Therapeutic Manipulation of the Enteric Microflora in Inflammatory Bowel Diseases: Antibiotics, Probiotics, and Prebiotics

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Crohn's disease, ulcerative colitis, and pouchitis are caused by overly aggressive immune responses to a subset of commensal (nonpathogenic) enteric bacteria in genetically predisposed individuals. Clinical and experimental studies suggest that the relative balance of aggressive and protective bacterial species is altered in these disorders. Antibiotics can selectively decrease tissue invasion and eliminate aggressive bacterial species or globally decrease luminal and mucosal bacterial concentrations, depending on their spectrum of activity. Alternatively, administration of beneficial bacterial species (probiotics), poorly absorbed dietary oligosaccharides (prebiotics), or combined probiotics and prebiotics (synbiotics) can restore a predominance of beneficial *Lactobacillus* and *Bifidobacterium* species. Current clinical trials do not fulfill evidence-based criteria for using these agents in inflammatory bowel diseases (IBD), but multiple nonrigorous studies and widespread clinical experience suggest that metronidazole and/or ciprofloxacin can treat Crohn's colitis and ileocolitis (but not isolated ileal disease), perianal fistulae and pouchitis, whereas selected probiotic preparations prevent relapse of quiescent ulcerative colitis and relapsing pouchitis. These physiologic approaches offer considerable promise for treating IBD, but must be supported by rigorous controlled therapeutic trials that consider clinical disease before their widespread clinical acceptance. These agents likely will become an integral component of treating IBD in combination with traditional anti-inflammatory and immunosuppressive agents.

The chronic idiopathic inflammatory bowel diseases (IBD) Crohn's disease and ulcerative colitis appear to be caused by an overly aggressive cell-mediated immune response to luminal commensal bacteria in genetically susceptible individuals.[1,2] Therefore, it is rational to consider therapeutic approaches that eliminate the bacterial antigens and adjuvants that constantly drive the pathogenic immune response as a potentially important component of treating IBD, in concert with anti-inflammatory and immunosuppressant agents. Although use of narrow- and broad-spectrum antibiotics, probiotics (beneficial bacteria), prebiotics (dietary components that foster the growth of beneficial bacteria), or the combination of each of these approaches has considerable logic, unfortunately, there is quite limited documentation of efficacy of these agents in properly designed controlled trials. This clinically oriented review discusses the rationale, available evidence for the use of antibiotics, probiotics, and prebiotics, and their mechanisms of action in the treatment and prevention of Crohn's disease, ulcerative colitis, and pouchitis, and attempts to provide an objective assessment for the current and potential therapeutic role of those approaches. The reader is referred to several comprehensive reviews for additional viewpoints on this controversial topic and more extensive documentation of the literature.[3–7]

Rationale for Treatment

There is considerable indirect evidence that components of the complex microecology of the distal ileum and colon contribute to the pathogenesis of Crohn's disease, ulcerative colitis, and pouchitis (Table 1).[1,8–10] Luminal bacterial concentrations reach 10^7 to 10^8 organisms/g luminal contents in the terminal ileum, 10^{11}/g in the colon, and $10^{10–11}$ in ileal pouches.[11] These areas of highest anaerobic bacterial populations are involved preferentially in clinical IBD. Moreover, Crohn's disease reproducibly responds to diversion of the fecal stream and recurs after restoration of bowel continuity or infusion of luminal contents into the bypassed ileum[12,13] and pouchitis does not occur before ileostomy takedown. These observations suggest that luminal contents provide the stimulus for intestinal inflammation. Increased concentrations of *Enterobacteriaceae* and *Bacteroides* species adhere to the mucosa of patients with Crohn's disease and

Table 1. Clinical Evidence for Bacteria in the Pathogenesis of IBD

Evidence	Crohn's disease	Ulcerative colitis	Pouchitis
Disease in area of ↑ bacterial concentration	Terminal ileum, colon	Colon	Ileal pouch
↑ mucosal adherence	Yes	Yes	?
↑ mucosal invasion	Yes	Yes	?
↓ inflammation with bypass, bowel rest	Yes	No	Yes
Response to antibiotics	Colon only	No	Yes
Protection by probiotics	?	Yes	Yes
Pathogenetic immune response to bacteria	Yes	Yes	?
Exacerbation by pathogens	Yes	Yes	?

↑, increased; ↓, decreased; ?, not studied.

ulcerative colitis[14] and invade the mucosa, especially adjacent to ulcers and fistulae.[15] Genetic susceptibility in a subset of Crohn's disease patients is determined by polymorphisms in NOD 2/CARD 15, which is a cytoplasmic receptor for muramyl dipeptide, a specific component of peptidoglycan in bacterial cell walls.[16–18] These NOD 2/CARD 15 polymorphisms lead to defective nuclear factor κ B activation and appear to result in inefficient epithelial (and possibly macrophage) clearance of invasive bacteria[19] and perhaps defective defensin production and secretion.[20] Furthermore, IBD patients exhibit loss of immunologic tolerance to commensal bacteria, with both increased T-cell and humoral immune responses.[21,22] Finally, IBD patients have altered composition of commensal enteric bacteria with increased *Bacteroides,* adherent/invasive *Escherichia coli, Enterococci,* and decreased *Bifidobacterium* and *Lactobacillus* species (Table 2).[23] Conclusive evidence for a primary role for nonpathogenic enteric bacteria is provided by the lack of enterocolitis in genetically engineered germ-free (sterile) mice, rats, and guinea pigs that reproducibly develop intestinal inflammation and T helper-1(TH1) or T helper 2(TH2) immune responses to cecal bacterial antigens within 1–4 weeks after colonization with commensal gut bacteria.[1] Host susceptibility, mediated by genes regulating mucosal immune responses, barrier function, and microbial defenses, determine response to commensal bacteria. Wild-type mice and rats exist in harmony with the same bacteria.[24,25] Importantly, commensal enteric bacterial species have selective abilities to induce immune-mediated colitis: some are pathogenic, some neutral, and some protective; these selective responses appear to be host and even regional specific in various mouse models.[24–26] These results suggest that luminal commensal bacteria provide the antigenic stimulation for pathogenic immune responses in IBD, that genetic predisposition is critical to developing dysregulated immune responses to bacteria, and that the relative balance of beneficial vs. aggressive commensal enteric microflora determines mucosal homeostasis vs. inflammation (Table 2). This latter concept provides the rationale for selective therapeutic manipulation of the enteric bacterial population by antibiotics, probiotics, and prebiotics.

Antibiotics

Antibiotics are widely recognized to have an essential role in treating the septic complications of IBD, including the intra-abdominal and perianal abscesses and fistulae of Crohn's disease, as well as superinfection with pathogens and postoperative wound infection (Table 3). Most clinicians also use broad-spectrum antibiotics as adjuvant treatment of fulminant colitis and toxic megacolon to decrease bacterial translocation. Small bowel bacterial overgrowth is more common than usually per-

Table 2. Balance of Protective Vs. Aggressive Enteric Commensal Microbial Species

Beneficial	Aggressive/detrimental
Lactobacillus species	Selected *B. species*
Bifidobacterium species	*Enterococcus faecalis*
Selected *E. coli* strains	Adherent/invasive, toxigenic *E. coli* strains
Streptobacillus salivarius	*Eubacterium* and *Peptostreptococcus species*
Saccharomyces boulardii	*Fusobacterium varium*
Clostridium butyricum	Intestinal *Helicobacter species*

Table 3. Complications of IBD Requiring Antibiotic Treatment (With or Without Definitive Therapy)

Intra-abdominal, hepatic, or perianal abscesses, inflammatory phlegmon
Fistulae (perianal, enteroenteric, enterocolonic, enterocutaneous, and enterovesicle)
Anal fissures
Small intestinal bacterial overgrowth secondary to strictures, loss of ileocecal valve, enteroenteric and enterocolonic fistulae
Postoperative infections
Toxic megacolon
Secondary infections (*C. difficile,* and so forth)

Table 4. Mechanisms of Action for Antibiotics in IBD

Decrease luminal and adherent mucosal bacterial concentrations
Selectively eliminate detrimental luminal bacterial subsets (alter ratio of beneficial to aggressive commensal bacteria)
Decrease tissue invasion, treat microabscesses and secondary bacterial proliferation adjacent to mucosal ulcers and fistulae
Decrease bacterial translocation and systemic dissemination of viable bacteria

ceived,[27,28] particularly with loss of the ileocecal valve, clinically significant stricturing with partial small bowel obstruction and delayed transit, and enterocolonic fistulae. The use of antibiotics in these clinical settings is empiric, although uncontrolled trials do show efficacy of high-dose (20 mg/kg) metronidazole (see later).[29]

Antibiotics as primary or adjuvant treatment of active luminal Crohn's disease and ulcerative colitis are more controversial. Although the therapeutic rationale for using antibiotics active against enteric commensal bacteria is strong, based on the convincing data incriminating endogenous luminal bacterial adjuvants and antigens in the pathogenesis of experimental enterocolitis and IBD, and the mechanisms of protection are rational (Table 4), controlled clinical trials supporting use of these agents in IBD patients is meager at best.[1,3,30,31] This review briefly summarizes representative published studies (Table 5) and attempts to derive broad conclusions based on available data. Readers are referred to a more extensive recent review of this topic for details of individual trials.[3]

Crohn's Disease

Broad-spectrum antibiotics are used to treat Crohn's disease by experienced investigators,[32] but large-scale rigorously controlled trials have never been performed.[3,30,33] In contrast to these uncontrolled observations, use of metronidazole (10 or 20 mg/kg/day) is reasonably well documented to be beneficial for primary or adjunctive therapy of colonic Crohn's disease, but not for isolated small intestinal involvement. However, results for unselected patients are less conclusive. In a randomized placebo-controlled trial, Sutherland et al.[34] reported that treatment with metronidazole for 16 weeks significantly decreased the Crohn's disease activity index (CDAI) from entry levels in unselected patients but had no difference in the rates of remission and only 53% of patients completed the trial. Benefit was dose dependent, with 20 mg/kg having greater efficacy than 10 mg/kg. In a multicenter Scandinavian trial, therapeutic response to metronidazole (800 mg/day or approximately 10 mg/kg)

Table 5. Representative Antibiotic Trials in IBD

Author	Date	Antibiotic	Duration of treatment	Result
Crohn's disease—primary therapy				
Ursing[35]	1982	Metronidazole 800 mg/d	16 wk	No difference from sulfasalazine
Sutherland[34]	1991	Metronidazole 10 or 20 mg/kg	16 wk	Superior to placebo ($\downarrow$ CDAI), no difference in remission
Colombel[41]	1999	Ciprofloxacin 500 mg 2 × day	6 wk	No difference from mesalamine
Arnold[42]	2002	Ciprofloxacin 500 mg 2 × day	6 mo	Superior to placebo ($\downarrow$ CDAI)
Prantera[43]	1996	Ciprofloxacin 500 mg 2 × day + metronidazole 250 mg 4 × day	12 wk	No difference from prednisolone
Greenbloom[36]	1998	Ciprofloxacin 500 mg 2 × day + metronidazole 250 mg 3 × day	10 wk	Uncontrolled, 68% remission
Leiper[45]	2000	Clarithromycin 250 mg 2 × day	4 wk	Uncontrolled, 64% response, 48% remission
Steinhardt[37]	2002	Ciprofloxacin 500 mg 2 × day + metronidazole 250 mg 3 × day	8 wk	No improvement over budesonide alone (33% vs. 38% remission)
Crohn's disease—prevention of postsurgical relapse				
Rutgeerts[38]	1995	Metronidazole 20 mg/kg	12 wk	$\downarrow$ clinical relapse 1 yr vs. placebo
Rutgeerts[39]	1999	Ornidazole 1 g/d	52 wk	$\downarrow$ severe endoscopic relapse vs. placebo
Ulcerative colitis—primary therapy				
Turunen[58]	1999	Cipro 500 mg 2 × day	6 mo	Superior to placebo
Mantzaris[59]	1997	Cipro 500 mg 2 × day		No benefit vs. placebo
Casellas[134]	1998	Amoxicillin 1 g/ Clavaline acid 250 mg	5 days	$\downarrow$ mucosal IL-8 and eicosanoids vs. placebo
Pouchitis				
Madden[64]	1994	Metronidazole 400 mg 3 × day	4 wk	Superior to placebo
Shen[66]	2001	Metronidazole 20 mg/kg or Cipro 500 mg 2 × day	2 wk	Both effective, Cipro > metronidazole
Gionchetti[67]	1999	Cipro 500 mg 2 × day and Rifaximin 1 g 2 × day	15 days	89% response, 33% remission, uncontrolled

was similar to that of sulfasalazine[35] as primary treatment of Crohn's disease. In both studies, metronidazole was effective for colonic and ileocolonic Crohn's disease, but not for isolated ileitis.[34,35] Similarly, the combination of ciprofloxacin and metronidazole is more effective for Crohn's disease affecting the colon than for isolated small intestinal disease.[36,37] Rutgeerts et al.[38] reported that high-dose metronidazole (20 mg/kg/day) begun immediately after resection and continued for 3 months significantly decreased the incidence of deep mucosal ulceration in the neoterminal ileum 6 months after surgery and the clinical recurrence rates at 1 year, with a trend toward protection over the 3 years of observation. Although this provocative report suggests a role for anaerobic luminal bacteria in the postoperative recurrence of Crohn's disease, toxic doses (20 mg/kg/day) of metronidazole were used and treatment was continued only for 3 months after surgery. Similarly, the related antibiotic ornidazole used continuously for 1 year significantly decreased severe recurrent ulcers in the neoterminal ileum at 3 and 12 months.[39] Although most antibiotics only transiently alter luminal bacterial concentrations owing to proliferation of resistant strains, chronic use of metronidazole eliminated *Bacteroides* species in Crohn's disease patients for at least 6 months.[40] Moreover, the clearance of fecal *Bacteroides* species correlated with disease remission. Metronidazole has significant side effects that include nausea, anorexia, dysgeusia, dyspepsia, and peripheral neuropathy that limit use of this agent in approximately 20% of patients.

Other single and combination antibiotic regimens can treat Crohn's disease effectively. Ciprofloxacin is comparable with mesalamine 4 g/day[41] and superior to placebo[42] in treating active Crohn's disease. The primary outcome of the latter study of patients with moderately active, resistant Crohn's disease was a decrease in CDAI rather than the more standard induction of remission. However, the mean CDAI level at the end of ciprofloxacin treatment was 112, well below the remission cut-off level of 150, in contrast to a mean CDAI of 205 in the placebo group. The combination of ciprofloxacin and metronidazole can be effective in Crohn's colitis,[36,43] although a retrospective analysis did not show benefit of the combination over either agent alone, with each approximately 70% effective.[44] Prantera et al.[43] reported no difference in the outcome of Crohn's disease patients treated with ciprofloxacin plus metronidazole (46% remission rate) vs. methylprednisolone (63% remission) for 12 weeks. However, more antibiotic-treated (27%) than steroid-treated (11%) patients failed therapy. A similar remission rate was obtained in a 10-week open-label study of combined ciprofloxacin and metronidazole; best results were seen in patients concomitantly treated with corticosteroids and without previous surgery.[36] Based on these results, Steinhart et al.[37] examined the ability of metronidazole plus ciprofloxacin to improve the effects of ileal-release budesonide in patients with ileal or ileo-right colonic Crohn's disease. The overall response rates were no different (38% budesonide + placebo vs. 33% budesonide + antibiotics), with increased toxicity in the antibiotic group (20% vs. 0% withdrawal rates), but analysis by disease site showed better antibiotic responses in the small number of patients (N = 33) with colonic involvement (53% remission with antibiotics vs. 25% remission with placebo, $P > 0.10$). Multiple studies with clarithromycin as part of an antimycobacterial regimen showed some benefit (see later), but interpretation was difficult because of the multiple drugs involved. Uncontrolled use of clarithromycin alone induced 64% response and 48% remission rates in resistant Crohn's disease patients treated for 4 weeks.[45] A pilot study suggested that rifaximin may have a role in treating Crohn's disease based on 78% response and 59% remission rates after 4 months of treatment.[46]

Several different combinations of antimycobacterial agents have been used in Crohn's disease patients to explore the hypothesis that mycobacterial species cause this disorder. The majority of trials using conventional triple or quadruple antimycobacterial treatments have been negative,[47] but *M. paratuberculosis* is resistant to these traditional agents. Combinations of rifabutin and a macrolide, usually clarithromycin, with or without clofazamine, appear more promising.[48–50] These small uncontrolled studies report responses ranging from 58% to 83%, with some patients showing mucosal healing and sustained remissions off anti-inflammatory medications. However, up to 20% of patients withdrew from these trials owing to drug side effects. These studies have been inconclusive regarding the role of *M. paratuberculous* owing to the broad spectra of activity of these antibiotics, failure to document selective responses in those patients with evidence of *M. paratuberculosis* infection by either polymerase chain reaction or serology, and lack of documentation of clearance of tissue infection in patients who respond to treatment. Ongoing, large, controlled trials in Australia and the United States will help settle these issues.

The same antibiotics used to treat luminal Crohn's disease have been reported to be beneficial in the treatment of perianal Crohn's disease.[3,51] Unfortunately, no controlled studies have been performed and fistulae tend to recur in many patients after cessation of treatment.

The largest studies reported 62% to 83% closure rates to metronidazole 20 mg/kg.[29,52] The combination of metronidazole and ciprofloxacin improved perianal fistulae in 9 of 14 patients (64% response rate) and closure in 3 of 14 (21%) patients.[53] Although these uncontrolled results are not definitive, many clinicians, including this author, use metronidazole, ciprofloxacin, the combination of these agents, or tetracycline as the first-line treatment of perianal Crohn's disease in conjunction with surgical drainage of associated abscesses. 6 Mercaptopurine/azathioprine, infliximab, or tacrolimus can be reserved for patients not responding to antibiotics. Clinicians must warn patients about the likelihood of peripheral neuropathy with high-dose metronidazole therapy. Paresthesias are dose and time dependent, with relatively low frequency at 10 mg/kg/day, but an incidence of up to 50% with higher doses.[54] Paresthesias usually are reversible if metronidazole is stopped when first detected but can be irreversible if treatment is continued despite symptoms.

Ulcerative Colitis

Although antibiotics cannot be advocated for treating ulcerative colitis based on available trials, several preliminary studies suggest their possible benefit as adjuncts to standard anti-inflammatory therapy. Brief tobramycin therapy had long-term benefits[55,56] and 90% of patients treated for 18 months with trimethoprim/sulfamethoxazole improved.[57] Turunen et al.[58] showed a modest additive effect for ciprofloxacin in the long-term management (6 mo) of ulcerative colitis, particularly in steroid-treated patients, although short courses of intravenous or oral ciprofloxacin were not effective.[59,60] However, the nonabsorbable broad-spectrum antibiotic rifaximin may have some promise,[61] consistent with the observation that the addition of multiple broad-spectrum antibiotics occasionally produce dramatic benefits in patients with fulminant refractory ulcerative colitis, despite the absence of demonstrable pathogens.[62] A small trial in ulcerative colitis patients showed that 3 intermittent courses of rifaximin did not permanently alter the colonic microbiota, although resistant *Bifidobacterium* species were found.[63]

Pouchitis

Overgrowth of commensal bacteria have been postulated to cause pouchitis in patients with ileal pouch-anal anastomoses, based on the predictable response to short-term treatment with metronidazole and other antibiotics in most patients.[64,65] In a controlled trial with a cross-over design, 73% of patients with active pouchitis responded to metronidazole vs. a 10% response to placebo.[64] Both high-dose metronidazole (20 mg/kg) and

ciprofloxacin (1000 mg/day) treatment for 2 weeks significantly decreased objective pouch inflammation, with better results and less toxicity seen in the ciprofloxacin-treated group.[66] The combination of ciprofloxacin plus rifaximin is effective in the majority (89%) of patients with refractory pouchitis.[67]

Lessons From Animal Models

In several rodent models metronidazole or ciprofloxacin can prevent onset of experimental colitis, but not reverse established disease, whereas broad-spectrum antibiotics both prevent and treat active inflammation.[68–72] Of interest, metronidazole and ciprofloxacin had selective protective effects in different colonic regions,[70] suggesting that different bacterial species cause disease in various colonic segments. Broad-spectrum antibiotics are effective in almost all models of acute and chronic colitis and ileitis in mice, rats, and guinea pigs.[72–75] However, in human leukocyte antigen (HLA)-B27 transgenic rats even broad-spectrum antibiotics have only transient benefit, with recurrence of colitis 1 month after cessation of treatment; probiotics could prevent this relapse of disease.[76] These results indicate that commensal enteric bacteria have synergistic activities in the induction and perpetuation of intestinal inflammation. Therapeutic implications of these studies are that most forms of clinical IBD should respond to treatment if the proper combination of broad-spectrum antibiotics are used, antibiotics should be individualized for the region involved (ileum, cecum, distal colon), and that sequential treatments with antibiotics followed by probiotics should be examined, as has been quite effective in chronic pouchitis.[77]

Conclusions

These diverse studies strongly implicate enteric commensal bacteria in the pathogenesis of Crohn's disease and pouchitis, and suggest that both aerobic and anaerobic bacteria contribute to the inflammatory response. There is presumptive evidence that metronidazole, ciprofloxacin, or the combination of these antibiotics are effective in Crohn's colitis and ileocolitis, but not in isolated ileal disease. The author uses antibiotics in Crohn's colitis, ileocolitis, and perianal fistulae in conjunction with 5-aminosalicylic acid (5-ASA), corticosteroids, and, in appropriate settings, immunosuppressive agents rather than as single agents. However, use of antibiotics as primary therapy of Crohn's disease is poorly documented, owing to significant defects in the study design, high dropout rates, and inadequate numbers of study subjects in the available studies. Equivalency studies are underpowered for definitive results and comparisons frequently are made against sulfasalazine or me-

Table 6. Randomized Double-Blind Trials of Probiotic Agents in IBD

Author (date)	Probiotic	Clinical situation	Result
Crohn's disease			
Plein, Hotz (1993)[135]	*Saccharomyces boulardii*	Maintenance of remission	↓ diarrhea vs. placebo
Malchow (1997)[93]	*E. coli* Nissle 1917	Maintenance of remission	↓ relapse vs. placebo
Guslandi (2000)[136]	*S. boulardii*	Maintenance of remission (probiotic + mesalamine vs. mesalamine alone)	↓ relapse vs. mesalamine alone
Prantera (2002)[94]	*Lactobacillus GG*	Postoperative prevention	No benefit
Ulcerative colitis			
Kruis (1997)[85]	*E. coli* Nissle 1917	Maintain remission	Equal to mesalamine (1.6 g)
Rembacken (1999)[86]	*E. coli* Nissle 1917	Maintain remission	Equal to mesalamine
Kruis (2001)[87]	*E. coli* Nissle 1917	Maintain remission	Equal to mesalamine
Ishikawa (2003)[88]	Bifidobacteria-fermented milk	Maintain remission	Superior to placebo
Pouchitis			
Gionchetti (2000)[77]	VSL#3	Maintain remission chronic pouchitis	Superior to placebo
Mimura (2002)[83]	VSL#3	Maintain remission chronic pouchitis	Superior to placebo
Gionchetti (2003)[84]	VSL#3	Prevention after ileostomy closure	Superior to placebo

salamine, which themselves have questionable efficacy in Crohn's disease. The use of antibiotics in ulcerative colitis currently is not justified based on available studies, although broad-spectrum regimens need to be explored. Although proper trials of antibiotics in pouchitis have not been conducted, widespread clinical efficacy of most antibiotics active against enteric bacteria validate their use.

Rigorous multicenter studies for optimal antibiotic regimens in Crohn's disease (primary therapy and prevention of relapse) need to be conducted using broad-spectrum agents or combinations of narrow-spectrum agents. It will be important to carefully consider responses in subsets of disease using genetic, clinical, or serologic markers. When used in Crohn's disease of the colon, treatment should be for 2–3 months duration and the patient must be alerted to the risk for peripheral neuropathy with sustained use of high doses of metronidazole.

Probiotics

Probiotics are viable microorganisms with beneficial physiologic or therapeutic activities. Originally derived from cultured foods, especially milk products, these protective bacteria and yeast include the lactic acid bacilli, Lactobacillus and Bifidobacterium, a nonpathogenic *E. coli* strain (*E. coli* Nissle 1917), *Saccharomyces boulardii, Clostridium butyricum,* and *Streptococcus salivarius* subspecies *thermophiles*. More recently, genetically engineered bacteria that secrete immunosuppressive substances such as interleukin-10 (IL-10) have been studied.

Limited clinical trials suggest that selected probiotic species, alone or in combination, can prevent recurrent intestinal inflammation and possibly treat active IBD,

with best results in pouchitis, and, to a lesser extent, ulcerative colitis (Table 6). These results have generated considerable enthusiasm, as evidenced by more recent reviews than primary studies.[6,78–82]

Pouchitis

The most convincing evidence of the clinical efficacy of probiotics in clinical IBD is provided by a small, prospective, double-blind, placebo-controlled trial showing that daily administration of a combination of 4 *Lactobacillus* species, 3 *Bifidobacterium* species, and 1 *S. salivarium* species (VSL 3) for 9 months prevented relapse of chronic pouchitis after induction of remission by antibiotics.[77] The relapse rate of VSL 3–treated patients was 15% (3 of 20 patients) vs. 100% in the placebo-treated group (N = 20). Moreover, every patient relapsed within 3 months of stopping VSL 3. These results have been replicated[83] and extended by decreasing the frequency of pouchitis by prospectively administering VSL 3 beginning at the time of pouch closure.[84] In this prophylactic study, 2 of 20 patients (10%) receiving 1 packet of VSL 3 containing 900 billion bacteria/day for 1 year developed pouchitis vs. 40% of placebo-treated patients. Moreover, median stool frequency in subjects not developing pouchitis was significantly less (5 vs. 8 stools/day) in VSL 3 vs. placebo-treated patients.[84]

Ulcerative Colitis

Several probiotic preparations show promise in ulcerative colitis, although results are not as convincing as in pouchitis. *E. coli* Nissle 1917 was reported to be as effective as low-dose 5-ASA in preventing relapse of ulcerative colitis.[85–87] A small study showed that administration of Bifidobacteria-fermented milk decreased the rate of relapse of ulcerative colitis to 3 of 11 treated

subjects vs. 9 of 10 controls.[88] Uncontrolled pilot studies have indicated that VSL 3 maintained remission of ulcerative colitis in 75% of patients over 12 months[89] and reduced active inflammation in 87% of ambulatory patients with mild to moderate disease.[90] Similarly, uncontrolled administration of the nonpathogenic yeast *S. boulardii* for 4 weeks induced a clinical remission in 17 of 24 (71%) of patients with mild to moderate ulcerative colitis.[91] A provocative study by Borody et al.[92] addressed the hypothesis that ulcerative colitis patients have abnormal enteric bacteria. They showed reversal of refractory ulcerative colitis in 6 patients who were repopulated with fecal enemas from normal patients.

Crohn's Disease

Results of probiotic trials in Crohn's disease are mixed. Malchow[93] found that *E. coli* Nissle was superior to placebo in preventing relapse of Crohn's disease after induction of remission by standard medical therapy, but Prantera et al.[94] reported no benefit of *Lactobacillus GG* administered for 1 year in preventing postoperative relapse of symptoms or endoscopic lesions in the neoterminal ileum. However, a pilot study of *Lactobacillus GG* reported benefit in pediatric patients with active Crohn's disease with successful tapering of steroids in 3 of 4 patients.[95]

Lessons From Animal Models

Animal models are useful to study optimal treatment protocols and to evaluate mechanisms by which probiotic microbial agents mediate their protective effects. A number of *Lactobacillus* and *Bifidobacterium* species attenuate experimental colitis in IL-10 knockout mice, including native murine *L. reuteri*,[96] VSL 3,[97] *L. plantarum*,[98] *L. salivarius*, and *B. infantis*.[99] Host background appears to be important because *L. plantarum* but not *Lactobacillus GG* can prevent colitis in HLA B27 transgenic rats.[76] This study and many others indicate that various probiotic species have different protective effects in selected hosts and inflammatory conditions. Of note, probiotic bacteria were able to prevent recurrence of colitis in HLA B27 transgenic rats after induction of remission with broad-spectrum antibiotics, but could not by themselves induce a remission of established disease.[76] These results suggest that probiotic agents may be more effective in preventing relapse of disease than in inducing remission and that sequential administration of antibiotics to induce a remission followed by probiotics to maintain quiescent disease may be an effective long-term synergistic approach to clinical management of IBD patients. This possibility is supported by clinical trials in refractory pouchitis patients.[77] Finally, a novel approach

Table 7. Mechanisms of Action of Probiotics

Inhibit pathogenic enteric bacteria
Decrease luminal pH
Secrete bacteriocidal proteins
Colonization resistance (occupy ecologic niche)
Block epithelial binding—induction of MUC 2
Inhibit epithelial invasion—Rho-dependent and -independent pathways
Improve epithelial and mucosal barrier function
Produce short chain fatty acids, including butyrate
Enhance mucus production
Increase barrier integrity
Alter immunoregulation
Induce IL-10, transforming growth factor β expression and secretion
Stimulate secretory immunoglobulin A production
Decrease tumor necrosis factor expression

to probiotic therapy is suggested by prevention and treatment of colitis in 2 murine models by daily administration of *Lactococcus lactis,* engineered to secrete recombinant IL-10.[100] This approach is now in pilot trials in IBD patients.

Mechanisms of Protection by Probiotics

Multiple mechanisms of action have been suggested to explain the protective effects of probiotic agents in intestinal inflammation (Table 7). These can be classified broadly as suppression of growth or epithelial binding/invasion by pathogenic bacteria, improved epithelial barrier function, or immunoregulatory activities. A detailed description of these mechanisms is beyond the scope of this clinically oriented review, but several recent examples of each category are provided. *Bifidobacterium infantis* suppressed growth of *Bacteroides vulgatus*,[101] an organism associated with IBD and experimental colitis.[24,102] Similarly, Bifidobacterium-fermented milk decreased fecal *B. vulgatus* concentrations in ulcerative colitis.[88] Suppression of the growth and function of enteric pathogenic bacteria has been attributed to decreased luminal pH via production of short chain fatty acids, secretion of bactericidal proteins, and prevention of epithelial adherence. *E. coli* Nissle 1917 inhibited both in vitro epithelial adhesion and invasion by an adherent/invasive *E. coli* strain isolated from Crohn's disease patients.[103] Parallel results using *L. plantarum* and enteroadherent *E. coli* suggested that up-regulation of MUC-2, a primary intestinal mucin glycoprotein, by this probiotic bacterial species prevented pathogenic bacterial adherence.[104] In addition, *S. thermophilus* and *L. acidophilus* prevented entry of enteroinvasive *E. coli* by rho-dependent and -independent mechanisms.[105] These results were confirmed by Mattar et al.,[106] who postulated that stimulation of mucins would decrease bacterial

translocation. Indeed, oral or rectal administration of *L. plantarum* to mice treated with trinitrobenzene sulfonic acid decreased the translocation of commensal enteric bacteria to mesenteric lymph nodes and spleens.[107] Similarly, *Lactobacillus GG* decreased *E. coli* translocation in rabbits.[108] Although the mechanisms are not clear, mucosal permeability was decreased by administration of *L. reuteri* or VSL 3 to IL-10−/− mice.[96,97] A number of studies showed that several probiotic agents can induce protective cytokines, including IL-10 and transforming growth factor β, and suppress proinflammatory cytokines, such as tumor necrosis factor, in the mucosa of patients with pouchitis and Crohn's disease,[109–111] in IL-10−/− mice[99] and in isolated splenocytes.[112] Obviously, induction of IL-10 is not the sole mechanism of protection because multiple probiotic agents prevent and treat colitis in IL-10−deficient mice.[96–99]

Recent data indicate that nonviable components of probiotics can mediate their beneficial effects. Both secreted proteins and DNA of VSL 3 can block nuclear factor κ B and p38 mitogen-activated protein kinase activation and prevent apoptosis of epithelial cells.[113,114] Interestingly, different effects are seen with DNA from different bacterial species within the VSL 3 mixture.[113] Nonmethylated DNA (CpG) from VSL 3 and a randomly selected *E. coli* strain can suppress experimental colitis in several models; this suppression is mediated through TLR 9, which binds CpG.[115]

Conclusions

Several probiotic preparations have promise in preventing relapse of chronic pouchitis and ulcerative colitis, and possibly in the treatment of mild to moderately active ulcerative colitis and prevention of postoperative pouchitis. Results in Crohn's disease are not yet clear owing to conflicting results and a paucity of trials. This approach is quite appealing because of a lack of toxicity and to the enthusiasm of patients wishing to use natural physiologic approaches to treating IBD. Animal models suggest that optimal results may be achieved by prophylactic use of probiotics after induction of remission by antibiotics, which can suppress detrimental bacterial populations. Thus, the combinations of antibiotics and probiotics can restore the normal predominance of beneficial enteric bacteria. The optimal composition, dose, and length of probiotic treatment in various IBD clinical settings need to be determined by large, well-designed, placebo-controlled, prospective trials. In addition, it is clear that all *Lactobacillus* and *Bifidobacterium* species are not equally beneficial, each may have individual mechanisms of action, and host characteristics may determine which probiotic species and even strain may

be optimal in a given setting. Thus, one cannot consider probiotics as a generic form of therapy. Therefore, results must be interpreted accordingly and trials designed to consider phenotypic and genetic subsets of patients.

Prebiotics

Prebiotics are dietary substances, usually nondigested carbohydrates, that stimulate the growth and metabolism of protective commensal enteric bacteria.[116,117] Lactosucrose, fructo-oligosaccharides, inulin, bran, psyllium, and germinated barley extracts foster the growth of *Lactobacillus* and *Bifidobacterium* species and stimulate production of short chain fatty acids, especially butyrate.[118,119] Thus, these prebiotic food additives have the potential to restore the deranged balance of beneficial vs. aggressive commensal bacterial species in the distal ileum of IBD patients by several mechanisms. These physiologic substances stimulate growth of protective lactic acid bacilli, which secondarily suppress detrimental species by decreasing the luminal pH and by inducing colonization resistance, blocking epithelial attachment, and secretion of bactericidal substances. In addition, increased substrate availability and enhanced numbers of metabolically active Lactobacilli and Bifidobacteria increase bacterial fermentation, with resultant butyrate production that improves epithelial barrier function. The net result of prebiotic administration is functionally equivalent to administering probiotic bacteria. Although this is an extremely attractive concept for treating intestinal inflammation, experimental support for these nutriceuticals is even less extensive than that for probiotics.

Human IBD

Several small controlled studies of prebiotics have been reported in ulcerative colitis patients, but none have been performed in Crohn's disease. Germinated barley foodstuff, a glutamine- and hemicellulose-rich extract of spent beer-brewing constituents, stimulates growth of *Bifidobacterium* and *Eubacterium* species and production of butyrate and other short chain fatty acids.[120] Four-week administration of 20−30 g of germinated barley extracts to patients with mild to moderate ulcerative colitis decreased clinical and endoscopic evidence of inflammation in both a pilot and a small placebo-controlled trial.[121,122] This treatment increased fecal concentrations of *Bifidobacterium* species and *Eubacterium limosum*. A longer-term (24 wk) open-label study of 21 patients reached similar conclusions.[119] Psyllium, long used by clinicians to maintain stool consistency in both constipation and diarrhea, is a form of prebiotic by virtue of its ability to serve as a metabolic substrate for bacteria. Administra-

tion of psyllium was superior to placebo in decreasing symptoms of patients with inactive ulcerative colitis, with a consistent increase in fecal Bifidobacterium concentrations, and decreased free water in the stool.[123]

Lessons From Rodent Models

A number of prebiotic preparations have been shown to inhibit experimental colitis and to increase luminal concentrations of lactic acid bacteria. Madsen et al.[96] showed that lactulose enhanced growth of endogenous *Lactobacilli* and attenuated colitis in IL-10−deficient mice. Similarly, feeding inulin[124] or germinated barley extracts[125–127] inhibited dextran sodium sulfate (DSS)-induced colitis and increased luminal concentrations of short chain fatty acids, including butyrate, *Lactobacilli,* and *Bifidobacteria.* Kanauchi et al.[127] reported a decrease in serum IL-6 levels and mucosal signal transducers and activators of transcription factor (STAT-2) and nuclear factor κ B activity after germinated barley foodstuff treatment of mice with DSS-induced colitis. Cherbut et al.[128] reported that fructo-oligosaccharides, which promote the growth of lactic acid bacteria and increase production of butyrate and lactate, decreased trinitrobenzene sulfonic acid–induced colitis in rats. Administration of high doses (10^{11} colony forming units/day) of intragastric lactic acid bacteria or intracolonic butyrate and lactate had similar protective effects, suggesting that the beneficial effects of prebiotics were mediated by induction of short chain fatty acids and growth of luminal probiotic bacteria. However, Moreau et al.[129] reported no benefit of fructo-oligosaccharides but a protective effect of type 3 resistant starch in the DSS rat colitis model. Similarly, galacto-oligosaccharides had no protective effect in trinitrobenzene sulfonic acid–treated rats, although colonic *Bifidobacteria* concentrations were increased.[130] Hoentjen et al.[131] showed that the combination of inulin and fructo-oligosaccharides significantly decreased gross and histologic inflammation and cecal IL-1β concentrations while increasing cecal transforming growth factor β secretion in HLA-B27 transgenic rats. These results suggest that the combination of several prebiotic compounds may be effective, analogous to cocktails of various probiotic bacteria. Studies in gnotobiotic rats colonized with human enteric bacteria compared dietary administration of inulin, fructo-oligosaccharide, or the combination of both prebiotic agents.[132] Fructo-oligosaccharides stimulated growth of *Bifidobacterium* and *Lactobacillus* species more effectively, whereas inulin induced higher luminal concentrations of butyrate. Thus, combinations of prebiotics may have additive effects owing to multiple mechanisms of action. Similar to probiotic bacterial species, each prebiotic substance has unique characteristics and therapeutic applications. Finally, nondigestible oligosaccharides suppressed in vitro growth of *C. difficile,* independent of the activities of *Bifidobacterium,*[133] suggesting that prebiotics may be beneficial in treating bacterial toxin–induced colitis.

Conclusions

Prebiotics offer an exciting potential treatment for IBD patients, with the strong theoretic rationale of stimulating growth of endogenous protective lactic acid bacilli and production of cytoprotective butyrate. This approach promises to be safe, physiologic, and cost effective, and therefore could be considered for long-term prophylactic use and possibly applied to high-risk individuals or those with subclinical disease. However, studies of this topic are just beginning and considerable data need to be generated to provide clinical proof of efficacy in different clinical situations before these agents can be applied to widespread clinical use. Because each agent has unique properties, comparative studies of various prebiotic compounds and combinations of agents, various doses, and duration of treatment need to be performed. Certain compounds (i.e., lactulose) have important side effects, including diarrhea and gas formation, which may limit their use in IBD patients. In addition, consideration should be given to altering the patient's diet because increasing the amount of dietary fiber and natural dietary oligosaccharides while decreasing refined carbohydrates similarly may restore a healthy balance of protective vs. detrimental enteric bacterial species. Finally, the interesting approach of combining probiotic and prebiotic agents (synbiotics) has considerable appeal and may decrease the required dose, duration, or frequency of probiotic administration, thereby decreasing costs and improving compliance (adherence) to treatment.

Conclusions and Future Directions

Although the rationale for therapeutic manipulation of the luminal microbiota in IBD is uncontested, current data for therapeutic efficacy do not withstand rigorous scrutiny or fulfill current evidence-based standards for using antibiotics, probiotics, and prebiotics in the treatment of IBD. Clinical trials consistently have been underpowered to show equivalency or superiority, many have design flaws that preclude definitive results, or use outcomes, such as mean CDAI, that do not conform with widely accepted criteria for disease response or remission. Thus, enthusiasm outstrips scientific support for these therapeutic approaches. However, although cur-

Table 8. Current and Potential Uses of Antibiotics, Probiotics, and Prebiotics in IBD Patients

	Reasonable evidence	Suggestive evidence	Potential use
Antibiotics	CD colitis, ileocolitis, (active), pouchitis	Postoperative prevention CD	UC (broad spectrum)
Probiotics	Chronic pouchitis (prevention of relapse) UC (prevention of relapse)	Pouchitis (postoperative prevention)	UC (active) CD (postoperative) CD (active)
Prebiotics	None	None	UC, CD, pouchitis (active, prevention)

CD, Crohn's disease; UC, ulcerative colitis.

rent data do not strongly support use of these agents, most trials and clinical experience suggest therapeutic benefit of antibiotics, especially metronidazole, ciprofloxacin, the combination of these 2 agents, and, possibly, clarithromycin, in the adjunctive treatment of active Crohn's disease involving the colon and possibly in the postoperative neoterminal ileum (Table 8). Likewise, metronidazole, ciprofloxacin, and rifaximin effectively treat pouchitis. Currently approved antibiotics are not effective in active ulcerative colitis, although broad-spectrum, nonabsorbable agents show promise in early trials. Existing probiotic agents, either alone (*E. coli* Nissle) or in combination (VSL 3), can prevent relapse of ulcerative colitis and chronic relapsing pouchitis, respectively. The latter combination of 8 probiotic species appears promising in prevention of pouchitis with prophylactic use after ileal pouch–anal anastomosis and in the treatment of active ulcerative colitis. Prebiotics have great potential but have not been adequately tested yet in IBD to reach valid conclusions. Large, well-designed, multicenter, controlled, clinical trials need to be organized and conducted to examine the possible indications listed in Table 8 to determine whether these agents will have a place in the IBD treatment armamentarium.

Although current clinical support is weak, therapeutic manipulation of luminal microecology has considerable potential as a physiologic nontoxic approach to treating and, perhaps even more importantly, maintaining medically or surgically induced remission and ultimately preventing onset of disease in high-risk individuals. As additional susceptibility genes are defined for both Crohn's disease and ulcerative colitis, genetic testing will be used widely to screen family members and possibly the general population. Nontoxic means to treat disease at a preclinical stage or to prevent onset of inflammation in high-risk individuals will need to be developed. Probiotics and/or prebiotics may be optimal agents for prophylactic use because of their physiologic nature and lack of obvious toxicity. Similarly, these approaches may be highly amenable for life-long use to prevent recurrence in patients with IBD once a remission occurs.

Optimal application of antibiotics, probiotics, and prebiotics will depend on a clear understanding of which individual agent is indicated for a defined patient subset. Current data suggest that metronidazole and ciprofloxacin are not effective for treatment of Crohn's ileitis, but can benefit Crohn's patients with colonic involvement. It may be that different bacterial populations are responsible for disease in various intestinal regions, as suggested by selectively monoassociated IL-10–deficient mice.[25] Furthermore, different bacteria may have dominant effects in different genetic backgrounds. Thus, optimal use of various antibiotics, probiotics, and prebiotics may depend on identifying patient subsets by genetic, phenotypic, stool microbiologic, serologic, or T-cell immune response criteria. Finally, antibiotics may be used optimally to eliminate certain aggressive (or pathogenic) enteric bacterial populations, followed by exogenous probiotics or prebiotics to fill the open ecologic niche, thereby permanently altering the balance of enteric microbiota for long-term efficacy. The concept of synbiotics (simultaneous use of probiotics and prebiotics) has considerable conceptual appeal and may decrease the dose, frequency, or duration of probiotic administration, thereby minimizing the expense of treatment. Similarly, concomitant use of antibiotics, probiotics, and/or prebiotics in combination with immunosuppressives to block pathogenic immune responses, growth factors to accelerate mucosal healing and to optimize mucosal barrier function, and granulocyte-macrophage colony-stimulating factor to potentiate bacterial killing and clearance provide the best hope for altering the insidious progression of idiopathic IBD.

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