Probiotics: Promise, Problems, and Progress

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ABSTRACT

The efficacy of probiotics in acute enteric infections and post-antibiotic syndromes is now established and there is emerging evidence for a role in necrotizing enterocolitis, irritable bowel syndrome, and some forms of inflammatory bowel disease. However, clinicians should be aware of several problems and pitfalls in assessing probiotic usage. There is a pressing need for regulatory constraints on soft claims and quality control of probiotic products. However, the future is bright; enhanced understanding of the molecular details of host–flora interactions within the gut promises to yield new therapeutic targets and the potential to move from "bugs to drugs."

INTRODUCTION

The Nobel Prize awarded to Warren and Marshall in 2005 is a timely reminder that the solution to chronic disease may not reside solely within the host. A cure for peptic ulcer disease would never have emerged if attention had not shifted toward the interface between the host and the microbial environment. Although the flora is an essential health asset, conferring protection against infections, priming mucosal immunity, and producing vitamins, nutrients, and other bioactives, some components of the flora may become a liability depending on host susceptibility. Thus, the distinction between pathogens and commensals will vary depending on the context. Normally, the microflora is critical for mucosal homeostasis; it exerts developmental and regulatory influences on the structure and function of the gut and is a rich repository of metabolites that can be "mined" for therapeutic benefit.[1-3]

Exploiting the flora with probiotics and prebiotics is becoming a realistic therapeutic and prophylactic strategy for infectious, inflammatory, and even neoplastic diseases within the gut. A *probiotic* is usually defined as a live microorganism that, when consumed in adequate amounts, confers a health benefit on the host. A *prebiotic* is a nondigestable food ingredient (frequently an oligosaccharide) that can beneficially influence health by selectively altering the enteric flora, and a *synbiotic* is a mixture of pro- and prebiotics. In practice, the definition of probiotics is continually under revision as more is discovered about the mechanism of host–flora interactions. For practical purposes, probiotics are most simply defined in operational terms as commensal organisms that can be harnessed for therapeutic benefit. The most commonly used probiotics are lactobacilli and bifidobacteria, although other bacteria, such as nonpathogenic *Escherichia coli* and even nonbacterial organisms, such as *Saccharomyces boulardii,* have been used for probiotic effect. It is also noteworthy that the scope for harnessing microbes for therapeutic effects in inflammatory bowel disease is not limited to targeting host–bacterial interactions; helminths and helminthic antigens are currently being investigated with encouraging results in animal models of inflammation and in humans.[4-5]

Recently, the less restrictive term, *pharmabiotics*, has been used to encompass all forms of microbial manipulation in therapeutics, including pre- and probiotics, engineered strains, live and dead organisms, and metabolites or components thereof.[1]

Regrettably, the field of probiotics has been impeded by a bewildering array of unsubstantiated or soft claims for efficacy which may distract consumers and dissuade clinicians from distinguishing the science amid the snake oil. Therefore, our intent here is to present a brief overview of the clinical relevance of host–flora interactions and the status of probiotics in gastroenterology. This is followed by a review of selected publications during 2005 that extend the field or illustrate challenges for the future. For reviews of earlier work and for evidence for probiotics in extragastrointestinal conditions, the reader is referred elsewhere.[1-3, 6-8]

THE SCIENCE BEHIND THE STORY SO FAR

Where Is the Evidence for Efficacy?

The best evidence for probiotics in any condition is the treatment and prevention of enteric infections and postantibiotic syndromes. Even the most ardent skeptics concede that several meta-analyses, including a favorable Cochrane review, have confirmed efficacy in acute infectious diarrhea and prevention of antibiotic-associated diarrhea with probiotics.[6,9,10] In very low birth weight infants, probiotics reduce the incidence and severity of necrotizing enterocolitis. In experimental animal models of inflammatory bowel disease, numerous reports have shown the prophylactic effects of probiotics, and this has been linked with reduced proinflammatory cytokines and induction of regulatory cytokines.[11-12] However, the role of probiotics in human inflammatory bowel disease is more complex. The most impressive reports have been in patients with pouchitis, where controlled trials showed the efficacy of a cocktail of 8 bacterial strains (VSL#3),[13-14] although the wider, open-clinical experience with probiotics in similar patients seems to be more varied.[15, unpublished] Whether this relates to variability in patient populations or in the quality and choice of probiotic preparation, is unclear. In ulcerative colitis, the *E coli* Nissl 1917 strain has been reported to be equivalent in efficacy to mesalazine in maintenance of remission.[16] Induction of remission of acute ulcerative colitis has also been reported with the same strain,[17] and with a synbiotic[18] in 2 small studies. Results have been even less impressive in Crohn's disease, where controlled studies did not find efficacy for either *Lactobacillus rhamnosus* (GG)[19] or *Lactobacillus johnsonii* (LA1)[20] as maintenance therapy in Crohn's disease.

Is There a Biologically Plausible Mechanism of Action?

Probiotics exert their beneficial effects by mimicking normal microbe–microbe and host–microbe interactions. The mode of action depends on the setting. In acute infections and postantibiotic diarrheal syndromes, probiotics mimic the commensal flora by competitive interactions, antagonism of pathogens, and pro-

duction of antimicrobial factors.[21] Extensive evidence supports the role of probiotics in enteric clearance of pathogens and in reduction of bacterial translocation.[22]

In other clinical settings, host–microbe signaling is probably more relevant to probiotic action. It is now well established that mucosal homeostasis requires continual signaling from bacteria within the lumen of the gut. Thus, not only are bacterial signals required for optimal mucosal and immune development, they are actually required to maintain and condition the mucosa for responses to injury.[23,24] Incoming signals from the flora engage with pattern recognition receptors, such as Toll-like receptors (TLRs), on enterocytes, dendritic, and other host immune cells. The bacterial signals include surface proteins, metabolites, and bacterial DNA,[25] which are recognized by different combinations of TLRs. In this way, the host immune system distinguishes commensals from danger signals generated by episodic pathogens. Oral consumption of probiotics mimics this process and is associated with immune engagement and demonstrable systemic immunologic changes.[11,26] It seems that probiotics induce regulatory T cells and a restoration of cytokine balance in experimental models of enterocolitis.[12]

Transduction of bacterial signals with TLRs into immune responses is an area of vigorous investigation and promises to reveal new targets for therapeutic intervention. For example, the transcription factor nuclear factor-κB (NF-κB) is the pivotal regulator of epithelial and immune responses to invasive pathogens, but nonpathogenic bacteria can attenuate inflammatory responses by delaying the degradation of IκB which is counter-regulatory to NF-κB.[27] Other signal transduction pathways probably account for the anti-inflammatory effects of probiotics or commensals. For example, the anaerobe *Bacteroides thetaiotaomicron* antagonizes the proinflammatory effects of NF-κB within the epithelial cell by enhancing the nuclear export of its transcriptionally active subunit (RelA), in a peroxisome proliferator activated receptor-γ-(PPAR-γ)-dependent manner.[28]

In summary, probiotic therapy is more complex than manipulating the host flora or replacing "bad" bacteria with "good' bacteria. Rather, it is a question of mimicking the flora and exploiting host–flora signaling pathways.

Strain Selection and Quality Control—Problems and Pitfalls

Several unresolved issues continue to delay progress in the clinical evaluation of probiotics and may account for mixed results in different studies. First, not all probiotics are the same. To discuss probiotics only in generic terms is as superficial and misleading as referring to "pills" rather than specific drugs for precise indications. There are clear distinctions between different bacterial strains which may translate into variability in efficacy in different clinical conditions.[1] Guidelines for probiotic strain identification and functional characterization have been generated by the Joint Food and Agricultural Organisation (FAO) of the United Nations and the World Health Organisation (WHO).[29] At present, there are no in vitro biomarkers that predict in vivo probiotic performance in any condition. Comprehensive comparisons of probiotic performance using different strains are needed in specific disease states.

Second, the dose range for humans has not been determined and may vary with different probiotics, in part influenced by survival during gastric transit. In addition, the optimal vehicle and formulation for delivery of probiotics is an important variable,[26] but remains to be defined in many cases. Both formulation and vehicle may be critical to the shelf life of a living organism and the inclusion of a prebiotic in the vehicle could enhance probiotic numbers in the hind gut due to bifidogenic effects. Third, and of more immediate concern is the absence of an international standardized system for verification of probiotic product quality, composition, stability, and shelf life. In a recent study, neither the number of organisms nor the identity of the strains was accurate on the label of some products.[30] Fourth, in addition to improvements in labeling and quality control, there is a pressing need for more stringent regulation of unsubstantiated health claims. Fifth, individual variability in composition of the enteric flora may be a determining factor for optimal probiotic strain selection. While combinations of probiotic strains may be an appropriate strategy to accommodate individual variations in host flora and different clinical indications, synergy rather than antagonism within any given cocktail of bacteria needs to be demonstrated. Finally, it is unclear whether the optimal use of probiotics in conditions such as pouchitis[13,14] may require prior use of an antibiotic, notionally to clear the microbial niche.

ADVANCES IN 2005

The following commentary focuses on noteworthy studies published within the past year. It is not a comprehensive review; rather, the work has been selected because it extends the field or illustrates new challenges.

Success With a Serious Disease—Necrotizing Enterocolitis (NEC)

Necrotizing enterocolitis (NEC) is a life-threatening gastrointestinal disease seen most commonly in very low birth weight (VLBW) (<1500g) infants. Factors contributing to the pathogenesis include immaturity of intestinal and immune function, enteral feeding, and gas-forming bacteria. In a randomized, controlled clinical trial in which 367 VLBW infants were fed prophylactically with either a probiotic combination of *Lactobacillus acidophilus* and *infantis* with breast milk or breast milk alone, the probiotics were associated with a significant reduction in incidence of NEC without significant adverse events.[31] Although mindful of theoretical risks of probiotics in premature babies, adverse events have not been a major problem and several commentators have acknowledged the importance of this and similar studies.[32,33] The results are consistent with earlier preliminary reports and with a subsequent controlled trial of a probiotic mixture (*B infantis, Streptococcus thermophilus,* and *Bifidobacterium bifidus*) in 145 VLBW neonates which showed that the probiotics were again associated with reduced incidence and severity of NEC.[34] Probiotics are a conceptually appealing approach to prevention of NEC; they appear to be safe and more effective than other strategies. There is also an argument for administering the probiotics to the mothers before delivery in addition to infants in conjunction with mother's milk.[35]

Irritable Bowel Syndrome (IBS)—Progress at Last!

Changing concepts that accommodate evidence for immune activation in IBS and the recognition of postinfectious IBS as a distinct entity, have prompted investigation of new therapeutic strategies.[36] Three main conclusions were derived from a controlled clinical trial of a lactobacillus (*L Salivarius*) and a bifidobacterium (*B infantis* 35624) in 75 patients with IBS.[37] First, the study showed that not all probiotics are the same; the bifidobacterium but not the lactobacillus, had a statistically signifi-

cant beneficial effect on composite symptom scores and on pain perception. Second, patients with IBS were found to have a reduced ratio of anti- to proinflammatory cytokines; and finally, this was normalized after consumption of the bifidobacteria but not the lactobacilli. While the cytokine disturbances do not necessarily explain the symptoms of IBS, they offer a biomarker that can be studied in a disorder previously conspicuous for an apparent absence of objective biomarkers.[38] In an accompanying editorial,[39] a larger study with a dose-response and a global symptom score was called for. This has been performed in 362 patients and *B infantis* 35624 was again found to exert a statistically significant dose-dependent improvement in symptoms.[40] This work should be replicated provided there is due attention to the importance of strain selection, and the field needs to move to establishing mechanisms of probiotic action in IBS.

Disappointment in Crohn's Disease

As expected, probiotics do not work in all conditions; but surprisingly, in Crohn's disease, where evidence implicating the contribution of the flora to the pathogenesis is strong, controlled trials of probiotics have been negative.[19,20] In 75 children with Crohn's disease, a randomized, double-blind trial of *Lactobacillus GG* in addition to standard maintenance therapy over 2 years versus placebo, did not significantly reduce the occurrence of relapses or alter the length of remission.[19] In addition, another lactobacillus (*L johnsonii LA1*) did not achieve statistical significance in reducing endoscopic recurrence of Crohn's disease, postoperatively, in a randomized double-blind, placebo-controlled trial of 98 patients.[20] Are we dealing with the wrong probiotic, the wrong dose, or the wrong indication? Are probiotic combinations needed? Variability in the composition of the flora both quantitatively and qualitatively along and over the cross-sectional axis of the gastrointestinal tract suggests that depending on the topographic distribution of the lesions in Crohn's disease, a single probiotic may not be equally suited to different subsets of patients.

Designer Probiotics—Another Step Forward, More To Do

Although naturally occurring probiotics may have insufficient efficacy in Crohn's disease, genetically engineered organisms (GMOs) for site-specific delivery of therapeutic molecules to the intestinal lesions is a realistic proposition. The potential for these "turbo probiotics" is limited only by one's imagination. Proof of principle has already been shown with a GMO (*Lactococcus lactis*) designed to deliver either the anti-inflammatory cytokine, interleukin-10 (IL-10), or the cytoprotective agent, trefoil factor, in animal models of enterocolitis.[41] The main safety concern surrounding GMOs is the theoretical public health risk if such organisms are excreted into the environment. This has already been addressed by insertion of the therapeutic transgene into the thymidylate synthase (*thy* A) gene locus. Without this enzyme, the organism is dependent on thymine or thymidine in the local microenvironment, but these are not readily available within the external environment, thereby limiting the viability of the excreted organism. In addition, the transgene would be eliminated from the bacterial genome if the engineered organism re-acquires the *thy* A gene from the wild-type strain.[42] However, an additional problem with the choice of recombinant *L lactis* relates to its bioavailability due to limited survival during gastrointestinal transit. This has been overcome with development of an enteric-coated formulation containing freeze-dried viable GM *L lactis*[43] which has already been used in an open trial of 10 patients with Crohn's disease.[43,unpublished] A carefully designed, controlled clinical trial is now needed.

An Unexpected Finding in the Flora

The modern runaway pandemic of obesity is usually considered in terms of the balance of energy intake and expenditure, but it now appears that the microflora has a contributory role. Intestinal bacteria are net contributors to human and animal nutrition, producing vitamins and facilitating the digestion of polysaccharides with enzymes that are not encoded for in our genome. Gordon and colleagues have shown that the microflora represents an environmental factor regulating fat storage by a pathway that involves microbial signals that negatively control the expression of fasting-induced adipocyte protein (Fiaf) in enterocytes.[44] This is a circulating inhibitor of lipoprotein lipase. More recently, the same investigators have shown that obesity affects the diversity of the intestinal flora, raising the possibility that manipulating the flora, particularly at an early age, may be useful for controlling energy balance in those who are at risk of obesity.[45] In mice that develop obesity because of deficiency in the leptin gene (*ob/ob*), the composition of the gut flora in the distal intestine was found to change with increasing adiposity. A major reduction in Bacteroidetes (also known as Cytophaga-Flavobacterium-Bacteroides) with proportional increase in Firmicutes (the majority of which are clostridia) was observed. While the work is at an early stage and the underlying mechanisms are unclear, these results highlight the importance of the metabolic activity of the commensal flora, and raise the intriguing vista of modifying the flora in the struggle against obesity or perhaps even prophylactically controlling the composition of the flora colonizing neonates.

LOOKING AHEAD

To fulfill the promise of pharmabiotics, normal host–flora interactions need to be better understood. The scope and importance of the metabolic activity of the intestinal flora has been shown by its apparent role in obesity. It is also likely that host–diet–flora interactions are involved in colorectal carcinogenesis. Despite remarkable advances, the incidence of this great killer has changed little, perhaps because a key component of the pathogenesis residing within the lumen of the colon has received inadequate attention.

"Mining" the flora for metabolites that have an impact on host physiology is a promising source of new therapeutics. Examples include the production of antimicrobial peptides,[21] conjugated linoleic acid,[46] and immunoregulatory molecules that control the maturation of the host immune system.[47] Similarly, once the molecular mechanisms of probiotic action in IBS and other disorders are understood, the potential to move from "bugs to drugs" will become a reality. For some conditions, such as aggressive IBD, engineered probiotics may be required. In designing such organisms, much can be learned from the sophisticated mechanisms deployed by pathogenic bacteria to overcome or evade host defenses. Bad bugs may even be used to do good things.[48] The anti-inflammatory properties of probiotics can also be improved by directed mutation rather than by genetic modification. For example, the d-alanine content of lipoteichoic acid, a component of the bacterial cell wall, has been found to modulate the immune response and determine protec-

tion conferred by a mutant *Lactobacillus plantarum* in a murine model of colitis.[49]

What practical advice can one give to clinical gastroenterologists now? As shown by 2 Nobel laureates, it is no longer acceptable to study gastrointestinal physiology and pathophysiology outside the context of the microbial residents within the gut. Gastroenterologists have exploited colonic bacterial metabolism for decades to generate active metabolites from prodrugs such as sulfasalazine. They now need to be aware of an expanding scope for the flora in clinical medicine. However, clinicians should continue to uphold traditional principles of patient management and evidence-based medicine when choosing pharmabiotic strategies. These should be selected only on the basis of solid science. Consumers should avoid products that lack true scientific backing.

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