Physiological Basis for Novel Drug Therapies Used to Treat the Inflammatory Bowel Diseases

I. Pathophysiological basis and prospects for probiotic therapy in inflammatory bowel disease

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Shanahan, Fergus. Physiological Basis for Novel Drug Therapies Used to Treat the Inflammatory Bowel Diseases. I. Pathophysiological basis and prospects for probiotic therapy in inflammatory bowel disease. *Am J Physiol Gastrointest Liver Physiol* 288: G417–G421, 2005; doi:10.1152/ajpgi.00421.2004.—Mechanisms underlying the conditioning influence of the intestinal flora on mucosal homeostasis, including development and function of immune responses, are attracting increasing scientific scrutiny. The intestinal flora is a positive asset to host defense, but some of its components may, in genetically susceptible hosts, become a risk factor for development of inflammatory bowel disease (IBD). It follows that strategies to enhance assets or offset microbial liabilities represent a therapeutic option; therein lies the rationale for manipulation of the flora in IBD. In addition, the diversity of regulatory signalling among the flora and host epithelium, lymphoid tissue, and neuromuscular apparatus is an untapped reservoir from which novel therapeutics may be mined. Moreover, the capacity to engineer food-grade or commensal bacteria to deliver therapeutic molecules to the intestinal mucosa promises to extend the scope of microbial manipulation for the benefit of mankind.

bacteria; intestinal flora; Crohn’s disease; ulcerative colitis; mucosal immunity

THE CHRONIC INFLAMMATORY BOWEL DISEASES (IBD), which are comprised of Crohn’s disease and ulcerative colitis, cause much personal suffering and disablement for patients and represent a substantial economic burden on healthcare resources. Of the three major contributory factors to the pathogenesis of IBD, genetic susceptibility, environmental triggers, and immune activation, only the latter is targeted by most current therapeutic strategies. Notwithstanding remarkable advances in biological and other immunomodulatory therapy over the past decade, the enthusiasm for such drugs is tempered by various issues such as expense, toxicity, and incomplete efficacy in many patients. Sustained therapeutic responses in IBD may require more comprehensive approaches including modification of the bacterial microenvironment.

The lesson of *Helicobacter pylori* and chronic peptic ulcer disease is a sobering reminder that the solution to some chronic disorders cannot be resolved by exclusive investigation of the host response. Lasting cure of peptic ulceration would never have been achieved by strategies directed solely at suppressing the host response with gastric acid suppressants. Rather, it was the host-*Helicobacter* interaction that held the answer. Similarly, host-flora interactions underpin the pathogenesis of chronic disorders such as IBD. In most instances, these appear to involve components of the normal commensal flora rather than infections with specific pathogens (4, 42).

Commensal bacteria within the human gastrointestinal tract vary widely in proinflammatory capacity, with some having apparent anti-inflammatory properties. Therefore, optimal modification of the intestinal ecosystem with probiotics has emerged as a realistic therapeutic opportunity for IBD (36, 41). Traditional descriptions of probiotics as “friendly” or “good” bacteria betray a naivété that has been eroded by a more intriguing picture involving the host response and modification of mucosal immunoinflammatory responses to the microenvironment (13). Although there is mounting evidence from meta-analyses for probiotic efficacy in several clinical conditions, particularly in *Clostridium difficile*, rotavirus, and other mucosal infections (11), the evidence for efficacy in IBD is less clear and largely based on studies in pouchitis and experimental animal models. Nonetheless, there is intriguing circumstantial evidence for manipulation of the gut flora in IBD. The intent here is to present an overview of probiotic rationale and promise for the future in IBD; the emphasis is on current concepts of mechanisms and the potential for therapeutic “mining” of the flora. Other sources are recommended for reviews on clinical and experimental efficacy with probiotics (11, 36, 41).

LIMITATIONS OF DEFINITION AND SELECTION CRITERIA FOR PROBIOTICS

Definitions of probiotics are evolving as understanding of their effects on human physiology increases. At present, the term describes “live micro-organisms, which when consumed in adequate amounts, confer a health benefit on the host” (33, 51). However, this definition may be too restrictive. Live bacteria may not be an absolute requirement for therapeutic efficacy; bacterial constituents, such as CpG DNA, account for some of the anti-inflammatory effects of probiotics, whereas secreted metabolites, such antimicrobial peptides (bacteriocins), contribute to others. Although several authorities, including the Joint Food and Agricultural Organization of the United Nations and the World Health Organization (33, 51), have described selection criteria for probiotic organisms, it is noteworthy that there is currently no in vitro predictor of probiotic performance (18, 41). Lactobacilli and bifidobacteria have traditionally been the most common candidates, but nonpathogenic *Escherichia coli* and nonbacterial organisms, such as *Saccharomyces boulardii*, or even nematode parasites have been used for probiotic effect (40, 42).

Most commercially available probiotics meet minimum selection criteria including acid and bile resistance and survival during gastrointestinal transit, but an ideal probiotic strain for...
any given indication has not been defined. In addition, rigorous strain-strain comparisons of probiotic performance have yet to be performed in a single disease setting. Different probiotics are unlikely to be equally suited to all indications; selection of strains for disease-specific indications will be required. Optimal selection of a probiotic may even need to take into account individual variations in host diet and composition of gut flora. In this respect, the apparent influence of human genetic variability on intestinal bacterial composition is particularly intriguing (47).

Finally, the likely emergence of genetically or otherwise-modified food-grade commensal bacteria may have to be accommodated into the probiotic concept, as discussed below. This, coupled with clarification of the molecular determinants and mechanisms of probiotic action, will hasten a transition “from bugs to drugs.” Perhaps the neologism “pharmabiotic” may be a more appropriate generic or umbrella term to encompass any form of therapeutic exploitation of the commensal flora including the use of whole organisms (probiotics), food ingredients that influence the composition of the flora (prebiotics), combinations thereof (synbiotics), dead or live organisms, or biologically active bacterial metabolites.

**PROKARYOTIC-EUKARYOTIC SIGNALLING WITHIN THE GUT**

Because probiotics may be considered operationally as commensal organisms that can be exploited for health benefit, the mechanisms of probiotic action are likely to be reflected in the normal host-flora signalling processes. The most tangible evidence for bacterial-derived regulatory influences on intestinal structure and function have been derived from comparative studies of germ-free and colonized animals (42) (Table 1).

At birth, immunologic organs are developed, but mucosal immune responses require education and fine tuning of cytokine balances and T cell repertoires. This is achieved with microbial exposure by bacterial colonization and sporadic mucosal infections. Without the flora, mucosal lymphoid tissue is rudimentary; induction of mucosal immune responses and tolerance is suboptimal (42).

Although the molecular details underpinning the regulatory exchanges between the flora and mucosal structures are unclear, they can now be explored with modern technology such as laser microdissection and gene array analysis (15, 16). When applied to animals colonized with only a single bacterial strain, *Bacteroides thetaiotaomicron*, this combined approach has illustrated the impact of bacterial-derived signalling on the expression of host genes controlling mucosal barrier function, nutrient absorption, angiogenesis, and development of the enteric nervous system. Similarly, the sequencing of the bacterial genome for several commensal (52) and probiotic strains (30) will help reveal properties that are essential for desired probiotic effects on host function.

The incoming bacterial signals include secreted chemotracants such as the formylated peptide f-met-leu-phe, cell wall constituents such as peptidoglycans and lipopolysaccharide, and bacterial nucleic acids (CpG DNA). These maintain the mucosal immune response in a state of “controlled” or physiological inflammation, a state of tolerance or constrained responsiveness to the commensal flora but on ready-alert for rapid response to episodic challenge with pathogens. This requires exquisitely precise regulation and accurate discriminatory responses to danger microbial signals versus those from harmless commensals. In this respect, the mucosal immune response is a sensory organ, the afferent and efferent limbs of which comprise a network of connectivity among lymphoid, epithelial, neuronal, stromal, and endocrine components of the intestine (39).

Sampling of the microbial environment across the epithelial “barrier” occurs at three main sites. First, M cells that overlie lymphoid follicles transport particulate and some microbial antigens to subjacent antigen-presenting cells (dendritic cells, B cells, and macrophages). Second, surface enterocytes transport soluble antigens and serve as afferent sensors of danger within the luminal microenvironment by producing chemokines that alert the host innate and acquired immune responses and direct them to breaches in mucosal barrier with infection (21). A bidirectional IgG-dependent system transepithelial transport of antigen has recently been demonstrated (8). In addition, specialized Paneth cells within the epithelium may also exhibit microbial discriminatory responses in relation to production of defensins. Third, dendritic cells throughout the mucosa have a pivotal role in mucosal immunosensory functions (44). These antigen-presenting cells have been identified within intestinal epithelium in rodents (26), and in vitro modeling suggests that subepithelial dendritic cells extend into the lumen between the surface enterocytes without disrupting tight junctions (34).

Compelling evidence has shown that intestinal dendritic cells can ingest and retain intact live commensal bacteria and transit to the mesenteric lymph node where immune responses to commensals are induced locally (25). Thus the mesenteric lymph node acts as a gatekeeper, preventing access of commensal bacteria to the internal milieu and protecting the host from harmful systemic immune reactivity. As expected, the immunosensory function of dendritic cells exhibits marked plasticity and versatility of responses (17). Moreover, dendritic cells are heterogeneous, with tissue-specific specialization in the gut (19, 20). These cells are the decision makers determining the balance of T effector cell responses (T$_{H1}$ and T$_{H2}$ effectors) versus regulatory T cell responses (T$_{reg}$/tolerance). Thus they provide the switch for the host response to danger from pathogens and determine the nature of that response (Fig. 1). The apparent paradox of probiotic efficacy in TH1- and TH2-mediated inflammatory disorders may be accounted for if the mechanism of probiotic action is activation of T$_{reg}$ cells.

In addition to presenting intact live bacteria to the immune system, dendritic cells respond rapidly to danger signals from the microbial environment via pattern recognition receptors (PPRs), which include Toll-like receptors (TLRs) (1) and C-type lectins. PPRs are also expressed on epithelial cells and may be differentially altered in ulcerative colitis and Crohn’s
disease (5, 6). Multiple TLRs are probably used simultaneously by immunocytes to recognize the features of a specific microbe. TLR2 recognizes lipoproteins and peptidoglycans and triggers the host response to gram-positive bacteria and yeast; TLR4 mediates responses to LPS primarily from gram-negative bacteria; TLR1 and TLR6 participate in activation of macrophages by gram-positive bacteria, whereas TLR5 and TLR9 recognize flagellin and bacterial (CpG) DNA, respectively. Bacterial DNA and oligonucleotides containing unmethylated CpG dinucleotides stimulate lymphocytes, whereas eukaryotic DNA and methylated oligonucleotides do not (49, 50).

**PROKARYOTIC-REGULATED EPITHELIAL ANTI-INFLAMMATORY RESPONSES**

A miscellany of host responses to commensal or probiotic bacteria has been observed in different experimental settings (Table 2). Some of these were expected, even predictable. Their therapeutic significance is uncertain. However, with the resurgence of interest in host-flora interactions, hitherto unknown anti-inflammatory mechanisms have emerged. Thus distinct mechanisms of bacterial regulation of epithelial responses in inflammation have been reported.

Transduction of bacterial signals into host immune responses probably proceeds along several pathways, but the transcription factor, NF-κB, is the pivotal regulator of epithelial responses to invasive pathogens (42). Separate mechanisms of prokaryotic regulation of NF-κB-mediated responses within epithelial cells have recently been described for nonpathogenic and/or commensal bacteria (22, 28). For example, some non-pathogenic bacteria can attenuate inflammatory responses by delaying the degradation of IκB, which is counterregulatory to NF-κB (28). Although conventional probiotic bifidobacteria and lactobacilli do not appear to use this mechanism, other signal-transduction pathways are likely to emerge to account for their anti-inflammatory effects. Thus the commensal anaerobe *Bacteroides thetaiotaomicron* has been shown to attenuate inflammation by antagonizing NF-κB within the epithelial cell, and this is achieved by enhancing the nuclear export of the transcriptionally active RelA subunit of NF-κB in a peroxisome proliferator-activated receptor γ-dependent manner (22). As the molecular details of these prokaryotic regulated anti-inflammatory events become clear, they may be translated into new therapeutic targets.

**PROBIOTICS AND IMMUNITY**

Although the host immune modulation by probiotics may be expected to mimic some of the effects of the indigenous flora, several caveats of therapeutic relevance are noteworthy. First, probiotics are not a generic form of therapy (36); different probiotics have distinct properties, and not all models of experimental colitis respond to the same probiotics (11). In particular, different species of lactobacillus have been shown to exert distinct patterns of dendritic cell activation, and at least...
one species appears to inhibit dendritic cell activation by others within the same genus (7). This has implications for the therapeutic use of multispecies combinations of probiotics. Second, there appears to be regional variability in immunological effects of probiotic organisms. Although lactobacilli characteristically induce Th1-type cytokines, including IL-12 and TNF-α, from peripheral blood mononuclear cells (27), exposure of mucosal tissue ex vivo to different lactobacilli, but not other bacteria, led to downregulation of TNF-α production (3). Third, probiotics can attenuate inflammatory disease without any apparent impact on gut flora. This was shown by the efficacy of subcutaneously administered Lactobacillus salivarius 118 to IL-10 knockout mice (43). Moreover, the probiotic effect was not specific to colitis, and an anti-inflammatory effect was also observed in a murine model of arthritis after subcutaneous delivery of the probiotic. This emphasizes the role of the host response in determining probiotic efficacy and indicates that mucosal delivery may not be essential. Fourth, in certain murine models of IBD, bacterial CpG DNA mediates the anti-inflammatory effect of probiotics by signalling through host TLR9 receptors (31, 32). These studies question whether live organisms are an absolute requirement but, as pointed out by others (13), are not fully conclusive. Once again, bacteria vary and differ in immunostimulatory DNA content (13, 30). More importantly, CpG DNA motifs may have opposing effects in experimental models of intestinal inflammation depending on the timing of its administration. In contrast to the proinflammatory effect of CpG DNA before the onset of inflammation, exposure to CpG DNA during acute inflammation has been shown to exacerbate disease in a murine model of IBD (29). Finally, although engagement with the host immune system is central to probiotic mechanisms, metabolites other than CpG nucleotides may influence selection criteria in certain settings. Bacterial production of short-chain fatty acids as nutrients for the colonic epithelium is well established but less well known is the production of conjugated linoleic acid (CLA) by some probiotic organisms. Among its health benefits, CLA has important anti-inflammatory properties (9).

TURBO PROBIOTICS–ENGINEERING DESIRED FUNCTION

The exploitation of microbes is no longer limited to their role as cell factories for production of human therapeutics. Commensal and food-grade bacteria can be engineered for delivery of anti-inflammatory cytokines or other biologically active molecules and vaccines to the gut. Proof of principle and efficacy have been demonstrated with Lactococcus lactis, engineered to secrete IL-10 locally within the gut in murine models of IBD (45). Other examples of genetically modified (GM) microbes include the delivery of single-chain antibodies for pathogen-specific passive immunity (2, 23) and bacterial-derived trefoil factors to promote healing and repair in the inflamed murine gut (48).

Public health concerns about the release of GM organisms into the environment have replaced technological constraints as the major hurdles to be overcome with GM bacteria (14). One approach to contain GM bacteria after their excretion from the host is to substitute the therapeutic transgene for the thymidylate synthase (thy A) gene within the bacterial genome (46). Because this enzyme is required for DNA biosynthesis by methylating uracil or uridine to make thymine or thymidine, the organism becomes dependent on the latter, which are available within the gut but not within the external environment. This leads to bacterial cell death and containment of the GM organism outside the host. An appealing aspect of this strategy is the elimination of the transgene from the bacterial genome in the event of the engineered organism reacquiring the thy A gene from the wild-type strain. The importance of this type of biocontainment for avoiding unforeseen consequences of transgenic organisms escaping into the environment has been emphasized in a recent report by the National Academics Research Council (reviewed in Ref. 14).

In conclusion, the metabolic activity of the indigenous gut flora represents a rich repository from which novel therapeutic agents can be “mined.” Therapeutic manipulation of the intestinal flora with any form of pharmabiotic is, at present, suboptimal because of incomplete understanding of the normal flora and host-flora interactions. Although the antimicrobial actions of probiotics and their prophylactic efficacy against infectious diseases are now well established, the anti-inflammatory properties of the indigenous commensal/probiotic flora are perhaps more intriguing. Mechanisms of probiotic action vary depending on the experimental or clinical context and depending on differences in the host and in the bacterial strain, but engagement with host immunity is central to probiotic action in IBD.

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REFERENCES

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