billion LAB per dose, or if supplemented twice or three times daily 800 to 1200 billion LAB/d).

Total flora replacement

Total flora replacement (TFR) was introduced as a treatment alternative in severe *C difficile* infections. It is based on transfer of fecal flora from a healthy individual, often a close relative, to a severely sick patient, who is prepared for the supplementation by oral polyethylene glycol lavage and broad-spectrum antibiotic treatment. TFR has been used occasionally in severe *C difficile* cases, but also in severe constipation, IBS, and IBD. The patients usually receive, after a preceding washout, about 200 to 300 mL of fresh feces dissolved in an equal amount of saline solution. The process is repeated for about 5 to 7 days [75,76].

PROBIOTICS AND SYNBIOTICS IN GASTROINTESTINAL DISEASE

Infectious diarrhea

Diarrhea is one of the most common expressions of disease. In the developed world, mainly elderly and immunocompromised individuals are affected. In

the developing world, diarrhea affects children with 6 to 12 episodes per year compared with about 2 episodes annually in children in the developed world. It is estimated that more than 3 million children die globally each year as a result of severe diarrhea. Reversing dehydration is the most effective treatment, but there is a strong need for complementary treatment. Prebiotics, probiotics, and synbiotics have the potential to be that complement because in addition to being relatively inexpensive and without serious side effects, they potentially can control infection and modulate motility. A meta-analysis based on 23 controlled studies totaling 1917 patients concluded that the risk of diarrhea was reduced by 3 days (relative risk 0.66) and the mean duration of diarrhea was 30.5 hours [77]. The effects are seemingly especially pronounced in rotavirus diarrhea, in which mean duration of diarrhea was reduced by 38.1 hours. The study identified great variations in results with the use of different probiotics. A combination of *L acidophilus* and *L bifidus* seemed to be the most effective. In contrast to most other regimens tried, *S thermophilus* and *L bulgaricus* seemed to have no effect on diarrhea, which is in line with observations in other conditions.

Antibiotic-associated diarrhea and *Clostridium difficile* colitis

Approximately one fourth of all antibiotic-associated diarrhea episodes involve *C difficile*. When 50 *C difficile* patients received 10 billion viable *S boulardi* organisms, recurrence was observed in 33 of 50 patients [78]. Prophylactic supplementation to antibiotic-treated patients of 20×10^6 colony-forming units/d of LGG was tried in a randomized trial involving 267 patients, but no difference was observed; diarrhea developed in 39 (29%) of LGG-treated patients and in 40 (30%) controls [79]. Twenty-nine patients with more than one verified episode of *C difficile*–associated diarrhea were supplemented with either 5 billion *L plantarum* 299V (LP299V) or placebo in addition to metronidazole [80]. No significant difference in outcome was observed; 4 of 11 LP299V-treated patients and 6 of 9 controls showed signs of recurrence. No study is yet reported with the use of more complex compositions, such as VSL#3 or Synbiotic 2000. TFR has been used in 84 patients: 36 patients with *C difficile*–associated diarrhea, 22 with *C difficile* colitis, and 26 with pseudomembranous colitis [75,76]; 72 of 84 patients (86%) showed immediate resolution of the problems. None of the patients as reported had signs of relapse during follow-up lasting 5 years. Cure is reported to have been achieved with a single-shot treatment in 33 of 36 patients (92%).

Crohn's disease

CD is a disease proven to be refractory to treatment [79]. With increasing knowledge about the inflammatory cascade, several possible treatment options have been suggested. So far antagonism of tumor necrosis factor (TNF)-α with the monoclonal antibody infliximab has proved to be the most successful. Future treatments are likely to be compared with infliximab as the gold standard or suggested as a supplement to infliximab treatment. Single-strain probiotics, such as *S boulardi* [81,82], *E coli* Nissle [83], and LGG [84,85], have

been tried with no or minimal success. An abstract published in 2000 claimed an effect with VSL#3, but the expected complete article was never published [86]. Synbiotic 2000 has been tried in two controlled trials. After an initial treatment with infliximab, 63 patients were randomized to receive either Synbiotic 2000 or placebo daily [87]. Median time to relapse was 9.8 and 10.1 months. In the other study, 20 patients were supplemented with Synbiotic 2000, and 9 patients received placebo; no difference was observed in disease activity scores after 3 months (R. Eliakim, personal communication). [Q11] Bioecologic treatment has not been able contribute to improved outcome in this group of patients. One cannot exclude, however, that supply of significantly larger doses of probiotic and synbiotic compositions could prove effective.

Ulcerative colitis

Of single-strain probiotics, mainly *S boulardi* [88] and *E coli* Nissle [89–91] have been tried, both with some, but far from satisfactory, improvements. In one *E coli* Nissle study involving 114 patients, 44 of 59 *E coli* Nissle–treated (75%) and 39 of 57 mesalamine-treated patients reached remission. Relapses during the study period occurred in 26 of 57 (67%) *E coli* Nissle–treated patients and 32 of 59 (73%) mesalamine-treated patients [90]. Subsequent reviewers have pointed out that the relapse rate in the mesalamine group was significantly higher than expected from the literature [92]. It also has been suggested that the study groups were heterogeneous with respect to severity of disease, and that the dose of mesalamine used in the study was lower than usually used. In the last study with *E coli* Nissle, 327 patients with quiescent UC were treated during 1 year with either *E coli* Nissle or mesalamine [91]. The relapse rate was 45% with *E coli* Nissle and 36% with mesalamine. A study using VSL#3 provided much more cutting-edge results [93]. For 1 year, 20 patients with UC in remission and intolerant to mesalamine treatment received daily morning and evening 3 g of pure VSL#3 bacteria, equivalent to, at that time, almost "astronomical amounts" of 1200 billion LAB. Four of the patients showed significant relapse after 3, 5, 5, and 7 months; 1 was lost to follow-up, the remaining 15 were still in remission after 12 months. Rectal instillations with Synbiotic 2000 reconstituted in saline were given to 10 patients during 2 weeks. One patient withdrew after 1 week; during the 3 weeks of observation, the remaining patients showed dramatic improvements in diarrhea scores, visible blood in stool, nocturnal diarrhea, urgency, and consistency of stool (Table 1) [94]. Two patients reported significant bloating and flatulence, but no other side effects were reported. Only 10 IBD patients, 9 UC patients, and 1 CD patient are reported as treated with TFR in the literature [75,76]. All TFR-treated IBD patients are reported to have had IBD for more than 5 years and to have been refractory to conventional treatments. The patients are claimed all to have experienced a complete reversal of disease and to have all anti-inflammatory treatments concluded after 6 weeks and, most important, to remain remission-free after periods of observation of 1 to 13 years. It also is claimed that on follow-up endoscopy and histology the mucosa had shown a normal

Table 1
Changes in urgency, episodes of diarrhea, nightly diarrhea, visible blood on stool, and consistency of stool during 2 weeks of repeat enemas with synbiotics and 3 weeks' study

	Day 1	Day 7	Day 14	Day 21
Urgency	1.9	1.2*	1*	1†
Episodes of diarrhea	2.4	1.3	0.9*	0.8*
Nightly diarrhea	0.5	0.1†	0‡	0‡
Visible blood	2.2	1.2†	0.8‡	0.8‡
Consistency of stool	1.1	0.9†	0.7‡	0.8‡

*$P < .05$.
†$P < .01$.
‡$P < .001$.
Data from Pathmakanthan S, Walsh M, Bengmark S, Willemse PJA, Bardhan KD. Efficacy and tolerability treating acute distal ulcerative colitis with synbiotic enemas: a pilot trial. [**Q21**]

appearance. This small but significant improvement with TFR is unprecedented. There is nothing to assume that even TFR would lead to more than a temporary colonization. Most likely the guest flora disappear within 2 to 4 weeks. With present knowledge, permanent neocolonization would not occur in adults. The most likely explanation seems at this stage to be that the guest flora has managed to eliminate an unknown, uncultivatable pathogen underlying the disease.

Pouchitis

LGG was tried in two studies of pouchitis. Twenty patients with a previous history of pouchitis and endoscopic signs of inflammation were randomized to either 1 billion LGG or placebo. No differences were observed in disease activity or total anaerobes and aerobes in mucosal biopsy specimens or in feces after 3 months of treatment [95]. Prophylactic supplementation of LGG immediately after surgery with construction of ileoanal anastomoses was tried in one study [96]. Comparison was with historical controls: 2 of 39 versus 8 of 78 developed single-episode pouchitis, 1 versus 12 had recurrent episodes of pouchitis, and 0 versus 7 had chronic pouchitis. Cutting-edge improvements were reported from a controlled study with supplementation of 1200 billion VSL#3 for 9 months. Only 3 of 20 VSL#3-treated patients in contrast to 20 of 20 control patients had remission of pouchitis [97]. Similar results were obtained in a second study in collaboration with British gastroenterologists; 17 of 20 (85%) VSL#3-treated and 1 of 16 controls remained in remission after 1 year of treatment [98]. In a third study, supplementation of VSL#3 was started immediately after the surgical construction of ileoanal anastomosis with the hope to prevent pouchitis from developing. Only 2 of 20 (10%) patients in the VSL#3-treated group compared with 8 of 20 (40%) in the control group ($P < .01$) developed pouchitis [99].

Irritable bowel syndrome

IBS is likely the most common gastrointestinal disorder in the Western world. It is characterized by altered bowel habits, dysmotility, and abdominal pain or

discomfort. The pathogenesis is obscure, but a study suggests that most patients (34 of 44) have a significant ($P < .001$) infiltration with mast cells in the mucosa compared with controls [100]. Especially the number of mast cells located close to (within 5μ) nerve fibers are significantly increased in IBS patients and significantly related to degree of abdominal pain and discomfort. Probiotics have been shown to modulate the enteroendocrine cell population in the rat intestine [101]. Several attempts to affect the disease with probiotic supplementation are reported in the literature. Twenty-four patients were randomized to receive either 5 billion LGG or placebo twice daily during 10 weeks, but no difference between the two groups was observed in bloating, pain scores, or bowel movements [102]. In a well-controlled study, 78 patients were supplemented with 10 billion of either *L salivarius* UCC4331 or *B infantis* 35624 or placebo [103]. Two different composite scores based on the three cardinal symptoms–pain/discomfort, bloating/distention and bowel movement difficulty–were calculated, and both showed statistically significant improvement with probiotic supply, the most pronounced effect seen with supply of *B infantis*. The abnormal ratio of the cytokines interleukin-10 to interleukin-12 was reported normalized in the *B infantis*–treated group. Dual-strain probiotics were tried in a study in which 50 patients were randomized to receive a combination of 5 billion of *L plantarum* Lp01 and 5 billion of *B breve* Br0 during 1 month. Reductions in pain and overall symptom scores were reported as 50% and 25%; no statistical analysis was regarded possible [104].

VSL#3 in a dose of 450 billion LAB/d was tried in a controlled study in 25 patients at the Mayo Clinic [105]. After 8 weeks, no differences were observed between VSL#3-treated and controls in mean gastrointestinal transit time, abdominal pain, gas, and urgency. The single-strain/single-fiber synbiotic based on *L plantarum* 299V and oat fiber was tried in three studies [106–108]. Twenty of 20 patients in the *L plantarum* 299V–treated group and 11 of 20 in the placebo group reported resolution of their abdominal pain [106]. A similarly designed study in 2 × 30 patients found the most modest influence of *L plantarum* 299V treatment–reduction in flatulence but no influence on bloating [107]. The third study reported no effect at all of *L plantarum* 299V treatment [108]. Total flora replacement also has been tried in a few patients, but so far the experience is at best anecdotal.

Helicobacter pylori infections

In sharp contrast to other microbes, a few *Lactobacillus* strains have the ability to tolerate low pH and survive and grow in the otherwise hostile-to-microbes environment of the stomach [109]. This ability could offer unique opportunities to prevent overgrowth of *H pylori,* the main cause of chronic gastritis and peptic ulcers and an important risk factor for gastric malignancies. A study compared 17 different LAB and their ability to inhibit growth of 10 different *H pylori* strains [110]. All strains inhibited the growth at low pH, but at a pH of 6.0, all strains except *L acidophilus* CRL639 lost that ability. This observation speaks against simultaneous use of H_2-blocking agents. A total of 120 patients were

randomized to receive *L acidophilus* or placebo as supplement to a 7-day course of triple-agent therapy (rabeprazole, clarithromycin, amoxicillin) [111]. The eradication rates were 52 of 59 (88%) and 42 of 58 (72%; $P = .03$). When the study was repeated with LGG, however, no such triple therapy–potentiating effect could be observed [112,113]. Daily consumption of 4×50 mL of supernatant from a whey-based culture with *L acidophilus* is reported to reduce significantly breath test results [114], and to decrease density of *H pylori* in the stomach [115]. Subsequent studies with *L acidophilus* [69] and a variety of other *Lactobacillus* and *Bifidobacterium* species [116,117] seem, however, to show universal suppression of *H pylori* by various LAB, which seemingly is independent of strains used [117].

Chronic liver disease

Endotoxin-induced activation of macrophages is assumed to be responsible for increased levels of circulating TNF-α and soluble TNF receptor observed in cirrhotics and the expression of toll-like receptors TLR2 and TLR4. Circulating endotoxin, TNF-α and soluble TNF receptor levels, peripheral blood mononuclear cell expression of TLR2 and TLR4, and in vitro TNF-α production by peripheral blood mononuclear cells when stimulated with endotoxin or *S aureus* enterotoxin B were measured in 36 cirrhotic patients supplemented Synbiotic 2000 and 32 controls [118]. Supplementation of synbiotics resulted in significant up-regulation of peripheral blood mononuclear cell expression of TLR2.

In a subsequent study, 55 patients with minimal hepatic encephalopathy were randomized to receive Synbiotic 2000 ($n = 20$), only the fiber in Synbiotic 2000 ($n = 20$), or placebo ($n = 15$) for 30 days [47]. Patients with cirrhosis and minimal hepatic encephalopathy were found to have substantial derangements in the gut microecology, with significant fecal overgrowth of potentially pathogenic *E coli* and staphylococcal species. Synbiotic treatment significantly increased fecal content of non–urease-producing *Lactobacillus* species and significantly reduced endotoxemia and potentially pathogenic flora. The observed alterations in gut flora were accompanied by a significant reduction in blood ammonia levels and reversal of minimal hepatic encephalopathy in half of the patients; the Child-Turcotte-Pugh functional class improved in nearly 50% of cases. Treatment with only fermentable fiber was beneficial in a substantial proportion of patients. Interventions aimed at reducing intestinal levels of endotoxin-containing, gram-negative bacteria have been suggested also to improve systemic hemodynamic disturbance in cirrhosis, but effects on hepatic blood flow were not reported. A study by the same group performed in 15 cirrhotic patients showed a significant reduction (median 17.5%, range 1.4–65%) in indocyanine green retention at 15 minutes (ICG_{R15}) in the cirrhotic patients after 7 days of supplementation with Synbiotic 2000 ($P = .003$) [119].

Orthotopic liver transplantation

Two prospective randomized trials with supplementation of synbiotics also have been performed. In the first study, the single-strain/single-fiber synbiotic

preparation based on 1 billion *L plantarum* 299 and 10 g oat fiber (L299) was compared with 1 billion heat-killed L299 and 10 oat fiber (H299) and with selective digestive tract decontamination (SDD) [120]. The study comprised 95 patients divided into three groups: (1) SDD four times daily for 6 weeks ($n = 32$), (2) L299 ($n = 31$) during 12 postoperative days, and (3) H299 ($n = 32$) during 12 postoperative days. The same enteral nutrition was supplied to all patients. There were no deaths. Signs of infections occurred in 48% (15 of 32) of SDD patients, in 34% (11 of 32) of H299 patients, and in 13% (4 of 31) of L299 patients ($P = .017$). The numbers of postoperative infections were SDD, 23; H299, 17; and L299, 4. The numbers of patients requiring hemodialysis were SDD, 8; H299, 4; and L299, 2. In a subsequent double-blind randomized study, 33 patients were supplemented with the multistrain/multifiber Synbiotic 2000, and another 33 patients received only the four fibers in the synbiotic composition [121]. The treatment started on the day before surgery and continued until day 14 after surgery. Only one patient in the Synbiotic 2000–treated group (3%) showed signs of infection (urinary infection) during the first month compared with 17 of 33 (51%) patients supplemented with only the four fibers. The infecting bacterium with Synbiotic 2000 was *Enterococcus faecalis* in 1 patient compared with 11 in the fiber-only group. The group supplied fiber only also had *E coli* infection in three patients, *Enterobacter cloacae* infection in two patients, *Pseudomonas aeruginosa* infection in two patients, and *S aureus* infection in one patient. The use of antibiotics was also significantly shorter in the Synbiotic 2000–treated group.

Perioperative and critical care

In a prospective randomized study, the effect of the single-strain/single-fiber synbiotic based on *L plantarum* 299 in a dose of 1 billion LAB/d and 10 g of oat fiber (L299) was compared with a similar amount of heat-killed *L plantarum* 299 and oat fiber (H299) and parenteral nutrition (PN) in 3×30 patients undergoing abdominal operations, including liver resection, pancreas resection, gastric resection, colon resection, and intestinal bypass [122]. The L299 and H299 groups had significantly fewer infections (3 of 30 patients in each group, 10%) compared with the PN group (9 of 30 patients, 30%; $P > .001$). An even larger difference was observed when the subgroup of gastric and pancreatic surgery patients was analyzed separately: None of the L299 patients, one of eight H299 patients (12%), and three of six (50%) PN patients had infections. A similar study was done in patients undergoing abdominal cancer operations. Postoperative infections were observed in 1 of 15 patients (6.7%) when the multistrain/multifiber Synbiotic 2000 was supplemented in a dose of 40 billion LAB/d, in 3 of 15 patients (20%) supplemented with only the fibers in Synbiotic 2000, and in 7 of 15 patients (47%) supplemented with standard enteral nutrition (Han Chun Mao, personal communication). Significant improvements in prealbumin, C-reactive protein, serum cholesterol, serum endotoxin, and white cell blood count also were observed. A more recent, not-yet-published, study in acute extensive trauma patients reported a dramatic

decrease in number of chest infections with supplementation of 40 billion LAB-containing Synbiotic 2000 (1 of 14 patients, 7%) compared with only fibers (11 of 28 patients, 39%), peptide (10 of 21 patients, 48%), or glutamine (12 of 37 patients, 32%). Equally the total number of infections were decreased: Synbiotic 2000, 2 of 14 patients (14%); only fiber, 16 of 28 patients (57%); peptide, 11 of 21 patients (52%); and glutamine, 19 of 37 patients (51%) (L. Kompan, personal communication). [Q12]

SUMMARY

Prebiotic, probiotic, and synbiotic treatment is still in its infancy. Although remarkable effects have been observed, extensive studies are necessary to understand the many mechanisms behind the observed effects. Compared with acute conditions, chronic diseases seem much more resistant to attempts to affect the course by modification of microbiota. Many of the significant effects observed in experimental animals with induced chronic diseases have not been repeated in patients with chronic diseases, especially patients with IBD. Induced chronic diseases are more acute than chronic, which might explain the differences in sensitivity to modulation of microbiota. Also, there is a tendency to use much higher doses in animals than has hitherto been the case in humans. The experience with total flora replacement cannot be ignored; dramatic effects sometimes have been observed in the patients receiving transfer of extensive amounts of normal human flora and fibers. Dramatic effects also have been observed with the introduction of VSL#3, which contains more strains and is provided in much larger amounts–sometimes several times more than has hitherto been the case. It is disturbing, however, that the producers have provided no information about the criteria for selection of the strains in the composition. With the lack of such information, one has to assume that the selection has been at random. It is hoped that such information will be made available. As pointed out by Shahanan [123]: "as with all medications, it is preferable that the properties and behavior of an individual component of probiotic cocktails be fully determined in vitro, with synergistic and antagonistic activities identified, before they are adopted for widespread routine use." Most likely several of the strains in the composition, such as the yogurt bacteria, do not add to the efficacy of the probiotic cocktail. Nevertheless, VSL#3 has provided the direction for future avenues in the development of more efficient tools for modification of microbiota. Most likely, future research will focus on attempts to use synergistic effects by combining several strains and attempts to improve efficacy by the use of much larger doses than previously used. Most likely doses of 5 trillion LAB/d will be tried. In an excellent review, Sartor [124] concluded that "the interesting approach of combining probiotic and prebiotic agents (synbiotics) has considerable appeal." The extensive experience in acute diseases and the limited experience in chronic liver disease with synbiotic treatment seem to support such an assumption. As stated by Sartor [124] in his comprehensive review, "current data for therapeutic efficacy do not withstand rigorous scrutiny or fulfill current

evidence based rationale for using antibiotics, probiotics and prebiotics in the treatment of IBD," and "clinical trials have consistently been underpowered to show equivalency or superiority, many have design flaws that preclude definite results, or use outcomes such as disease activity index, that do not conform with widely accepted criteria for disease response or remission" Too often "enthusiasm outstrips scientific support for these therapeutic approaches." It is crucial that studies be performed by different and independent groups on all continents with their different living conditions.

Recommended standards for clinical trials and specifically for IBD are available [125,126]. It seems that none of the IBD-related studies have attempted to meet these standards. Almost 100 years have passed since Metchnikoff [127] suggested health benefits from external health-promoting bacteria and almost 50 years since Eiseman et al [128] reported successful treatment with fecal enemas in antibiotic-associated pseudomembranous enterocolitis. Clinicians since have not administered the inheritance well.

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