SELECTED SUMMARIES

Henry J. Binder, M.D.
Selected Summaries Editor
Yale University School of Medicine
New Haven, Connecticut 06520-8019

PROBIOTIC THERAPY WITH *E. COLI* FOR ULCERATIVE COLITIS: TAKE THE GOOD WITH THE BAD

Rembacken BJ, Snelling AM, Hawkey PM, Chalmers DM, Axon ATR (Centre for Digestive Diseases and Department of Microbiology, University of Leeds, Leeds, England). Non-pathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis: a randomized trial. Lancet 1999;354:635–639.

Based on the hypothesis that resident pathogenic bacteria contribute to the inflammatory process in ulcerative colitis, an equivalence study comparing an oral preparation of nonpathogenic *Escherichia coli* with mesalamine was performed. The study had 2 primary objectives: (1) to compare the therapies for rate of remission and time to remission in active colitis and (2) to compare the therapies for rate of relapse and time to relapse after achieving remission.

A total of 116 patients with active ulcerative colitis were recruited for the study. The investigators defined active ulcerative colitis as patients with at least 4 liquid stools daily over the previous 7 days, with at least erythema on sigmoidoscopy, and histological confirmation of active colitis. At each assessment, a clinical activity index, a modification from Rachmilewitz (BMJ 1989;298:82–86), was performed. During the induction of remission phase of the study, eligible patients were randomized in a double-blinded, double-dummy design to receive either mesalamine, 800 mg 3 times daily, or a nonpathogenic strain of *E. coli* (serotype O6:D5:H1) named Nissle 1917 (Mutaflor; Ardeypharm GmbH, Herdecke, Germany), 2 capsules twice daily (2.5×10^{10} viable bacteria per capsule). Before treatment, all patients were treated with a 1-week course of oral gentamicin, 80 mg 3 times daily, to suppress native *E. coli* flora. The randomization was stratified for mild, moderate, or severe disease activity according to criteria by Truelove and Witt (BMJ 1955;2:1041–1048) to ensure adequate representation of disease severity in each treatment group.

To induce remission, all patients were treated by different protocols depending on their respective disease activity groups. Patients with mild proctitis were treated with hydrocortisone acetate enemas per rectum twice daily. Patients with mild-to-moderate disease extending proximal to the sigmoid colon without systemic disturbance were treated with 30 mg/day prednisolone. Patients with severe disease received 60 mg/day prednisolone as inpatients. Any patient whose clinical condition worsened or who did not achieve remission in 12 weeks was excluded from the trial. Remission was defined as generalized well-being as well as no more than 3 formed stools daily and endoscopic and histological confirmation of inactive disease.

The follow-up portion of the study pertains to objective 2, the maintenance phase of the study. The doses of mesalamine and *E. coli* were reduced to 1.2 g/day and 2 capsules daily, respectively. The steroid preparation (either enema or oral) was tapered over 2 weeks (enema) or 4 months (oral). The patients were then followed up until relapse or 12 months maximum. Confirmation of relapse was made endoscopically and histologically.

For the statistical analysis, the investigators assumed the *E. coli* preparation would not have superior remission rates compared with mesalamine, and therefore performed only 1-sided testing of the null hypothesis. The study was designed with a power of 80% to exclude, with 90% (1-sided) confidence, a difference of >20 percentage points in favor of mesalamine. The investigators assumed a relapse rate of 20% at a year to reach a statistical power of 80% when calculating the number of patients needed in the trial.

The results of the trial were that 75% and 68% of the mesalamine and *E. coli* groups achieved remission, respectively. The relapse rate in both groups was markedly higher than the investigators anticipated, 73% for mesalamine group and 67% for *E. coli* group. The time to relapse was not significantly different between the groups. The investigators concluded that *E. coli* seems to be as effective as mesalamine in maintaining remission of ulcerative colitis.

Comment. The above study has several significant flaws that limit the importance of the findings; specifically, this is a heterogeneous group of patients (mild to severe in illness severity), treated with various corticosteroid formulations in addition to the study medication. The mesalamine doses used were relatively low (1.2–2.4 g/day), and only a small number of patients were in remission at the end of the study, resulting in an underpowered equivalence study. Nonetheless, the hypothesis of the study is interesting and should stimulate further research into the use of probiotics in inflammatory bowel disease (IBD). We briefly review some of the scientific rationale supporting

the role of bacteria in IBD, discuss the results of 3 other probiotic trials, and conclude that while the possibilities for probiotics are tantalizing, there is not yet a role for these agents in routine clinical practice.

Luminal contents have long been suspected in the initiation or perpetuation of the inflammatory state. The terminal ileum, cecum, and rectum are areas of relative stasis, providing prolonged mucosal contact with luminal contents. Fecal diversion has been shown to prevent ileal postoperative relapse among patients with Crohn's disease (Lancet 1991;338:771–774), and the disease quickly recurs upon reexposure (Gastroenterology 1998;114:262–267).

The evidence supporting the role of luminal bacteria exists in both animal models as well as observations in human disease. Animal models of IBD have shown that colitis does not occur in a germfree environment, lending powerful evidence that the critical component of the fecal stream is the bacteria (Am J Pathol 1997;150:91–97, J Immunol 1998;160:385–394, Infect Immun 1998;66:5224–5231). Importantly, colitis has been shown to be transferable by activated T cells against bacterial antigens in the C3H/HeJBir mouse model (J Exp Med 1998;187:855–864).

In human IBD, studies indicate that the disease occurs in areas of highest bacterial contents. A variety of immunohistochemical data (Gastroenterology 1995;108:1396–1404), specific bacterial stimulation of T-cell clones (Gut 1999;44:812–818), and assays for mucosal antibody specific for luminal bacteria (Gut 1996;38:365–375) suggest specific bacteria or bacterial products in the pathogenesis or perpetuation of chronic intestinal inflammation. Clinical data supporting the use of antibiotics in IBD disease would strengthen the implications of luminal bacteria as instigators. However, antibiotics have only a limited role in the management of Crohn's disease (Gut 1991;32:1071–1075), and a benefit of antibiotics in ulcerative colitis has been difficult to demonstrate (Gastroenterol Clin North Am 1989;18:51–56).

The potential mechanisms behind bacterial initiation or potentiation of chronic inflammation must be addressed before one can speculate on the potential mechanisms of action of "beneficial" bacteria. When considering hypotheses implicating bacteria and IBD, enhanced mucosal permeability may play a pivotal role in maintaining a chronic inflammatory state. This enhanced permeability may be a primary event (i.e., an inheritable genetic predisposition in some families (Gastroenterology 1996;110:1395–1403) or a secondary event either as a result of direct contact with pathogenic bacteria (Gastroenterology 1996;110:1429–1437), or a consequence of intestinal inflammation.

The hydrophobic epithelial barrier, composed of an interaction between mucin glycoproteins and the cellular expression of trefoil peptides (Gastroenterology 1995;109:516–523), normally prevents the epithelial influx of hydrophilic bacterial products (Am J Physiol 1992;262:G171–G177). A defective epithelial barrier may even cause a loss of tolerance to normal resident enteric bacteria (Clin Exp Immunol 1995;102:448–455). Once these hydrophilic bacterial products have gained access to the submucosa, they can drive a variety of proinflammatory signaling pathways causing further recruitment and further disruption of epithelial tight junctions (Gastroenterology 1993;105:60–66).

How might altering the enteric bacterial flora affect intestinal inflammation? Data from experimental models imply that certain luminal bacteria are more pathogenic than others. *Bacteroides* species have been found to be particularly pathogenic in many experimental models (Infect Immun 1999;67:2969–2974), whereas *Lactobacillus* species seem to have a beneficial effect (Gastroenterology 1999;116:1107–1114). Mechanisms by which *Lactobacillus* species promote intestinal healing include the secretion of inhibitory products with antimicrobial activity (Microb Ecol Health Dis 1989;2:131–136) and suppression of bacterial adherence and translocation of other more pathogenic bacterial species (Gastroenterology 1999;116:1107–1114). Evidence supporting the beneficial role of *Lactobacillus* species in humans includes *Lactobacillus* species decreasing the colonic pH and the growth of pathogenic bacteria (Appl Environ Microbiology 1993;59:15–20), induction of growth factors (Ann Med 1990;22:37–41), and enhanced synthesis of antibodies to microbial pathogens (Pediatr Res 1992;32:141–144). It seems that in experimental models of colitis, altering the enteric flora toward a higher concentration of beneficial bacteria with bacteriostatic or anti-inflammatory properties may improve host colitis.

The term probiotic was first used to describe a substance or organism that contributes to the intestinal microbial balance of farm animals (Science 1965;47:747–748). More recently, probiotics have been defined as "living organisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition" (Trends Food Sci Technol 1995;6:241–245). Which and how many of these living organisms are chosen seem mysterious. It seems that normal gut commensals are the logical choice only because they are presumed to be harmless and may proliferate and persist in the gut. Packaging, shelf life, delivery systems, and other formulation issues are challenges for the future.

Data from controlled clinical trials supporting the health-promoting claims of probiotic bacteria therapy for colitis are sparse. The current trial by Rembacken et al. follows an earlier larger study reporting equivalence for maintenance of remission over 3 months with the same nonpathogenic *E. coli* preparation compared with 1.5 g mesalamine (Aliment Pharmacol Ther 1997;11:853–858). In the study by Kruis et al., 103 patients were treated over 12 weeks with either a nonpathogenic *E. coli* or 1.5 g/day mesalamine. No significant differences in relapse rate were found. In addition, 2 pilot studies have promoted the benefits of a new probiotic, VSL # 3 (Yovis; Sigma-Tau, Pomezia, Italy), that contains 300 billion/g of viable lyophilized bacteria of 4 strains of lactobacilli, 3 strains of bifidobacteria, and 1 strain of *Streptococcus salivarius* subsp. *thermophilus*. The first pilot study by Venturi et al. (Aliment Pharmacol Ther 1999;13:1103–1108) showed a significant increase in fecal concentrations of lactobacilli, bifidobacteria, and *S. salivarius* subsp. *thermophilus* when used for maintenance of remission in ulcerative colitis patients. Additionally, 75% of the patients maintained remission over the year on therapy. In the second trial (Gastroenterology 1998;114:A985), 40 patients with chronic pouchitis who initially achieved remission after combination antibiotic treatment were randomized to placebo or VSL #3 for 9 months. All 20 patients randomized to placebo relapsed; in contrast, 17 of 20 patients treated with VSL # 3 were still in remission at 9 months. Although these results are provocative and the scientific data supporting a pathological role of certain enteric bacteria are reasonable, it is still too soon to recommend routine use of probiotics in general clinical practice. The mechanistic hypotheses established at the bench have yet to be translated to the bedside. The choice of bacteria, the optimal dose of bacteria, and the duration of therapy all require further clarification. Continued investigation into the mechanisms by which "good" bacteria prevent or ameliorate the chronic inflammatory state is necessary. In the future, we may well take the "good" bacteria with "bad," but first, an appropriately selected agent must be studied in well-controlled randomized trials.

WILLIAM A. FAUBION, M.D.
WILLIAM J. SANDBORN, M.D.