Rationale and Efficacy of Manipulating Intestinal Bacteria in Chronic Intestinal Inflammation by Probiotics, Prebiotics, and Diet

R. Balfour Sartor, MD

Humans coexist with an incredibly complex diversity of bacteria, fungi, and viruses that increases in both complexity and number in the distal relative to the proximal GI tract. The microbiota of the stomach and duodenum is predominantly characterized by aerobic *Lactobacillus* and *Streptococcus* and *Candidus* species while the distal ileum and colon are colonized by up to 10^{11}-10^{12} predominantly anaerobic bacteria that comprise up to 1000 species.[1] These fecal bacteria consist of 16 divisions but the majority are Firmicutes (69%) and Bacteroidetes (17%), with smaller numbers of Actinobacteria (6%) and Proteobacteria (5%).

The gut microbiome is an integral part of our genetic landscape and humans can be viewed as a supra-organism composed of both human and microbial species, genomes, and metabolomes.[1] Comparative studies show that Firmicutes and Bacteroidetes dominate across all mammals, but there are profound dietary influences such that carnivores have the fewest divisions with the highest concentrations of Firmicutes and herbivores have the most complex microbiota.[2] The ratio of Firmicutes and Bacteroidetes is altered by a diet leading to weight loss with a gradual decrease in Firmicutes and increase in Bacteroidetes over 52 weeks.[3] In addition to compositional changes, diet dramatically alters bacterial species' gene expression by activating those genes relevant to available dietary substrates.[4] Finally, intestinal bacteria are efficient metabolic factories that ferment dietary carbohydrates and fiber to protective short-chain fatty acids such as butyrate, which is the primary metabolic fuel of the colonocyte. Conversely, other bacterial species can produce potentially toxic metabolites such as hydrogen sulfide and oxygen metabolites from other dietary substrates.

Bacteria Involved in the Pathogenesis of Inflammatory Bowel Diseases (IBD)

Bacteria are integrally involved in the pathogenesis of IBD by providing the chronic antigenic and toll-like receptor agonists that activate effector adaptive and innate immune responses, respectively, in genetically susceptible hosts (Fig 1).[5,6]

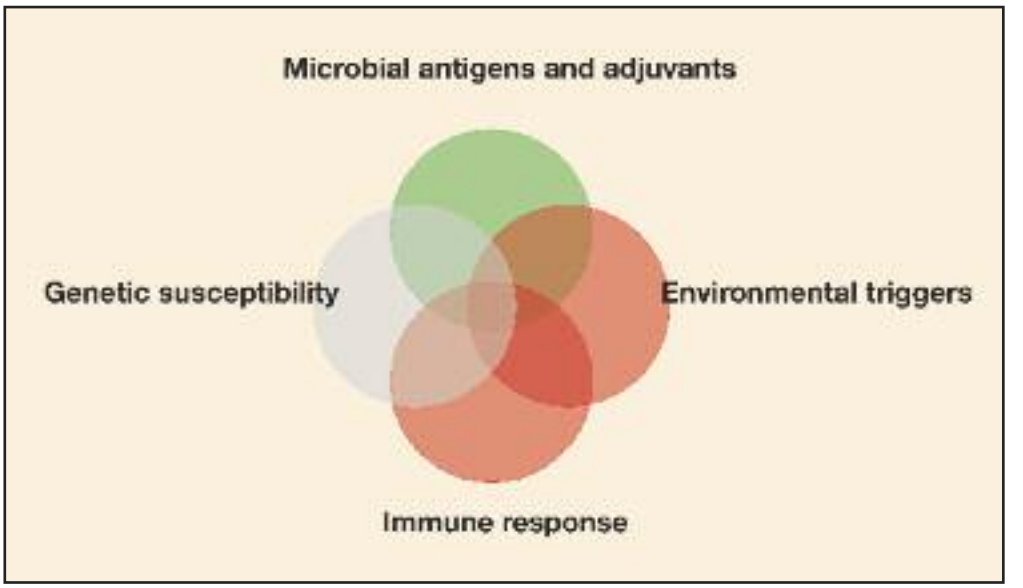

Fig 1. Pathogenesis of inflammatory bowel disease. Reprinted by permission of Wolters Kluwers Health. http://lww.com.

Developing evidence suggests that dysbiosis (abnormal microbial composition or function) can contribute to if not cause chronic intestinal inflammation.[5,7] This inflammation can be caused either by an abnormal composition of enteric bacteria with an elevated ratio of aggressive vs protective species, defective production of short-chain fatty acids and other protective microbial products, or enhanced production of hydrogen sulfide and nitrates that block butyrate metabolism and disrupt the mucosal barrier. Available data show a selective decrease in Bacteroidetes and Lachnospiraceae, including Clostridia groups IV and 14A.[5,7] Gnotobiotic rodent studies illustrate the essential nature of commensal enteric bacteria in chronic immune-mediated intestinal inflammation.[5] Selected colonization of genetically susceptible hosts indicates that various bacterial species have differential abilities to induce or prevent experimental colitis, signifying that all bacteria are not equal in this capacity.[8] In this setting some commensal bacterial species are aggressive, some are neutral, and some are protective, including multiple *Lactobacillus* and *Bifidobacterium* species and the commensal

Faecalibacterium prausnitzii.[9] Thus there is ample evidence that the relative balance of beneficial vs detrimental bacteria strongly influences intestinal inflammation vs homeostasis (Fig 2).

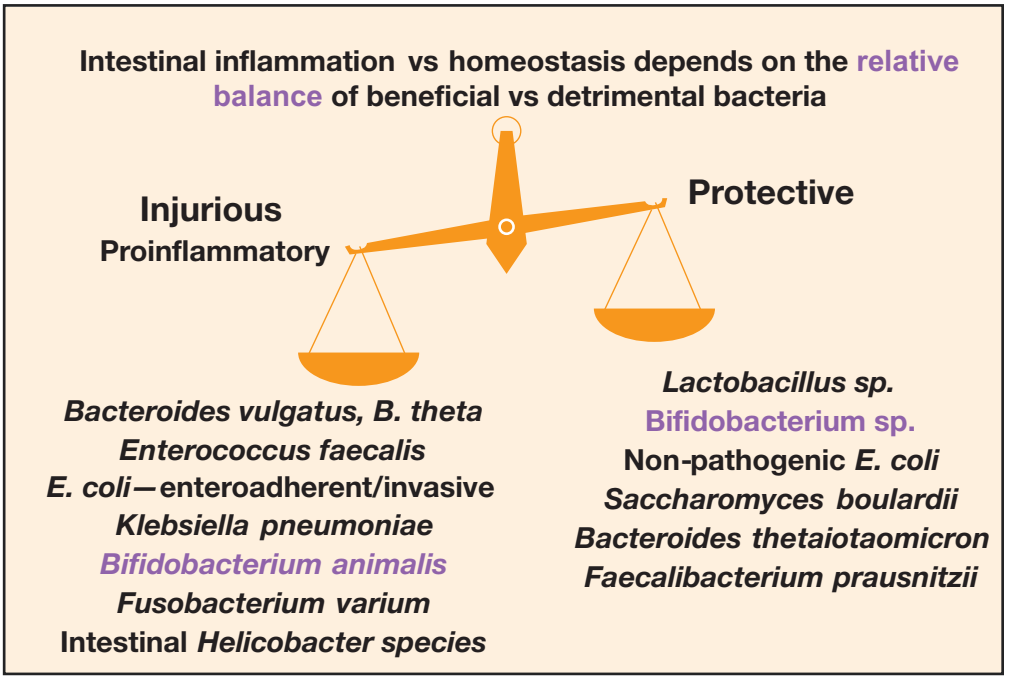

Fig 2. Relative balance of beneficial vs detrimental bacteria strongly influences intestinal inflammation vs homeostasis. *E. coli = Escherichia coli.*

Diet Affects Experimental Colitis

We observed that dietary iron, sucrose, fructose, and aluminum potentiates colitis in interleukin (IL)-10-deficient mice and that nonabsorbed oligosaccharides (prebiotics) attenuate colitis in HLA-B27 transgenic rats.[10-13] We hypothesize that dietary constituents affect enteric bacterial composition, gene expression, metabolic activity, and mucosal immune function. These changes induce chronic intestinal inflammation or promote mucosal homeostasis or tolerance. Postulated mechanisms include preferential stimulation of growth of detrimental bacteria by dietary sucrose, fructose, and iron, while poorly absorbed oligosaccharides (prebiotics) foster growth and metabolic activity of beneficial bacteria with decreased production of short-chain fatty acids. Dietary aluminum, iron, and animal fat stimulate pathogenic mucosal immune responses. We have demonstrated that dietary iron alters bacterial composition by expanding luminal *Klebsiella*, *Escherichia coli*, and *Citrobacter rodentium*. These enteric commensal species require iron for

growth.[10] Likewise, several adherent/invasive *E. coli* strains that are capable of causing experimental colitis in knockout mice preferentially grow with fructose but are incapable of metabolizing sucrose. Conversely, the protective enteric species *Faecalibacterium prausnitzii* does not grow with fructose, glucose, or sucrose but preferentially proliferates in the presence of maltose as a carbon source. Thus, it is quite likely that diet can contribute to the composition of intestinal bacteria with increased growth and function of aggressive species by refined sugars and iron and of protected bacteria by complex carbohydrates such as dietary fiber and commercially available prebiotics.

Clinical Efficacy of Probiotics in IBD

Probiotics have been used in multiple clinical trials in an effort to treat ulcerative colitis, Crohn's disease, and pouchitis.[14-16] These studies show some potential benefit of certain agents to prevent relapse of ulcerative colitis and possibly treat mild to moderate active ulcerative colitis, but no real benefit in Crohn's disease. However, a dramatic improvement in preventing relapse (maintaining remission) of chronically recurring pouchitis by VSL 3, a combination of eight different probiotic species, was noted.[17] Clearly there is a need for large, multicenter, double-blind, placebo-controlled trials for primary and adjunctive treatment of active ulcerative colitis and Crohn's disease with probiotics, as well as for the use of these agents in preventing relapsing steroid-treated ulcerative colitis and Crohn's disease and preventing pouchitis.

Certain conclusions can be reached: 1) Different probiotic species have different efficacies, 2) commercially available probiotics do not colonize the intestine over time despite chronic use, 3) components of probiotics can have biologic effects even when the parent organisms are nonviable, and 4) protected bacteria work by multiple mechanisms that include inhibiting growth and epithelial binding of pathogenic bacteria, improving epithelial barrier function and augmenting immunoregulatory effects by enhancing protective cytokines such as IL-10 and transforming growth factor beta (TGFβ) while blocking effector molecules such as tumor necrosis factor (TNF) and IL-12 p40. A relatively unexplored but exciting treatment option is to use genetically engineered probiotics or commensal bacteria to deliver protective molecules.[18]

Use of Prebiotics and Diet in Intestinal Inflammation

Prebiotics are dietary substances, usually nondigested carbohydrates, that stimulate the growth and metabolic activity of beneficial enteric bacteria. These substances can prevent intestinal inflammation by stimulating growth of protective

commensal bacteria such as *Bifidobacterium* species; enhancing production of short-chain fatty acids such as butyrate that have protective activities; decreasing stool pH, which inhibits growth of detrimental bacteria; and enhancing water-holding capacity of the stool.[5,15] The use of prebiotics in human IBD has been limited but several compounds, particularly fructooligosaccharides, including inulin, have demonstrable activity in experimental colitis.[13,15] In a pilot study, fructooligosaccharides appeared to have clinical benefit in active ileocolonic Crohn's disease with some immunoregulatory activities.[19]

Dietary therapy of IBD could improve intestinal inflammation by several mechanisms: changing bacterial composition and metabolism, preventing bacterial adherence to mucosa, upregulating epithelial expression of toll-like receptors, inhibiting aggressive immune responses, and promoting epithelial differentiation. In the process, nutritional therapy could have the combined benefits of treating and preventing nutritional deficiencies, improving the host immune response, and improving microbial composition and metabolism, with the net effect of improving clinical outcomes.

Conclusions

Antibiotics, probiotics, and prebiotics, and the combination of all three approaches, have great potential to treat active IBD and to prevent relapse, but each patient subset may respond selectively to various agents. This selective response may result in the need to individualize treatment for each patient. To date, manipulating the intestinal microbiota in human IBD has not substantially altered the underlying disease process or changed the natural history of these disorders. It is likely that we have not yet identified the optimal mix of probiotics and/or prebiotics or that individual patient responses have obscured overall results in groups of heterogeneous patients. There is no doubt, however, that dietary manipulation offers the most physiologic and least toxic approach to treating IBD and has tremendous potential for long-term use. Finally, it is likely that multiple human inflammatory infectious and hypersensitivity disorders might respond to therapeutic use of probiotics, prebiotics, and dietary manipulation (Table).

Table. Potential Role for Probiotics, Prebiotics, and Nutritional Therapy in Multiple Human Conditions

- IBD (pouchitis, ulcerative colitis, Crohn's disease)
- Irritable bowel syndrome
- Enteric infections
- Obesity
- Metabolic syndrome
- Nonalcoholic steatohepatitis
- Chronic obstructive pulmonary disease
- Cystic fibrosis
- Asthma, hypersensitivity disorders

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