Abstract
The dysbiosis theory of inflammatory bowel disease (IBD) posits that there is an alteration in the gut microbiome as an important underpinning of disease etiology. It stands to reason then, that administering agents that could impact on the balance of microbes on the gut could be impactful on the course of IBD. Herein is a review of the controlled trials undertaken to assess the use of antibiotics that would kill or suppress potentially injurious microbes, probiotics that would overpopulate the gut with potentially beneficial microbes or prebiotics that provide a metabolic substrate that enhances the growth of potentially beneficial microbes. With regard to antibiotics, the best data are for the use of nitroimidoles postoperatively in Crohn’s disease (CD) to prevent disease recurrence. Otherwise, the data are limited with the regard to any lasting benefit of antibiotics sustaining remission in either CD or ulcerative colitis (UC). A recent meta-analysis concluded that antibiotics are superior to placebo at inducing remission in CD or UC, although the meta-analysis grouped a variety of antibiotics with different spectra of activity. Despite the absence of robust clinical trial data, antibiotics are widely used to treat perineal fistulizing CD and acute and chronic pouchitis. Probiotics have not been shown to have a beneficial role in CD. However, *Escherichia coli* Nissle 1917 has comparable effects to low doses of mesalamine in maintaining remission in UC. VSL#3, a combination of 8 microbes, has been shown to have an effect in inducing remission in UC and preventing pouchitis. Prebiotics have yet to be shown to have an effect in any form of IBD, but to date controlled trials have been small. The use of antibiotics should be balanced against the risks they pose. Even probiotics may pose some risk and should not be assumed to be innocuous especially when ingested by persons with a compromised epithelial barrier. Prebiotics may not be harmful but may cause gastrointestinal side effects. Finally, the timing of ingestion of antibiotics and other dietary factors that may function as prebiotics, especially in early childhood, may be critical in shaping the gut microbiome and ultimately predisposing to or preventing IBD. Finding ways to impact on the gut microbiome to alter the course of IBD makes good sense, but should be undertaken in the setting of rigorously performed controlled trials to ensure that the interventions are truly effective and well tolerated.
**Introduction**

Elsewhere in this symposium, the role of the gut microbiome in inflammatory bowel disease (IBD) has been discussed. Herein, I will discuss clinical trials undertaken to manipulate the gut microbiome; specifically with agents that either suppress or kill bacteria (antibiotics), or that alter the ecological balance of the gut microbiome. The latter can be undertaken by substituting potentially benign or even healthful bacteria (probiotics) or by the ingestion of carbohydrates that can induce healthful bacteria or their metabolic byproducts (prebiotics). Finally, I will discuss how the use of agents that can manipulate the gut microbiome can potentially predispose to IBD or prevent the development of an ‘IBD-conducive’ gut microbiome. Altering the gut microbiome is an emerging science. While we have yet to find the optimal therapeutic approach to suppress or cure IBD by manipulating the gut microbiome, the increase in IBD worldwide may be secondary to adverse alterations in the gut microbiome [1, 2].

**Antibiotics**

Clinicians need no introduction to antibiotics as they have been used topically, orally and systemically for decades to cure infections and even to treat some chronic inflammatory diseases (such as the use of tetracyclines in treating rheumatoid arthritis). With diseases such as Crohn’s disease (CD) and ulcerative colitis (UC) involving active intestinal inflammation and especially in CD, where abscesses and purulent draining fistulas are common, it has been a long-considered hypothesis that antibiotics would be effective therapy. In fact, they have been widely used as therapy even in the absence of convincing evidence. In the absence of a specific organism to target, broad-spectrum antibiotics have been prescribed and in some instances formally studied.

A recent meta-analysis of antibiotics in IBD was supportive of a benefit of antibiotic use [3]. For active CD, there were 10 randomized controlled trials (RCTs), and antibiotics were superior to placebo (relative risk, RR, of active CD not in remission = 0.85; 95% confidence interval, CI: 0.73–0.99). There was moderate heterogeneity between results and a diverse number of antibiotics were tested (antimycobacterial therapy, macrolides, fluoroquinolones, 5-nitroimidazoles, and rifaximin) either alone or in combination. In perianal CD fistulas, there were three trials evaluating either ciprofloxacin or metronidazole. There was a statistically significant effect in reducing fistula drainage (RR = 0.8; 95% CI: 0.66–0.98) with no heterogeneity ($I^2 = 0\%$) and a number needed to treat of 5 (95% CI: 3–20). However, individually these studies were small and
underpowered. For quiescent CD, there were 3 RCTs evaluating maintenance of remission using different antibiotic combinations (all including antimycobacterials) compared to placebo. Antibiotics were significantly more likely to maintain remission than placebo (RR of relapse = 0.62; 95% CI: 0.46–0.84), with no heterogeneity ($I^2 = 0\%$). In active UC, there were 9 RCTs, and there was a significant benefit for antibiotics inducing remission (RR of UC not in remission = 0.64; 95% CI: 0.43–0.96). There was moderate heterogeneity ($I^2 = 69\%$) and the antibiotics used were quite different.

The problem with meta-analyzing antibiotics in IBD treatment is because of the various antibiotics studied with their varying antibacterial spectra. Should the conclusion from this meta-analysis be that it does not matter which antibiotic or antibiotic combination is chosen, as long as there is some disruption to the gut microbiome? One study that is worthy of closer attention is by Selby et al. [4] for a number of reasons. Firstly, it is the largest placebo-controlled antibiotic study published as a full report to date. Secondly, its premise was to use a combination of antibiotics that might be effective in treating *Mycobacterium avium* subsp. *paratuberculosis*, the organism that causes Johne’s disease, but has been the subject of much debate as to whether it is even a zoonosis let alone whether it causes CD [5]. The results early into the study (the active treatment phase) were positive, and since the antibiotics used treat not only mycobacteria but a wide spectrum of bacteria, it heightens the plausibility that altering the gut microbiome may be of therapeutic value.

Two hundred and thirteen patients were randomized to clarithromycin 750 mg/day, rifabutin 450 mg/day, clofazimine 50 mg/day or placebo, in addition to a 16-week tapering course of prednisolone [4]. Those in remission at week 16 continued their study medications in the maintenance phase of the trial. At week 16, there were significantly more subjects in remission in the antibiotic arm (66%) than the placebo arm (50%; $p = 0.02$). This is interesting because a study assessing different antibiotics (metronidazole and ciprofloxacin) together with a less potent steroid (budesonide) showed that the antibiotics had no beneficial effect [6]. Of 122 subjects entering the maintenance phase, 39% taking antibiotics experienced at least 1 relapse between weeks 16 and 52, compared with 56% taking placebo ($p = 0.054$). By 2 years, despite ongoing antibiotic therapy the impact was waning as relapses occurred in 26% of the antibiotic users and 43% of placebo users ($p = 0.14$). During the 3rd year, off of study medications, the rate of relapse was the same in the antibiotic and placebo groups. Hence, these antibiotics may have some effect while they are used. They are not ‘curing’ a specific infection, but more likely are suppressing some relevant microbe(s).

Metronidazole is an interesting agent to consider as it continues to be widely used clinically. Clinicians have adopted it as a mainstay of therapy when treating
CD-related abscesses (to cover anaerobic bacteria), perianal disease (mostly based on observational data) and even luminal disease. In the largest randomized controlled study of metronidazole in active CD, Sutherland et al. [7] randomized 105 subjects to receive metronidazole (10–20 mg/kg daily) or a placebo for 16 weeks. Only 51 subjects completed the 16 weeks of therapy. Subjects withdrew mainly because of either lack of efficacy or drug side effects. Significant improvements in the Crohn’s Disease Activity Index (CDAI) were noted as compared with placebo, although there was no statistically significant increase in the number of metronidazole-treated patients achieving remission. Subjects receiving 20 mg/kg daily of metronidazole had a greater degree of improvement than those on 10 mg/kg daily; however, there were more adverse events in this group. Analyzing the data by site of CD activity, no difference in CDAI decrease was found for those with isolated small bowel disease. However, of the 45 subjects with ileocolonic disease there was a significant reduction in CDAI in the metronidazole-treated group (p = 0.005) and of the 12 subjects with isolated colonic disease there was a borderline significant reduction in the metronidazole group versus placebo (p = 0.05). These latter findings led to speculation that metronidazole may be an effective drug in predominately colonic CD, although this has never been adequately tested in a sufficiently powered study.

Where metronidazole has gained some ground is in postoperative maintenance after ileal or ileocecal resection in CD. It is in this setting where Rutgeerts et al. [8] randomized 60 patients to receive metronidazole (20 mg/kg per day) or placebo for 3 months. At 3 months, the incidence of endoscopic recurrence was lower in the metronidazole group compared with placebo (52 vs. 75%; p = 0.09). In the metronidazole group, the clinical recurrence was significantly reduced at 1 year (4 vs. 25%; p = 0.044). In terms of reducing clinical recurrence over 2 and 3 years postoperatively, however, the reduction in rates seen at years 2 and 3 persisted but was not statistically significant. More recently, Rutgeerts et al. [9], in a placebo-controlled, double-blind clinical trial studied the effect of ornidazole 1 g/day in 80 subjects for 1 year. Ornidazole was chosen as it was thought to be associated with fewer side effects than metronidazole. At 1 year, the antibiotics group had a lower recurrence rate, 7.9%, compared to the placebo group, 37.5% (p = 0.0046; odds ratio, 0.14; 95% CI: 0.037–0.546). At 2 years follow-up, 30% in the ornidazole group versus 45% in the placebo group had clinical recurrence (p = 0.17), and by 3 years follow-up there was no difference in clinical recurrence rates between the groups. In both of these studies, there were issues with tolerance of the nitroimidazole antibiotics. Two main conclusions can be drawn, however. Firstly, there was a proof of principle that during the period of nitroimidazole use there was a reduction in disease recurrence, and hence the longer it can be used, likely the better. Secondly, manipulating the spectrum of
bacteria these drugs impact upon is valuable in CD. It may be that impacting on other bacteria with antibiotics that have different treatment spectra may also be valuable, but this needs to be studied.

**Pouchitis**

Pouchitis is a condition that can develop in persons who have undergone a total colectomy with creation of an ileal pouch anal anastomosis (IPAA). It can affect approximately 50% of persons at some time after IPAA and up to 10% may have chronic pouchitis problems. For most patients, gut microbiome manipulation has become a common treatment, but immunomodulatory therapy including antibodies to tumor necrosis factor is occasionally and successfully used. Clinicians have widely adopted the use of metronidazole or ciprofloxacin or a combination of both for the treatment of acute pouchitis or for prevention of relapses despite the absence of robust data proving the effectiveness of these agents [10]. For the treatment of acute pouchitis, ciprofloxacin was more effective at inducing remission than metronidazole but this was a study of 16 subjects and has not been published in full [11]. Another small study of 13 subjects by Madden et al. [12] suggested a benefit of metronidazole over placebo. Despite these agents becoming standard of care in pouchitis, there are little other data that prove their benefit.

**Summary**

It is difficult to channel the results of the meta-analysis touting the benefit of antibiotics in inducing remission in CD and UC into clinical action since a wide variety of antibiotics with varying antimicrobial spectra were studied. There is some suggestion that at least while they are being used, antibiotics may reduce the relapse rates in medically or surgically induced remissions. The best data are for the use of nitroimidazoles postoperatively in CD. Antibiotics are widely used to treat perineal fistulizing CD and pouchitis despite the absence of robust clinical trial data in either scenario.

**Probiotics**

Probiotics are defined as ‘live microorganisms that when administered in adequate amounts confer a health benefit on the host’ [13]. While the FDA has yet to declare probiotics as having a benefit on the host, in 2007, the FDA enacted
regulations requiring dietary supplements to be produced in a quality manner, to be free of contaminants or impurities, and to be accurately labeled. Degnan [14] has reviewed how the FDA approaches the regulation of probiotics based on whether it is intended to be used as a drug, as a dietary supplement, as a food or food ingredient, or as a medical food. To summarize, the intended use of a probiotic product will determine its regulatory categorization under the FDC Act. This categorization, in turn, will determine the regulatory status of the product. Over the past decade, there has been a heightened interest in the use of probiotics as therapy in IBD. It remains a grey area as to whether probiotics in IBD should be considered drugs or medical foods, but considering their relationship to antibiotics in terms of having an impact on the gut microbiome, the standard has become to study probiotics in IBD with similar rigor expected of studies of antibiotics – the randomized, double-blinded, controlled trial. Rigorous scientific evidence regarding probiotics in IBD has been lacking in terms of both efficacy and safety. In fact, it is generally assumed that these agents might be effective and that they are safe. To follow, I will only comment on randomized, controlled studies.

Ulcerative Colitis

Kruis et al. [15] studied *Escherichia coli* Nissle 1917 in comparison to mesalamine 1,500 mg per day for 12 weeks in patients in remission (n = 120). These subjects were on average 1 year from their last relapse. Over the course of this study, the relapse rate was similar (14 vs. 16%). It could be argued that the study was biased in terms of assessing patients with a low likelihood to relapse, and the comparison was to a suboptimal dose of mesalamine. This group undertook a second and larger *E. coli* Nissle 1917 study comparing it to mesalamine 1,500 mg/day for 12 months (n = 327) and found a comparable clinical relapse rate (36 vs. 34%) [16]. Finally, Rembacken et al. [17] enrolled subjects with active UC (n = 116) and treated them with a variety of therapies (considered standard medical therapy, remission was induced by either oral or rectal corticosteroids) and 1 week of gentamicin. While undergoing remittive therapy, subjects were randomized to *E. coli* Nissle 1917 versus mesalamine 2,400 mg/day. Once remission was achieved, subjects in the mesalamine arm received 1,200 mg/day. Subjects were followed for a maximum of 12 months. Remission rates were similar in the mesalamine (75%) and *E. coli* Nissle (68%) groups, and time to remission was also similar in both groups. Relapse rates were similar (mesalamine, 73% vs. *E. coli* Nissle 67%) and mean duration of remission was also similar in both groups. In summary, for maintenance of remission *E. coli* Nissle 1917 was com-
parable to what amounts to a suboptimal dose of mesalamine. A meta-analysis of 11 mesalamine versus placebo studies for maintenance of remission in subjects initially enrolled with active UC and a separate analysis of 11 studies of mesalamine versus placebo for maintenance of remission in quiescent UC, doses of at least 2,000 mg/day were more effective than doses less than 2,000 mg/day [18]. Hence, to understand if *E. coli* Nissle 1917 could replace mesalamine as a choice for maintenance of remission, a study using optimal doses of mesalamine should be undertaken. Nonetheless, since even lower doses of mesalamine can be effective in maintaining remission (compared to placebo) [18], there is reason believe that *E. coli* Nissle 1917 may have some effectiveness.

VSL#3 is a combination of 4 strains of *Lactobacillus* (*L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbruekii* subsp. *bulgaricus*), 3 strains of bifidobacteria (*B. longim*, *B. breve*, and *B. infantis*) and *Streptococcus salivarius* subsp. *thermophilus*. VSL#3 was compared to placebo in patients with mild to moderately active UC (*n*= 147) [19]. At week 6, more subjects randomized to VSL#3 had at least a 50% decrease in the UC Disease Activity Index (33%) compared to placebo (10%, *p* < 0.001). By week 12, more subjects randomized to VSL#3 achieved remission (43%) compared to placebo (16%, *p* < 0.001). Furthermore, significantly more patients given VSL#3 (52%) responded with a decrease in their UCDAI by at least 3 points compared to placebo (19%, *p* < 0.001). By week 12, significantly more subjects in the VSL#3 group achieved mucosal healing (32%) compared to the placebo group (15%, *p* < 0.03). While the trial was unbalanced at enrollment for concomitant medications (significantly more VSL#3 subjects used only mesalamine while significantly more placebo subjects used a combination of mesalamine and immunosuppressants), this trial was one of the first to show a benefit of a probiotic in inducing remission in UC. Since 96% of VSL#3 users were concurrently on mesalamine, it is possible that VSL#3 effectiveness is maximized with concurrent mesalamine use.

In a study with a similar design (but only 8 weeks’ duration), subjects on concomitant therapy with mildly to moderately active UC were randomized to VSL#3 or placebo (*n*= 144) [20]. The decrease in UCDAI scores of 50% or more was higher in the VSL#3 group (63%) than in the placebo group (41%, *p* = 0.03). In this study, however, outcomes on endoscopic scores were not different between the groups. Remission at 8 weeks was higher but not significantly so in the VSL#3 group than in the placebo group (44 vs. 32%, *p* = 0.13). Nearly all subjects in the study were on concomitant mesalamine.

In a pediatric VSL#3 study, 29 children with newly diagnosed UC were randomized to receive either VSL#3 (weight-based dosing) or placebo in conjunction with concomitant steroid induction and mesalamine maintenance treatment and followed up to 1 year [21]. The Lichtiger colitis activity index and a
physician’s global assessment were used to measure disease activity. Remission was achieved in 93% of those treated with VSL#3 and other IBD therapy and in 36% treated with placebo and other IBD therapy (p < 0.001). Overall, 21% patients treated with VSL#3 and other IBD therapy and 73% of subjects treated with placebo and other IBD therapy relapsed within 1 year of follow-up. At 6 months, 12 months, or at time of relapse, endoscopic and histological scores were significantly lower in the VSL#3 group than in the placebo group (p < 0.05). A combination of mesaline and VSL#3 may be effective in UC for both children and adults, but more data are required.

A series of studies have assessed lactobacilli and bifidobacteria species in UC with mostly negative results. In a 1-year study of subjects with left-sided colitis in remission and who had at least 1 relapse within the prior year, randomization was to either a combination of *L. acidophilus* La-5 and *B. animalis* subsp. *lactis* BB-12 or placebo (n = 32) [22]. No other concomitant medications including mesaline were allowed. At the end of 1 year, 25% in the probiotic group versus 8% in the placebo group were in clinical remission (p = 0.37) with a similar median time to relapse in both groups. The authors concluded that there was no effect of this probiotic combination on maintaining remission in UC, although the study was likely underpowered, and it is unknown if this probiotic in combination with mesaline would be of benefit. A large 1-year study (n = 157) evaluated two probiotics (*L. salivarius* subsp. *salivarius* UCC118 or a *B. infantis* 35624) versus placebo in UC administered 1 month after a steroid-induced remission. Subjects were allowed mesaline use. At the end of 1 year, approximately half of all patients were in remission with no difference between the groups. This study has not been fully published in manuscript form [23]. In a study of 20 subjects with active UC using concomitant mesaline randomized to 100 ml of bifidobacteria-fermented milk (containing a *Bifidobacterium* and *Lactobacillus* supplement) or placebo treated for 12 weeks, remission rates were 40% in the active treatment group and 30% in the placebo group [24]. While endoscopy and histology scores improved more in the treatment group than the placebo group, the sample size was simply too small to facilitate conclusive results.

### Pouchitis

More so than conventional forms of IBD such as CD and UC, antibiotics seem to have a beneficial effect in pouchitis, although this is based mostly on clinical experience rather than clinical trial evidence. However, relapse rates are high, and a minority develop intractable or chronic pouchitis. Forty patients with UC
after IPAA with known episodes of relapsing pouchitis (at least 3 episodes per year) were randomized while in remission to either VSL#3 or placebo [25]. Relapses up to 9 months occurred in 15% of VSL#3 subjects and 100% of placebo subjects (p < 0.001). In another study, 40 patients with UC within 1 week after ileostomy closure of an IPAA were randomized to VSL#3 or placebo and followed for 1 year [26]. Acute pouchitis was seen in 10% of the VSL#3 group compared to 40% in the placebo group (p < 0.05). In a third study, 36 patients with at least 2 episodes of pouchitis in the previous year were randomized while in remission to either VSL#3 or placebo. Remission was maintained in 85% of the VSL#3 group and 6% of the placebo group (p < 0.0001) [27]. In a 1-year open parallel arm study to prevent pouchitis, 31 patients were randomized to VSL#3 or no treatment [28]. No patients in the VSL#3 group developed pouchitis, while only 8.3% of the control group developed pouchitis (p = 0.24). Kuisma et al. [29] randomized 20 subjects with acute pouchitis to Lactobacillus GG or placebo for 3 months. Clinical response occurred in 10% of the Lactobacillus group and in 0 of the placebo group (p = 0.32). Overall, in randomized controlled clinical trials, VSL#3 seems beneficial to prevent pouchitis.

*Crohn’s Disease*

There has been a paucity of studies assessing the utility of probiotics for the induction of remission in CD. A Cochrane review published in 2008 assessing the use of probiotics to induce remission in CD found only 1 study with 11 subjects worthy of inclusion in their review and concluded that there was no evidence to support the use of probiotics for induction of remission in CD [30]. There have been little new data on this topic since.

A number of studies have assessed the utility of probiotics in maintaining remission in CD. Thirty-seven patients with CD who had undergone ileocecal resection were randomized in a double-blind study to either Lactobacillus GG or placebo. In those randomized to Lactobacillus GG at 1 year, clinical recurrence was diagnosed in 17%, and endoscopic recurrence was evident in 60%. This was not significantly different than the 11% with clinical recurrence and the 35% with endoscopic recurrence randomized to placebo [31]. Ninety-eight patients with CD who had undergone resection (of any intestinal type) within 3 weeks were randomized to either L. johnsonii LA1 or placebo. At 6 months, endoscopic recurrence was more apparent in the placebo group (64%) than the LA1 group (49%) but the difference was not significant. Clinical recurrence occurred in 7% in the placebo group and 9.3% of LA1 group [32]. Seventy patients undergoing ileocecal resection for CD were randomized postoperatively
to either *L. johnsonii* or placebo, and there was no difference in endoscopic recurrence rates at 12 weeks [33]. While these results were disappointing, interventions that may have an impact on postoperative recurrence should be studied up to at least 1 year. A large well-done multicenter study examining the role of VSL#3 in maintaining remission was negative and also has yet to be fully published [34].

A pediatric trial enrolled 75 patients who were in medically induced remission. They were randomized to receive either *Lactobacillus* GG or placebo for 2 years as an adjunct to standard maintenance therapy (including 20% who were using alternate day steroids for some period and 65% who were using thiopurines). The relapse rates were 31% for the *Lactobacillus* GG group versus 17% for the placebo group. The median time to relapse was similar between the groups as well [35].

In a Cochrane systematic review published in 2006, the authors’ conclusion was that of the 7 small controlled studies available for review, there was no evidence to suggest that probiotics are beneficial for the maintenance of remission in CD [36]. As reviewed above, no studies undertaken more recently would likely impact on changing this conclusion.

**Safety**

There is a general assumption that ingesting probiotics would be safe. Especially, since the epithelial barrier has been compromised in CD or UC this may not be uniformly true. For one thing, probiotics may interfere with the action of other drugs. Probiotic bacteria especially strains of lactobacilli, produce acetic, lactic and propionic acids that lower the local pH [37]. Hence, it is possible that ingestion of probiotics may interfere with the pH-dependent release of certain 5-aminosalicylates, rendering them less efficacious. Therefore, the presumption that it is safe to simply add them to standard of care therapy in IBD may be erroneous.

Several reports have directly linked cases of *Lactobacillus* and other bacterial sepsis to the ingestion of probiotics [38]. Probiotic bacteremia or fungemia have occurred in patients with underlying immune compromise, chronic disease or debilitation [38]. Having intestinal inflammation is considered a minor risk factor for probiotic sepsis [38]. Hence, patients with IBD with moderate to severe disease activity or any IBD patient on immunosuppressive medication may potentially be at risk from ingesting probiotics.

A multicenter randomized double-blind placebo-controlled trial of a probiotic mixture (containing *L. acidophilus*, *L. casei*, *L. salivarius*, *L. lactis*, *B. bifidus*,
*dum, B. lactis*) in 296 patients with severe acute pancreatitis reported no difference between groups for infectious complications but a significantly increased mortality rate among the probiotic group (RR = 2.53, 95% CI: 1.22–5.25). Nine patients in the probiotic group developed bowel ischemia compared with none in the placebo group [39]. Two other studies have shown nonsignificant increases in sepsis in critically ill patients. Recently, a 17-year-old boy with UC treated with corticosteroids and infliximab, who presented with *Lactobacillus* bacteremia 1 week after starting *L. rhamnosus* GG probiotics, 16S rRNA sequence analysis identified the organism from the patient’s blood culture and probiotic capsule as *L. rhamnosus* with a 99.8% match for both strains [40]. This case report highlights the potential risk of *Lactobacillus* bacteremia in immunosuppressed patients with severe active UC. These reports serve as stark reminders about the presumption of probiotic safety, particularly in inflammatory diseases.

**Summary**

While there is great promise for novel probiotics, physicians need to be circumspect when proof of efficacy and safety are lacking. There are promising results for *E. coli* Nissle 1917 in maintenance of remission in UC and the multispecies product VSL#3 in active UC and in preventing pouchitis. There is no evidence available to support the use of probiotics in CD. These same conclusions were reached in a recently published systematic review on the topic [41]. Since there are some safety concerns especially with an inflamed intestinal epithelial layer, it is important for probiotics to be rationally as opposed to randomly tested in IBD. For example, given the scientific evidence of the relative lack of *Faecalibacterium prausnitzii* in patients with ileal CD, and its beneficial effect in an animal model of colitis, there is a rationale for a placebo-controlled study of this agent [42].

**Prebiotics**

Prebiotic refers to a food substance that is not digested in the human small bowel and promotes selective growth of beneficial bacteria in the colon [43]. The role of diet in modulating the gut microbiome has not been fully studied, but carbohydrates such as oligofructose, inulin, and galacto-oligosaccharides are known to stimulate selectively the growth of bifidobacteria and lactobacilli in the colon (prebiotic effect) and thereby contribute to barrier function. Inulin and oligo-
fructose are fructans that are not hydrolyzed by pancreatic enzymes and escape digestion in the small bowel. Fructo-oligosaccharides (FOS; oligofructose plus inulin) are nondigestible polymers of fructose found naturally in artichokes, leeks, asparagus, onions, and bananas. Bifidobacteria have relatively high amounts of β-fructosidase, so they can selectively metabolize FOS. Lactobacilli can also ferment FOS. Fourteen subjects supplemented their diet for 2 weeks with a mix of 7.5 g of oligofructose and 7.5 g inulin. Fifteen subjects were recruited at the time of colonoscopy and given no supplement. Multiple endoscopic biopsies were taken from the caecum, transverse and descending colon, and rectum from both groups. The mucosal flora was cultured. In the prebiotics group, there was increased mucosal bifidobacteria (p = 0.01) and lactobacilli (p = 0.04) in both the proximal and distal colon [44]. FOS have also been shown to stimulate the growth of both fecal and mucosal bifidobacteria in persons with CD [45]. In 10 persons with CD, FOS reduced the Harvey Bradshaw score from 9.8 (SD 3.1) to 6.9 (3.4, p = 0.01), a score which is still considered active disease. In these subjects, the percentage of IL-10-positive dendritic cells increased, and the percentage of dendritic cells expressing TLR2 and TLR4 increased. The question arises if these specific prebiotics can enhance any other gut bacteria since there is little evidence that bifidobacteria or lactobacilli are useful probiotics in IBD. Some data exist showing that inulin can increase other microbes including *F. prausnitzii* [46], a firmicute that has been shown to be reduced in the gut of patients with higher relapse rates in CD [42].

In one of the first placebo-controlled trials of prebiotics in IBD, 103 patients with active CD were randomized to FOS versus placebo × 4 weeks [47]. The withdrawal rate due to side effects was 26% in the FOS group compared with 8% in the placebo group (p < 0.02). Clinical response rates of 22% for the FOS group versus 39% in the placebo were not significantly different (but certainly not better in the FOS group). In this study, even fecal bifidobacteria were not different between groups. In another study, 67 subjects with active CD were randomized to receive oligofructose-enriched inulin (longer chain than FOS) or placebo for 4 weeks and the fecal metabolome and microbiome were assessed [48, 49]. In those randomized to oligofructose-enriched inulin, there was a significant increase in fecal butyrate and acetaldehyde [48]. Butyrate can induce T cell apoptosis and suppresses IFN-γ-mediated inflammation in colonic epithelial cells to suppress colonic inflammation [50]. There was a significant decrease in fecal *Ruminococcus gnavus* and increase in *B. longum* [48, 50]. The authors claimed that there was greater clinical improvement in the oligofructose-enriched inulin group versus the placebo group, although this is difficult to fully discern from the two reports [48, 49]. Of note, 34% randomized to oligofructose-enriched inulin withdrew because of side effects compared to just 12% (p = 0.07) in the
placebo group. Hence, to date there has been no benefit of prebiotics in active CD and perhaps excess intolerance. Prebiotics remain to be tested in maintaining remission in CD.

While there have been more studies of probiotics in UC than CD, there have been few prebiotic studies in UC or pouchitis. In one study of 19 subjects with mildly to moderately active UC randomized to either FOS or placebo for 2 weeks, fecal calprotectin decreased in the FOS group but not in the placebo group. Interestingly, disease activity scores decreased in both groups [51]. In 20 subjects after IPAA without pouchitis randomized to either inulin or placebo for 3 weeks, there were increased levels of fecal butyrate and Bacteroides fragilis [52]. However, there was no difference in fecal bifidobacteria or lactobacilli and no difference in clinical symptoms.

Summary

Prebiotics are nonabsorbable carbohydrates that may alter the gut microbiome and in so doing alter the gut metabolome, the immune response and possibly then clinical outcomes in IBD. Studies are needed especially to prove whether the latter effect (improving clinical outcome) is true.

Timing Is Everything

While much work needs to be done to determine what species within the gut microbiome are the optimal targets to modulate IBD, it follows that choosing the optimal antibiotics, probiotics or prebiotics is currently in the domain of ‘best guesses’. While researchers chase the critical microbial species, another critical often overlooked variable is timing, that is timing of gut microbiome and gut immune response vulnerability and how this can be manipulated for both harm and gain. Norris et al. [53] who established the extraordinary DAISY Cohort, a cohort of newborns at risk for developing celiac disease showed that infants fed gluten for the time between 1 and 3 months had the highest rates of seroconversion on celiac antibody screening; those fed gluten after 7 months had the next highest rates of celiac antibody seroconversion, and those fed gluten between 4–7 months had the lowest rate. The implication was that as the infant’s gut microbiome and gut immune response evolves within the first year of life, there may be critical times at which certain changes to the gut milieu either through foods ingested or antibiotics administered or infections encountered may leave lasting imprints. The first year of life is a critical time in gut
microbiome development. With this in mind, Shaw et al. [54] explored the population-based University of Manitoba IBD Epidemiology Database to determine if children with IBD were more likely than matched controls to receive antibiotics within the first year of life. In fact they were, with an odds ratio of 2.9 (95% CI: 1.2–7.0). Because the most common diagnosis for which antibiotics were prescribed to children was otitis media and because the prescription drug database only was initiated in Manitoba in 1995, this group explored the diagnosis of otitis media in children with IBD compared to controls [55]. This analysis could be extended back to 1984, and they found that children with IBD were significantly more likely to have diagnoses of otitis media by 1 year of age with an odds ratio of 2.8 (95% CI: 1.5–5.1). They hypothesized that since nearly all diagnoses of otitis media were diagnosed by age 5, the otitis media was not an extraintestinal manifestation of IBD but rather a proxy measure for antibiotic use. This same group explored antibiotic use in the 2–5 years prior to IBD diagnosis amongst adults with IBD, and they found that persons with IBD were more likely to have used antibiotics with a dose-response effect between having at least 1, at least 2 or at least 3 antibiotic dispensations so that even in adulthood with a well-established gut microbiome perhaps based on genetics or other gut milieu factors antibiotic use at a vulnerable time can be impactful in ultimately contributing to the expression of IBD [56]. Others have also reported on the antecedent use of antibiotics posing a risk for development of IBD in adults and children [57–61]. In Finland, a population-based case-control study of children diagnosed with IBD versus controls reported that the risk of pediatric CD increased with the number of antibiotic purchases from birth to the index date of diagnosis and persisted when the 6 months preceding the case’s diagnosis were excluded (for 7–10 purchases vs. none, OR = 3.48, 95% CI: 1.57–7.34) [57]. In IBD cases overall, the effect was evident for any amount of prescriptions of 7 or more but was not evident for prescription purchases of 6 or less. The association between CD and antibiotic use was stronger in boys than in girls, and there was no impact of antibiotic exposure on the development of pediatric UC. In a Danish study of children born between 1995 and 2003, 117 were very young children diagnosed with IBD with a mean age at diagnosis of 3.3 for CD and 3.5 for UC [58]. The RR of IBD was 1.84 (95% CI: 1.08–3.15) for antibiotic users compared with nonusers. This association appeared to be an effect on CD alone (RR 3.41 95% CI: 1.45–8.02) and was strongest in the first 3 months following use, although this may be confounded by indication. The effect was also strongest among children with CD with more than 7 courses of antibiotics (RR 7.32, 95% CI: 2.14–21.99). Finally, in the UK Health Improvement Network (464 general practices) from 1994 to 2009, exposure to antibiotics before 1 year of age had an adjusted hazard ratio of 5.51 (95% CI: 1.66–
18.28) but decreased to 2.62 (95% CI: 1.61–4.25) and 1.57 (95% CI: 1.35–1.84) by 5 and 15 years, respectively [59]. Each antibiotic course increased the IBD hazard by 6% (4–8%).

What about dietary ingestion within the first year of life? What is it about breastfeeding that is touted to be beneficial [62]? Although nondigestible oligosaccharides are virtually absent from cow milk, they represent the third most abundant fraction after lactose and lipids in human milk [63]. Where short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) have been administered to infants in clinical trials, there has been generally a reduction in infections and allergic diseases [62]. Since 2002, studies have investigated the effect of scGOS + lcFOS on the composition of the intestinal microbiota in preterm, term, and weaning infants [64, 65]. These studies showed that scGOS + lcFOS affect the early microbial pattern in a way similar to human milk with an intestinal microbiota enriched with bifidobacteria and lactobacilli [64]. It has been shown that the microbial changes associated with scGOS + lcFOS during the first 6 months of life may have a long-lasting effect. Differences continue to be observed after the weaning period, at age 1 year, without further supplementation [63, 65]. These data show that dietary intervention in a period of life when the microbiome is still developing may have substantial consequences on microbial equilibrium. The impact of scGOS + lcFOS on microbial composition coincides with changes in SCFAs, lactate, and pH in the infant gastrointestinal tract that resemble the fermentation pattern generated by human milk [66].

What about prebiotics later in life? Until it is proven that increasing bifidobacteria and lactobacilli species in the colons of adults with IBD is beneficial, it may be misguided to administer prebiotics with this specific intent in mind. It is known that diet can impact on the gut microbiome even among the elderly [67]. Hence, it is unknown what impact prebiotics will have if diet is not regulated simultaneously. So, beyond the use of breast milk or prebiotic-enhanced supplements, the diet we administer to our children may also have an impact on their developing microbiome and in turn their developing intestinal immune response and in turn their expression of IBD.

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