Nutrition, Gut Microbiota and Immunity: Therapeutic Targets for IBD
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Nutrition, Gut Microbiota and Immunity: Therapeutic Targets for IBD

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Preface

Inflammatory bowel disease (IBD), including Crohn’s disease (CD) and ulcerative colitis (UC), are chronic debilitating diseases that occur in populations around the world. The diseases can manifest at any age, and therefore represent a clinical challenge for pediatricians, internists, family practitioners, and surgeons. The underlying etiology is multifactorial, where host genetic polymorphisms account for a minority of the risk for disease development, emphasizing the importance of environmental factors such as the gut microbiota. Supportive of this notion, epidemiologic associations show a significant increase in IBD incidence over the past few decades associated primarily with residence in industrialized nations.

Current therapeutic modalities for IBD are largely targeted at suppression of the innate and adaptive immune response. Commonly used therapies include mesalamine, corticosteroids, thiopurine analogues (azathioprine or 6-mercaptopurine), methotrexate, and anti-tumor necrosis factor-α agents. There is also limited use of natalizumab, a biologic targeting the α4-integrin adhesion molecules. While effective for many patients, these therapeutic strategies are not universally effective. Furthermore, they are each associated with the risk of serious and sometimes fatal adverse events.

An alternative approach to the treatment of IBD is to change the environmental factors that contribute to the etiology or perpetuation of inflammation. Leading targets for this alternative approach to therapy are the principal contents of the gastrointestinal tract – our diet and the human gut microbiota. Indeed, there is reason to believe that the composition of our diet and the gut microbiota might have a synergistic effect on inflammation related to IBD.

This monograph includes summaries of talks presented at the 79th Nestlé Nutrition Institute Workshop held in New York on the 28th and 29th of September 2013. The speakers in the symposium addressed our current understanding of the epidemiology and biologic underpinnings that manifest as CD and UC; the gut microbiota, its function, and how it may interact with...
nutritional status in perpetuating IBD; the potential for manipulation of the gut microbiota through the use of prebiotics, probiotics, antibiotics, and fecal transplantation, and the current role of and future prospects for nutritional interventions in the management of these diseases.

Despite advances in the treatment of IBD, a substantial proportion of patients experience relapse of the disease every year. Many of these patients still require surgery. Although surgery represents a cure for UC, it is associated with lifelong alteration in bowel function and risks of other complications. For CD, surgery is generally only a temporizing measure as disease recurrence is common. Given the incomplete effectiveness of our current immunosuppressive therapies and their associated toxicities, there is a real need for alternative treatment strategies. Altering key environmental exposures that drive the inflammatory response could open new avenues to treat these debilitating lifelong diseases.

James D. Lewis
Frank M. Ruemmele
Gary D. Wu
Foreword

Inflammatory bowel disease (IBD) currently affects 1 in 200 people in the United States. The incidence of IBD has been gradually increasing globally in the past several decades. While the explanation for this increase is not totally clear, environmental factors, including changes in the diet, may be a key factor.

The 79th Nestlé Nutrition Institute Workshop held in New York City in September 2013 carries on the theme from the 77th Nestlé Nutrition Institute Workshop, where world experts gathered in Panama City to present their latest findings on how nutrient status can modulate immunity and improve health conditions in pediatric patients. This workshop chaired by Prof. Lewis, Prof. Ruemmele and Prof. Wu focused on the complex relationship between nutrition, inflammation and the microbiome as it relates to IBD; this is arguably the hottest area of IBD research currently.

Previously, the theories on pathogenesis of IBD suggested a combination of genetic susceptibility and immune and external environmental factors. In recent years, the gut microbiota has greatly gained in importance and has been accepted as the 4th element in the pathogenesis of IBD. These relationships are complex and not independent since IBD patients may have a genetic susceptibility that leads to abnormal immune responses directed against the intestinal microbiota.

Currently, over 160 genetic susceptibility genes have been identified for IBD, the most prevalent of these are Nod2, an important intracellular pathogen recognition sensor, and ATG 16L1, important in autophagy, killing and processing of phagocytized bacteria. However, the function of many of the other genes identified has not been fully characterized.

The gut microbiota consists of both protective and aggressive microbes, and the balance between these populations is important, not only in the pathogenesis of IBD, but also in the ongoing inflammatory response. A better understanding of the complex interactions, particularly the role of the gut microbiota in the inflammatory process, holds the key for potential for targeted therapy in
the future. The ability to selectively alter the composition and thus the function of the gut microbiome through diet, prebiotic and probiotic therapy may be a very attractive treatment alternative for patients with IBD. There is already good evidence in the medical literature that total enteral nutrition is highly efficacious in inducing remission in pediatric Crohn’s disease. In these patients, there is a significant shift in the gut microbiota following the successful enteral therapy; however, the causal relationship has not been established to date.

On behalf of Nestlé Nutrition Institute and Nestlé Health Science, we would like to thank the Chairmen, Prof. James Lewis, Prof. Frank M. Ruemmele and Prof. Gary Wu for their diligent work in assembling such a distinguished group of researchers, clinicians and speakers. We would also like to thank all the speakers for their hard work in putting together such outstanding presentations. The energy throughout the meeting, the interest among the participants and the quality of the questions posed to the speakers were all testaments to the quality of the meeting and the importance of this topic. We hope that important collaborations will result from the many positive interactions during this workshop.

Finally, we would like to thank Natalia Wagemans, Mélanie Costinas, Bernice Hammer and Mélanie Pittier who worked tirelessly in the background to ensure the meeting ran smoothly and made it the resounding success that it was.

We look forward to a follow-up NNI Workshop in the near future to review the advances in this exciting field of research.

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Inflammatory bowel disease (IBD), including Crohn’s disease (CD) and ulcerative colitis (UC), are chronic debilitating diseases that occur in populations around the world. The diseases can manifest at any age, and therefore represent a clinical challenge for pediatricians, internists, family practitioners, and sur-
gences. The underlying etiology is thought to be multifactorial. There is a well-defined genetic contribution to the diseases, but this does not fully explain the epidemiology. Environmental factors, including the composition of the gut microbiota, are also important. This review will focus on the relationship between IBD and other environmental factors, such as diet, infections, and medications.

**Incidence and Prevalence of IBD**

Studies of the incidence and prevalence of IBD were recently summarized in a review by Cosnes et al. [1]. Within North America, the prevalence of CD is approximately 44–201 per 100,000, with increasing incidence until approximately age 30. Similarly, estimates of the prevalence of UC range from 37.5 to 238 per 100,000, with incidence increasing until approximately age 40. Although early research suggested a bimodal pattern of incidence with a second peak later in life, this has not been consistently observed.

There is wide geographic variability in the incidence and prevalence of IBD. High incidence rates have been observed in the United Kingdom, Northern Europe, Canada, and the United States. The incidence of IBD is generally lower in the Asia-Pacific region, with the exception of Australia [2]. In many regions, there is evidence of increased incidence and prevalence as one moves further from the equator [3]. Similar patterns have been seen with other immune-mediated diseases such as psoriasis and multiple sclerosis [4, 5].

Globally, there is evidence of increasing incidence of CD and UC over time [6]. Furthermore, the rising incidence of IBD in Western countries has generally predated that in developing nations. In general, the incidence of UC has risen before that of CD within any given area. For example, in 2012, the ratio of UC to CD in Asia was 2.0, while in Australia it was 0.5 [2].

The rising incidence of UC and CD across the world, but earlier in developed nations, has contributed to the hypothesis that ‘westernization’ of our lifestyle has led to the increased incidence of IBD. Before focusing on the specific evidence that supports an association between a Western lifestyle and the development of IBD, it is important to consider possible alternative explanations for the geographic patterns that have emerged. The most obvious alternative explanation is that improved access to healthcare and improved diagnostic tools led to more frequent diagnosis of IBD. It is possible that some patients with mild IBD who previously went undiagnosed throughout their entire life are now diagnosed because of greater availability of colonoscopy and cross-sectional imaging modalities. Increased awareness of IBD by clinicians could also contribute to rising incidence rates. Likewise, cultural norms may have evolved in some re-
gions, such that there is greater willingness to discuss one’s bowel symptoms. Each of these could contribute to an apparent increased incidence and prevalence even if there were truly no change in the epidemiology of these diseases.

Arguing against detection bias is the observation that incidence rates of numerous other immune-mediated diseases have also increased in a pattern similar to IBD [7–10]. Diseases such as asthma and psoriasis are diagnosed without the need for invasive or expensive tests. Given the frequent co-occurrence of immunologic diseases, it seems more likely that one or more environmental factors have contributed to the rising incidence rates of all of these diseases [11, 12].

This review will focus on several hypotheses related to the changing epidemiology of IBD, with a specific focus on environmental factors. Although the human gut microbiome can be considered an environmental factor, this will not be addressed in detail in this review, as it is the focus of another chapter in this book. Likewise, the important contribution of genetics to the epidemiology of IBD will not be discussed in detail since this will also be covered in another chapter.

**Emigration**

Some of the regional variation in incidence and prevalence of IBD is likely due to genetic factors. Increased access to care and diagnostic tests could also lead to higher incidence rates in more industrially developed nations. A recent systematic review demonstrated significantly higher incidence rates for both CD and UC among urban populations [13]. The strength of association was greater for CD than UC, although there was significant heterogeneity in each analysis without an obvious explanation. In addition to greater access to healthcare, environmental factors such as diet, pollution, climate, hygiene, and crowding may also contribute to these differences, and would be associated with urban residence.

Studies of people who move between regions of differing IBD incidence and prevalence provide an opportunity to assess the impact of environmental factors on the risk of developing IBD. Several investigators have examined the incidence of IBD within Israel because Jews residing in Western countries are known to have an increased incidence of IBD. In early studies from Israel, immigrants to Israel had higher incidence rates than did Israeli-born populations [14–16]. However, by late 1980s, the incidence and prevalence of CD was comparable among Jews in southern Israel regardless of whether the patient or the patient’s father was born in Israel, Asia, Africa, Europe or America [17]. In contrast, the prevalence was much lower among Arab Israelis, which could be due to genetic or environmental differences since during this time period the Israeli Bedouin population led a lifestyle ‘more characteristic of Third World countries’ [17].

A Review of the Epidemiology of IBD 3
More recent data from Sweden suggest that the incidence of IBD is generally lower in first-generation immigrants, but by the second generation is comparable to that of the Swedish population [18]. Taking advantage of a unique population-based registry, Li et al. [18] observed that the incidence of CD was significantly lower among all first-generation immigrants, and that this was most evident among immigrants from Africa (SIR 0.54, 95% CI: 0.37–0.77), Asia (SIR 0.64, 95% CI: 0.54–0.74), Baltic countries (SIR 0.45, 95% CI: 0.23–0.79) and Latin America (SIR 0.43, 95% CI: 0.28–0.63). Only among those from Latin America was there a significantly lower incidence rate of CD among second-generation immigrants. Generally, similar results were observed for UC, with second generation incidence rates for most immigrants being similar to that of the Swedish population, while second-generation immigrants from southern and Eastern Europe continued to have lower incidence rates. Notably, if both parents were immigrants from the same country, the second generation continued to have a lower incidence of CD. Whether this is due to greater retention of lifestyle customs from the parents’ native land or other reasons is unknown.

Leicester, UK, is home to a large south Asian immigrant population. In the 1990s, the incidence of UC in Leicester was higher among south Asian immigrants than among those of European ancestry [19]. The distribution of disease differed between first- and second-generation immigrants, with proctitis being most common among first-generation immigrants whereas extensive colitis was more common among the second generation. It is likely that the second generation would have assimilated to the Western culture more than their parents, supporting a hypothesis that environmental factors not only influence incidence rates but could also influence disease phenotype.

Dietary differences are often cited as a contributor to the increased incidence of IBD among immigrants from Asia or Africa to Western nations. However, not all studies support this hypothesis. For example, Carr and Mayberry [19] observed that immigrants from south Asia to Leicester, UK, who developed UC were more likely to follow a traditional vegetarian diet than immigrants who did not follow a vegetarian diet.

Barreiro-de Acosta et al. [20] took a different approach to this question by studying people from the Galicia area of Spain where people often emigrate to other countries to find work and then return to Spain at a later time. Patients newly diagnosed with IBD while living in Galicia were more likely to have emigrated to a foreign land and returned than control subjects. Furthermore, the association was qualitatively stronger among those who had emigrated to industrialized European countries (OR 1.91, p = 0.02) than to Latin America (OR 1.48, p = 0.32). The association was somewhat stronger for UC (OR 2.24, p < 0.01)
than for CD (OR 1.56, p = 0.15). These data support the hypothesis that changes in environmental exposures that result from emigration may influence the risk of developing IBD.

The changing incidence of IBD during the last half century has occurred too fast to be attributable to changes in the underlying gene pool. Far more likely is that changes to our environment have led to the ‘epidemic’ of immune-mediated diseases and IBD in particular. That immigrants from low-incidence regions to high-incidence regions typically continue to have lower incidence rates for the first generation but comparable incidence rates to natives of their new home by the second generation suggests that early exposure to environmental factors may be important.

**Obesity and Physical Activity**

People living in Western societies are typically less physically active and have easier access to food than our predecessors. This has led to an epidemic of obesity, including childhood obesity. One hypothesis is that the reduced physical activity and subsequent obesity could contribute to increasing incidence rates of IBD. Indeed, prior studies have documented that visceral adipose tissue produces inflammatory cytokines such as tumor necrosis factor-α and that obesity is associated with increased gut permeability [21]. Furthermore, several studies have suggested that obese patients with CD are more likely to undergo surgery [22, 23]. However, evidence linking physical activity and obesity to new-onset IBD is generally lacking. The strongest evidence comes from the European Investigation into Cancer and Nutrition (EPIC) study [24]. This prospective cohort study found no evidence that physical activity, total caloric intake or obesity was associated with new-onset CD or UC. The study included mostly middle-age and older adults, so it is possible that physical activity and obesity could still impact the incidence of these diseases in children.

**Diet and the Risk of IBD**

The bowel lumen is continually exposed to numerous antigens, including the food that we consume and the enormous population of organisms that compose the gut microbiome. Although obesity and higher total caloric intake do not appear to increase the risk of IBD, selected micro- and macronutrients, additives, or contaminants could influence the risk through a variety of pathways. Proposed mechanisms through which diet could influence the incidence of IBD in-
clude direct dietary antigens, altering the gut microbiome, influencing gene expression, and affecting gastrointestinal permeability [25].

Our first diet is typically breast milk or infant formula. Breastfeeding is more common in less developed areas where there typically has been a lower incidence of IBD. In addition to secreted immunoglobulins, breast milk contains a number of anti-infective and anti-inflammatory compounds, including caseins, whey proteins, growth factors, and milk fat globule proteins (reviewed in detail by Chatterton [26]). Furthermore, breastfeeding has been shown to alter the composition of the neonatal gut microbiota [27]. Numerous studies have examined the relationship between breastfeeding and the subsequent risk for IBD. A recent meta-analysis observed that breastfed newborns had a lower incidence of childhood onset IBD (pooled odds ratio 0.69, p = 0.02) [28]. Although separate analyses of CD and UC did not identify statistically significant reductions in the incidence, the magnitude of the effect for both diseases was comparable to that observed for IBD. These data support a role for the earliest dietary exposures in the risk of developing IBD.

Lending further support to the role of early diet in the risk for IBD is the observation that even among children, new onset of IBD is uncommon during the first year of life and therefore prior to the introduction of table food [29]. Following the introduction of table food, there is a dramatic alteration in the composition of the gut microbiome [30]. Given the likely important role of the gut microbiome in the etiology of IBD, it is logical that early-life food exposures could also impact the risk for IBD. Unfortunately, data on the relationship between early-life diet and risk for IBD are generally lacking.

Several investigators have examined the association of long-term dietary patterns and the risk of incident IBD [reviewed in 25, 31]. Most recently, the authors of a systematic review came to the conclusions that high dietary intakes of total fats, PUFAs, omega-6 fatty acids, and meat were associated with an increased risk of CD and UC; high fiber and fruit intakes were associated with decreased CD risk, and high vegetable intake was associated with decreased UC risk [31]. These are briefly discussed below.

One of the earliest hypotheses related to sugar intake. Although most studies have been potentially flawed by the challenges of recalling pre-illness dietary patterns and/or failure to account for confounders such as smoking, several investigators have observed associations between sucrose and other sugars and the incidence of CD and UC [32–35]. Subsequent studies have focused on other dietary components. Most studies have found a positive association between dietary protein and both CD and UC, although this was only statistically significant in a few [35, 36]. Numerous studies have also examined fiber, most not identifying an association with new-onset IBD [25]. Fruit intake was significant-
ly associated with a lower incidence of CD in several studies [37, 38], but not in any of 8 studies of UC [reviewed in 31]. Both meat and egg consumption have been associated with UC in several studies [33, 36, 39, 40], but these have generally not been associated with CD [31].

Dietary fat has been examined in multiple observational studies, but a few prospective studies deserve particular notice. The EPIC study collected data on dietary patterns at baseline and identified cases of UC diagnosed at least 18 months after the dietary assessment [41]. Compared to non-IBD patients, those with UC had higher consumption of the n-6 PUFA linoleic acid with evidence of a dose response. Those in the highest quartile of intake had more than a 2-fold increased risk of UC. Linoleic acid is converted to arachidonic acid and incorporated into cell membranes from which they can be released and converted to prostaglandins, leukotrienes, and thromboxanes. Linoleic acid is found in high concentrations in red meat, cooking oils and polyunsaturated margarines. In contrast, high intake of the n-3 PUFA docosahexaenoic acid was inversely associated with new-onset UC. Importantly, the Denmark-EPIC subcohort obtained gluteal adipose biopsies at baseline and were able to measure the n-6 PUFA arachidonic acid in the gluteal adipose tissue. Again, those in the highest quartile had the greatest incidence of subsequent UC (RR = 3.1 with a positive dose response) [42]. This study is particularly important as it used pre-disease tissue levels rather than relying on dietary recalls to assess exposure. Thus, it validated the findings from dietary recalls using pre-disease biomarkers. New data from the Nurses’ Health Study cohorts have partially reproduced the findings of the EPIC cohort [43]. A food frequency questionnaire was used to assess dietary patterns every 4 years, and participants were followed prospectively for 26 years. In this cohort, cumulative energy-adjusted intake of total fat, saturated fats, unsaturated fats, n-6 and n-3 PUFAs was not associated with incident diagnosis of CD or UC. However, greater consumption of long-chain n-3 PUFAs (docosahexaenoic acid, eicosapentaenoic acid, and docosahexaenoic acid) and a higher ratio of n-3:n-6 PUFAs appeared protective against development of UC.

There are a variety of hypotheses as to why a diet low in fiber and high in meat and refined sugars would be associated with an increased incidence of IBD. A high-fiber diet reduces intestinal transit time and therefore reduces the amount of time that the gut mucosa may come in contact with dietary antigens. However, this seems an artificially simple explanation given that over the course of a lifetime, there is extensive exposure of the gut mucosa to dietary antigens, regardless of the composition of the diet. Both CD and UC occur in regions of the gastrointestinal tract with the highest concentration of microorganisms, and the gut microbiome is believed to be a central contributor to the pathogenesis. Long-term diet is associated with alteration in the composition of the gut microbiota,
including bacteria, viruses, microeukaryotes, and archaea [44, 45]. However, it remains unknown what microorganisms are most influential in the etiology of IBD. Interestingly, the prebiotic inulin has been associated with an increase in the abundance of *Faecalibacterium prausnitzii* in 2 studies [46, 47]. *F. prausnitzii* has been associated with reduced relapse rates after ileocolonic resection [48]. Given that after surgery the intestine is temporarily ‘healed’, this raises the hypothesis that insoluble fibers may influence the risk of developing IBD through modulation of the gut microbiota. Diet could also influence the incidence of IBD by altering the composition of fecal bile acids. Fat consumption, particularly meat, leads to greater concentration of secondary bile acids [49]. In animal models, consumption of milk-derived saturated fat leads to the production of taurine-conjugated bile acids, which further leads to an increase in sulphate-reducing bacteria *Bilophila wadsworthia*, which in turn can produce greater amounts of the potentially mucosal toxic hydrogen sulfide and which may directly influence antigen-presenting cells leading to production of inflammatory cytokines [50, 51]. Finally, it is conceivable that the relationship between diet, the gut microbiome, and IBD may be mediated through a metabolic product of the microbiota [52]. A similar model has been proposed for other diseases, such as cardiovascular disease [53]. While data from animal models lend credence to this hypothesis for IBD [52], more definitive evidence in humans is needed.

**Infections and Antibiotic Exposure**

In addition to diet, other factors can influence the composition of the gut microbiome. Antibiotics and enteric infections have been a focus of several studies of risk factors for IBD. Much research has focused on atypical mycobacterial infections (*Mycobacterium avium* subspecies *paratuberculosis*, MAP) given the similarity of CD to Johne’s disease in cattle. Two systematic reviews of these studies observed that patients with CD were more likely than healthy controls or patients with UC to have evidence of prior MAP infection whether assessed by PCR or ELISA [54, 55]. However, several questions remain regarding the causal nature of this association. Most notable is whether CD or the genetics of CD could predispose patients to colonization or infection with MAP [56]. For example, Marks et al. [57] have demonstrated impaired ability of patients with CD to respond to breaches of the mucosa and local infection, which could contribute to granuloma formation. Furthermore, a well-designed clinical trial of long-term treatment with antibiotics targeted at *Mycobacterium* failed to produce long-term changes in the natural history of CD, albeit the participants in this study were not limited to those with documented prior MAP
Interestingly, the proportion achieving remission at week 16 was higher in the antibiotic arm than in the placebo arm, raising questions about the role of antibiotics in managing acute exacerbations of CD.

More recent studies have examined common enteric infections. Among residents of Denmark, those with culture-documented *Salmonella* or *Campylobacter* infection were 2.9 times more likely to be subsequently diagnosed with IBD than a group of matched controls. The association was stronger during the first year of follow-up, but a subsequent diagnosis of IBD remained statistically more common even after excluding the first year \[59\]. Similar results have been observed in a second cohort from the UK \[60\], where a culture-documented bacterial gastroenteritis was 1.7 times more common among patients with UC and 3.7 times more common among patients with CD than in nondiseased controls. In a cohort of military personnel, Porter et al. \[61\] observed that those with documented gastroenteritis were also more likely to be subsequently diagnosed with IBD, with a somewhat stronger association for CD than for UC. These retrospective cohort studies could produce biased results if patients with confirmed infection were more likely to subsequently undergo workup for persistent symptoms after the infection resolved (detection bias) or if patients with clinically undiagnosed IBD tend to develop more severe infections than those without IBD (spectrum bias). Alternatively, it is possible that patients with a genetic predisposition to develop IBD are also more likely to develop enteric infections. Many of the genes associated with an increased risk for IBD control are linked to the innate and adaptive immune response to microorganisms \[62, 63\]. Thus, enteric infections may be a marker for those at increased risk for IBD without being part of the causal pathway.

Antibiotic exposure has also been associated with an increased risk of developing IBD, particularly CD (table 1). To prevent the risk of recall bias inherent

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**Table 1. Selected studies of antibiotic exposures and the risk of subsequent IBD**

<table>
<thead>
<tr>
<th>First author</th>
<th>Selected classes</th>
<th>OR (95% CI)</th>
<th>Dose response for any antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Card [64]</td>
<td>cephalosporins</td>
<td>1.30 (0.81–2.09)</td>
<td>no</td>
</tr>
<tr>
<td></td>
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in asking people to recall their prior antibiotic exposure, several studies have relied on administrative or electronic health record data to define antibiotic exposure [64–68]. These studies have generally observed more frequent antibiotic exposure among patients who were later diagnosed with CD, but no association with subsequent diagnosis of UC (fig. 1). Because early antibiotic exposure may impact the long-term composition of the gut microbiome, several investigators have examined the impact of antibiotic exposure early in life. For example, Shaw et al. [68] observed that the odds of antibiotic exposure in the first year of life was 2.9 times greater among children subsequently diagnosed with IBD than among matched controls. Furthermore, there was evidence of a dose response with a stronger association among those with more courses of antibiotics.

Most studies of antibiotic exposure have focused on short-term use of antibiotics for common infections. Margolis studied the impact of long-term antibiotic exposure among patients with acne. Tetracyclines are the most commonly used antibiotics for acne, and exposure to tetracyclines was associated with a 60% increased incidence of CD but no significant increased incidence of UC. Thus, the association between antibiotic exposure and subsequent risk of CD but not UC appears to be unrelated to the indication for antibiotic therapy. Furthermore, this CD-specific association differs from that observed with prior enteric infections, where infection appears to be associated with both CD and UC.

No specific class of antibiotics has been consistently associated with incident CD (table 1). For example, while both Margolis and Card observed associations with tetracyclines, Kronman did not. Rather, Kronman observed an association with broad-spectrum penicillins, as did Hviid, but only in the first 3 months af-

Fig. 1. Association of antibiotic exposure with subsequent IBD diagnosis.
ter treatment. In contrast, Card observed no association with broad-spectrum penicillins. These inconsistencies potentially provide insight into the underlying biology behind the observed associations with CD. It seems unlikely that the association is related to a specific immunologic property of the antibiotics. One possibility is that the antibiotics alter the gut microbiota in a way that is specific to different populations. For example, the patterns of association were more similar among studies of children [65, 66] and among the studies of older patients [64, 67]. This may reflect a relationship between the risk of developing IBD and the different indications for antibiotics, which may differ by age.

Other Environmental Exposures of Interest

Mode of Delivery

Much like the data on breast- and formula feeding, Cesarean versus vaginal delivery has been explored as a risk factor for developing IBD. The rate of C-section has increased substantially in recent decades and is known to impact the composition of the neonatal gut microbiota [69]. Vaginal delivery exposes the newborn to maternal vaginal and fecal flora whereas the gut of infants born via C-section is initially colonized with maternal skin flora [70]. Additional differences persist to at least 4 months of age [69]. Recently, Bager et al. [71] found that C-section was associated with a small but significantly increased incidence of IBD. In exploratory analyses, they observed that the increased risk appeared to decline with age and was particularly elevated if the C-section was performed emergently rather than electively. No clear difference in effect was apparent based on whether the mother or father had IBD.

Appendectomy

The finding that appendectomy, particularly for acute appendicitis, is associated with lower incidence rates of UC has been highly reproducible [72]. Data on the relation between appendectomy and CD are less consistent. For example, a recent meta-analysis demonstrated that the risk of CD was higher in the first 5 years following appendectomy but no effect was evident beyond 5 years [73]. Furthermore, there was substantial heterogeneity among the studies. The reason for the observed association between appendectomy and incident IBD is unclear. The appendix has been considered by some to be a reservoir for repopulating the gut microbiota if needed. Recent data suggest that the composition of the microbi-
ta within the appendix differs from that in stool. While Firmicutes are the dominant phylum, the appendix contains genera from several phyla that are not routinely found in stool [74]. *Fusobacterium nucleatum* abundance appeared to correlate with severity of inflammation of the appendix [74, 75]. Interestingly, *F. nucleatum* has also been found in patients with IBD [76, 77], thus potentially suggesting a link between appendectomy and UC via the gut microbiota. We have previously observed a borderline association of appendectomy with composition of the gut microbiota based on presence of different bacterial genera but not when accounting for relative abundance [44]. The complex biology of *F. nucleatum* and its interactions with other bacteria in the pathogenesis of gingivitis raises interesting yet complex hypotheses that could explain the strong association of appendectomy status and subsequent risk of developing IBD [78, 79]. Further studies specifically addressing this hypothesis are warranted.

**Sun Exposure and Vitamin D**

As noted previously, the incidence of IBD is higher in areas further from the equator. A potential explanation for this association is lower sun exposure leading to lower levels of vitamin D. Of course, vitamin D stores also depend on dietary intake. In France, people living in areas of high sun exposure have a lower incidence of CD, although the same association was not observed for UC [80]. Sun exposure is only one source of vitamin D. Another group of investigators estimated vitamin D stores based on self-reported dietary intake and sun exposure in the Nurses’ Health Study cohort. Those women with the predicted highest vitamin D levels had lower risks of both CD and UC, although this was not statistically significant for UC [81]. Dietary intake of vitamin D was associated with a reduced risk of UC (10% risk reduction for each 100 IU per day of intake) and a nonsignificant 7% reduction in CD incidence.

**Smoking**

Smoking is perhaps the best studied of the environmental factors, and these studies have yielded some of the most consistent results (table 2) [82–84]. Current smokers have an increased risk of developing new CD and have a worse outcome following surgery than people who never smoke. In contrast, their risk of developing UC is reduced, as long as they continue to smoke. However, former smokers have a higher incidence of UC than people who never smoked. This creates a paradox. If current smoking reduces the incidence of UC, why
would stopping smoking increase the incidence of UC? Surprisingly, little is
known about this or why the epidemiology of smoking differs so dramatically
between CD and UC. A simple hypothesis is that smoking predisposes to ileal
disease and protects against colonic disease [85, 86]. However, the association
with ileal CD is not uniform, and many patients with colonic CD are smokers.
Granuloma are characteristic of CD, yet among patients with CD, smokers may
be less likely to have granuloma than nonsmokers [87]. Interestingly, smoking
is less common among patients with sarcoidosis, another granulomatous disease
[88]. Some have suggested that smoking contributes to microthrombi which in
turn may be part of the pathogenesis of CD. However, the mechanism behind
the association is likely more complicated. Timing and mode of delivery of
smoke exposure could also be important. Use of smokeless tobacco does not ap-
pear to predispose to CD [88], nor does passive smoke in utero or as a child [82].

Some data suggest that the effect of smoking varies among ethnic groups and
by age. Lakatos et al. [89] recently demonstrated that the association of current
smoking with CD and UC risk may be greater in young adults than in children
or the elderly. Interestingly, the association of smoking with CD among Israelis
has not been consistent with most other populations [90, 91]. This has led some
to hypothesize that smoking may have less of an effect in a genetically high-risk
population, although the association has been observed in other high-risk popu-
lations such as Sweden. Furthermore, some of the countries with low smoking
rates, such as Canada and Sweden, also have the highest incidence of CD [3].

**Conclusions**

CD and UC are chronic and often debilitating diseases without a known cure.
Understanding the environmental factors associated with the onset of IBD can
provide insight into the underlying etiology of these diseases, and perhaps lead to
ways to prevent or at least alter the natural history. Given the relatively low inci-

<table>
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<th>Meta-analysis groups</th>
<th>CD Pooled OR</th>
<th>UC Pooled OR</th>
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<td>1.30 (0.97–1.76)</td>
<td>1.79 (1.37–2.34)</td>
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<td>1.10 (0.92–1.30)</td>
<td>1.01 (0.85–1.20)</td>
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<td>Prenatal exposure [82]</td>
<td>1.10 (0.67–1.80)</td>
<td>1.11 (0.63–1.97)</td>
</tr>
<tr>
<td>Postoperative smoking – clinical recurrence [84]</td>
<td>2.15 (1.42–3.27)</td>
<td>not applicable</td>
</tr>
<tr>
<td>Postoperative smoking – surgical recurrence [84]</td>
<td>2.30 (1.29–4.08)</td>
<td>not applicable</td>
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idence of disease, it is generally impractical to consider interventions targeted at the entire population with a goal of preventing disease onset. However, such interventions could be useful in high-risk populations. To that end, there is a need for better ability to identify high risk of populations using genetics and other biomarkers. Smoking, antibiotic use, and diet are potentially reversible risk factors for IBD. Recommendations to avoid smoking are appropriate for all people for numerous reasons. Antibiotic use should be limited to appropriate indications, a recommendation that too is appropriate for all populations. Detangling the relationship between diet, the gut microbiome and IBD raises the potential to reduce the incidence of IBD through dietary modification, an approach that might be considered among those at the highest risk (e.g. children of parents with IBD).

Although much progress has been made in describing the epidemiology of IBD, many questions remain unanswered. For example, why does the incidence of IBD increase with age but plateau after the 3rd or the 4th decade of life? If diet is associated with incidence of IBD, can the risk be altered by dietary modification? Why does smoking have opposite relationships with CD and UC? Are antibiotics directly causal or do the genetic risk factors for IBD increase the risk of enteric and other infections? Breakthroughs in sequencing techniques have allowed substantial advances in our understanding of the genetic and microbial epidemiology of IBD. Additional breakthroughs in related fields can be expected in the next decade. Because of the increasing complexity of the science, major breakthroughs will likely require close collaboration of population epidemiologists, nutritional epidemiologists, geneticists, microbiologists, immunologists, and mucosal biologists.

Disclosure Statement

Dr. Lewis has served as a consultant for Takeda, Rebiotix, Amgen, Millennium Pharmaceuticals, Prometheus, Lilly, Shire, AstraZeneca, Janssen Pharmaceuticals, Merck, MedImmune, and AbbVie. He has served on a Data and Safety Monitoring Board for clinical trials sponsored by Pfizer. He has received research support from Bayer, Shire, Centocor, Nestle, and Takeda.

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Abstract
Although the randomized controlled trial has become the standard for regulatory approval of new drugs and devices in inflammatory bowel disease (IBD), the components of effective trial design and implementation are still evolving. While induction and maintenance of remission are the ultimate goals in the treatment of IBD, the conduct of trials intended to measure these outcomes has varied substantially over time, as have the definitions of disease remission. Significant progress has been made in recent years towards understanding patient- and disease-related factors that are essential considerations in clinical trial design. However, questions remain regarding the best methods for assessing disease activity, and importantly for establishing trial end points that are clinically relevant and likely to affect meaningful long-term improvements in disease outcomes. This chapter will discuss the current ‘best methods’ in IBD trial design and introduce potential future concepts for improving the efficiency of clinical trials as well as their utility to inform clinical practice management.

Random assignment of humans to determine the efficacy and safety of new treatments is a relatively modern concept that has undergone continuous evolution over the past 70 years. Since the first controlled trial of hydrocortisone therapy in ulcerative colitis (UC) was performed by Sidney Truelove in 1955 [1], the randomized controlled trial (RCT) has become a standard for approval of new drugs and devices in inflammatory bowel disease (IBD). During this period, trial design has improved tremendously as a result of multiple methodological advances. However, the landscape continues to provide new challenges to clini-
cians who are engaged in trial design and implementation [2]. This chapter provides a selective overview of clinical trial design issues in IBD and speculates on future directions in this field.

**The Randomized Controlled Trial: A Gold Standard for Evidence-Based Medicine**

The modern clinical trial was founded upon statistical principles laid out by Pearson and Fisher in the early 1900s. Subsequently, Bradford Hill proposed the then radical concept of randomly assigning patients to therapy that culminated in performance of the British Medical Research Council trial of streptomycin therapy for tuberculosis [3]. This initial ‘big idea’ has culminated in a paradigm whereby, in the current era, virtually all interventions with an intent to improve human health undergo testing in RCTs. The explanation for this dramatic change from ‘experience-based’ to ‘evidence-based’ medicine lies in two fundamental strengths of the randomized design. First, randomization allows blinding of the treatment assignment and thereby minimizes the effects of bias on outcomes. A large body of empiric evidence has demonstrated that unblinded studies systematically overestimate the true magnitude of a treatment effect [4]. Second, randomization equally allocates confounding variables between the experimental and control groups and thus minimizes their effect on the estimate of the between-group differences. This characteristic is especially advantageous in the context of IBD given that our understanding of the determinants of treatment outcomes in these diseases is quite limited.

**Selected Issues in the Design of IBD Trials**

**Trial Structures**

Historically, the concepts of ‘induction of remission’ and ‘maintenance of remission’ have served as the models for studies submitted to regulatory authorities for approval of new drugs. However, this construct requires establishment of a specified time at which induction ends and maintenance begins. Since (1) the optimum time at which the pharmacodynamic effect of a new agent occurs is not usually known in early drug development and (2) a need exists to pre-specify one time to serve as the basis for statistical evaluation of the primary end point, it is easy to see how early-phase induction studies often fail to achieve significance. Furthermore, the concepts of active disease and remission are arti-
ficial if symptoms are used to define disease activity, since many patients who lack symptoms will have active disease on endoscopy. Following participation in an induction trial, patients will often undergo re-randomization (fig. 1, A) into a maintenance study [5]. Some controversy exists as to whether responders, in distinction to remitters, are the most appropriate population in which to evaluate long-term therapy. On one hand, it is obviously not possible to evaluate maintenance of remission if patients are not in remission at entry. Alternatively, continuing to treat responders mirrors clinical practice, the notion being that many responders will become remitters over time with continued therapy. An alternative maintenance design evaluates induction and maintenance in the same study without re-randomization. This ‘treat-right-through’ scheme (fig. 1, B) [6] avoids the problem of highly variable response rates that encumbers estimation of the sample size of the re-randomization design but has other specific disadvantages. First, because two end points are evaluated, multiplicity of statistical testing must be accounted for in the analysis of data. Second, since these trials typically are of at least a year in duration, patients assigned to placebo must both ‘survive’ the induction period and persist into the maintenance phase. If patients with severe disease are selected for inclusion, few placebo patients may be available for the comparison to be valid. Finally, an alternative maintenance design that is frequently encountered is open-label induction followed by re-randomization to either continued active treatment or placebo (fig. 1, C) [7, 8].

Fig. 1. Trial designs in IBD.
Although this design can be criticized for potentially overestimating the efficacy of therapy, since only responders who have tolerated induction therapy are included, it has the advantage of simplicity and is similar to how clinicians behave in clinical practice.

Recently, several drug development programs have, in an attempt to shorten timelines, used integrated induction and maintenance designs [5]. This strategy carries multiple doses forward into a maintenance trial that is populated with patients who have responded to induction therapy with the study drug. Following an interim analysis of the induction trial and identification of an optimum dose, additional induction studies are initiated and the final maintenance dose(s) is selected. This design requires greater initial investment but has the potential to accelerate decision-making in drug development programs.

**Patient Populations**

Trial participants should be well characterized. We have learned through experience that inclusion of patients who lack objective evidence of inflammation results in high placebo rates, which predisposes to failure of the study to identify a treatment effect. Consequently, for induction of remission trials in UC, a sigmoidoscopy that, at a minimum, demonstrates the presence of friability is required for inclusion. Central reading of video recordings increases the efficiency of trials in UC by ensuring that this minimum criterion is met [9]. In Crohn’s disease (CD) studies, this issue is even more important. Recent induction studies that have mandated either an elevated serum concentration of CRP, an elevated fecal calprotectin concentration, or a centrally read endoscopic video demonstrating an ulcer as inclusion criteria have consistently observed placebo induction rates of less than 10% [10, 11]. Furthermore, in CD trials exclusion of patients with obstructive symptoms based on the presence of fibrotic strictures is potentially important. However, this specification can be a difficult problem to reconcile since our experience with capsule endoscopy has demonstrated that these lesions are often not detected by conventional imaging modalities [12]. The development of magnetic resonance enterography (MRE) as an alternative means of assessing disease extent and activity may provide a technological solution to this problem [13]. However, standardization of techniques and access to MR scanners currently limit the widespread application of MRE in CD trials.

Participants in clinical trials are usually receiving multiple drug therapies at the time of screening. Some of these medications must be stopped since their toxicities overlap with the investigational drug. For example both tofacitinib, an
investigational Janus kinase inhibitor under evaluation in IBD, and azathioprine can cause bone marrow suppression and should not be coadministered. Topical aminosalicylates and corticosteroid may interfere with sigmoidoscopic examination in UC trials and must be discontinued. Prednisone therapy is a potent confounder in both diseases that must be controlled. Limitation of the maximum acceptable dose to 20 mg per day and maintaining this dose throughout induction are conventional procedures for achieving this objective. Another approach, which has not been widely used is to uniformly force taper corticosteroids during induction, which has the potential to accentuate differences between treatment groups [14, 15].

Finally, all participants in IBD trials should be carefully screened for *Clostridium difficile* toxin prior to randomization, since this infection is common in patients with a disease exacerbation [16].

**Choice of Outcome Measures**

Traditionally, clinical symptom-based measures have been primarily used as outcome measures for both UC and CD. The Mayo Clinic score, a composite instrument that includes both symptom-based items and endoscopic assessment, is the most frequently used outcome measure in UC trials [2]. Various definitions of the binary outcomes of remission and or response have been utilized. However, in UC a relatively good correlation exists between symptoms and endoscopy [17]. Accordingly, it might be attractive to use endoscopy exclusively as an end point since this outcome can, through the use of validated central reading by experts, be verified by regulatory authorities. With the development of more effective treatment for UC, the criterion for endoscopic remission is likely to become more rigorous, with a Mayo Clinic endoscopic score of 0 as the standard in distinction to the conventional 0 or 1 criterion. Finally, there is increased interest in the notion of including histopathologically defined disease activity in the definition of mucosal healing since this outcome has been shown to convey important prognostic information. Unfortunately, no validated index of microscopic disease activity has been developed for use in UC [2].

In CD, the situation is more complex since a very poor correlation exists between symptoms and endoscopic disease activity. The Crohn’s Disease Activity Index (CDAI), a composite outcome based on symptoms, signs and laboratory parameters, has served as the primary outcome measure for CD trials for almost 40 years [18]. A CDAI score of less than 150 points is considered remission; a minimum clinically important difference in the score is defined as 50 points, and
response is usually defined as a change from baseline of either 70 or 100 points. Multiple problems exist with the CDAI. Although the instrument is highly sensitive to change, it has relatively large variances and thus is statistically inefficient. This characteristic is problematic for early-phase IBD studies. The total CDAI score is highly weighted towards the three patient diary card items (pain, stool frequency, general well-being) that are subjective and are not in any way specific for CD. Finally, although the CDAI includes items relevant to patients, it is not a patient-reported outcome as defined by the US FDA [19]. For these reasons, there is considerable interest in developing an alternative outcome measure for use in RCTs. However, an obvious alternative is not apparent at this time. Likewise, no validated patient-reported outcome currently exists, although multiple initiatives are underway to develop these instruments. Although two endoscopic instruments are to assess disease activity (CDEIS, SES-CD) [20, 21], the clinical interpretation of changes in these scores is poorly understood, and their operating properties have not been fully defined.

MRE assessment of disease activity may be a way forward; however, the two scores that have been developed for this purpose have not been validated in large-scale studies [22, 23]. Finally, although biomarkers such as CRP and fecal calprotectin hold promise as low-cost surrogate outcomes, highly variable measurements are present among individuals, so these measures have limited value for use in early-phase studies in either disease. Based on these considerations, it is probable that the CDAI or a derivative thereof will continue to be the outcome of choice in CD trials until new instruments are identified and validated.

Selection of a Comparator

Although placebo is the traditional comparator of choice in IBD trials, this situation is changing. In some indications for example, induction therapy for active mild to moderate UC, use of a placebo comparator is ethically problematic, and a new agent must be added on to background 5-ASA therapy. Active comparator studies are more difficult to conduct than placebo-controlled studies since it is usually more difficult to detect a treatment effect. Furthermore, in some instances the standard of care may not be an especially attractive comparator. Although corticosteroids are effective agents for induction of remission in both UC and CD, their use is associated with a high incidence of adverse events. Therefore, placebo-controlled trials for this indication remain ethically justifiable. An alternative approach to the active comparator superiority study is the noninferiority design [24] which intends to determine with reasonable certitude that the investigational drug is ‘no worse than’ the standard therapy. If the new
agent is superior in other ways to the established drug (tolerability, convenience, cost), it could then be considered a superior choice. These studies are challenging to perform and interpret from multiple perspectives. First, they are inherently large and costly to perform. Consider that a standard two-arm superiority trial designed to detect a 15% difference in remission rates would require randomization of approximately 300 patients. In contrast, a noninferiority trial that would rigorously exclude a 7.5% (one half of the clinically important difference) clinically inferior difference for the investigational drug would require assessment of 1,200 patients. A second important issue for noninferiority designs is the fundamental assumption that the established comparator will perform in a similar manner as was observed in the original clinical trial that established its efficacy. This is a large assumption that can often erode the validity of these studies. Finally, the noninferiority design is highly susceptible to poor study quality, i.e. failure to show a difference is likely to occur if outcomes are inadequately assessed and adherence with the study protocol is poor. For this reason, the most appropriate type of analysis for these trials is ‘per protocol’ in distinction to ‘intent to treat’ since the former is more sensitive to differences in treatment effects should they exist.

Future Directions

Over the past two decades, IBD trials have become both more sophisticated and larger. Phase III programs routinely evaluate up to 1,000 patients. Trial size is an important factor because larger trials provide a more precise estimate of treatment efficacy and adverse events than smaller studies, generate important information about subgroups of patients and are generally more methodologically rigorous than smaller trials [25]. This trend is likely to continue in the future. However, changes are evolving in the types of questions asked by IBD RCTs. Consequently, new trial designs are likely to emerge that address ‘real world’ questions of relative clinical effectiveness that go beyond simple comparisons of two medical or surgical treatments. Conceptually, these types of studies are necessary to provide high-quality experimental data at the systems level that decision-makers need to make appropriate policy decisions. For example, wide differences are present in the timing and use of combination therapy for the treatment of CD. These differences are largely due to variances in physician and patient perception of the therapeutic index of these drugs. A common explanation offered by late adapters of early combination therapy is that the results generated by large-scale RCTs are not generalizable to usual clinical practice.
One approach to address this concern is to obtain ‘real world’ data by performance of cluster randomization trials [26]. In these studies, the unit or randomization is an intact social unit – in the context of medical trials often a practice or a hospital – in distinction to an individual patient. Cluster studies are particularly useful to evaluate complex interventions such as treatment algorithms since they are able to avoid contamination that would compromise the validity of a patient-level randomized trial. However, an important consideration in the planning and analysis of these studies is the level of statistical inference, which must reflect the design. If statistical inferences at the individual patient level are relevant, clustering must be taken into account in the analysis since individuals within a given cluster are more likely to behave like each other than individuals in another cluster, i.e. a high intra-cluster coefficient exists. Accounting for this phenomenon typically results in much higher sample sizes than randomization of individual patients and is therefore statistically inefficient. However, the logistical advantages of the design may overcome this consideration for specific applications. In contrast, for cluster level analyses such an adjustment is not necessary since the inference is relevant for all of the individuals in the cluster, not individual patients. This approach is highly appropriate when policy questions are being evaluated. For example, if a health care system wishes to compare the clinical and economic consequence of performing high-intensity screening for dysplasia in UC with a less intensive strategy, analysis at the level of the cluster is probably most appropriate since policy would have to be implemented systematically to adopt the best strategy.

Cluster randomization trials have become relatively common in medicine. Recent examples in gastroenterology include an evaluation of a hospital-based education program to improve outcomes in gastrointestinal bleeding and an RCT that assessed the efficacy of multiple strategies for colorectal cancer screening. Two cluster randomization trials have been initiated in CD. The REACT 1 study [Feagan, pers. commun.] conducted at community-based practices in Canada and Belgium assigned practices to implement an accelerated step-care algorithm for the management of CD that compared the early introduction of combined immunosuppression with usual care. This 2-year study that involves participation of approximately 2,000 patients has been completed, and the data will be presented later this year. Similarly, the US-based REACT 2 trial, which is comparing a strategy of intensification of treatment based on endoscopic disease assessment with usual symptom-based management, has recently been initiated [Sandborn, pers. commun.]. The results of these large-scale trials should provide important new information on the optimal strategies for patient management in CD.
Conclusions

RCTs in IBD have undergone continuous evolution since Truelove’s original experiment more than 50 years ago. The data generated by these studies have resulted in an evidence-based approach to therapy, better decision-making by gastroenterologists and improved outcomes for patients. It is highly likely that the next generation of trials will build upon this legacy of success and provide answers to the many unanswered questions that currently exist in the treatment of these chronic diseases.

Disclosure Statement

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He has also served as a member of the Speakers Bureau at Abbott/AbbVie, Jn/Janssen, Takeda, Warner-Chilcott, and UCB Pharma, and of the Scientific Advisory Board at Abbott/AbbVie, Amgen, Astra Zeneca, Avaxia Biologics Inc., Bristol-Myers Squibb, Celgene, Centocor Inc., Elan/Biogen, Ferring, Jn/Janssen, Merck, Novartis, Novonordisk, Pfizer, Prometheus Therapeutics and Diagnostics, Protagonist, Salix Pharma, Takeda, Teva, Tillotts Pharma AG, and UCB Pharma.

References

Pathogenesis of IBD


The Intestinal Microbiota in Inflammatory Bowel Diseases

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Abstract

Abundant clinical and experimental evidence supports a role for resident microbiota in Crohn’s disease and pouchitis, and probably in ulcerative colitis (UC). These disorders occur in areas of highest bacterial concentrations. Pouchitis and Crohn’s colitis respond to antibiotics, while pouchitis and UC can be treated with probiotics. Serologic markers recognizing intestinal bacteria and yeast are present in the majority of Crohn’s disease patients and may predict disease aggressiveness. Abnormal profiles of fecal and mucosally associated enteric bacteria (dysbiosis) occur in Crohn’s disease, UC, pouchitis and experimental enterocolitis, with a proliferation of aggressive species that promote experimental colitis and a corresponding decrease in protective bacterial subsets. Many of these protective bacteria produce short-chain fatty acids, including butyrate, that promote epithelial barrier function, inhibit effector immune responses and induce regulatory T cell subsets. Furthermore, certain Clostridia species stimulate regulatory T cells that can inhibit intestinal inflammation. Animal models of chronic, immune-mediated enterocolitis convincingly demonstrate that enteric resident bacteria stimulate effector immune cells in susceptible hosts and that a subset of enteric bacteria has particularly aggressive activities, with host and bacterial specificity. Recent studies suggest parallel and perhaps complementary roles for enteric viruses, which have only very recently been identified.

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Introduction

Humans exist with intestinal bacteria, fungi and viruses, collectively referred to as microbiota, that outnumber our own mammalian cells 10-fold [1–3]. These organisms are metabolically active and reciprocally interact with intestinal mu-
curs, epithelial cells, and the mucosal and systemic immune systems. These bacteria increase in both concentration and complexity in a spatial gradient from aerobic conditions of the stomach, duodenum and jejunum, where *Streptococcus*, *Lactobacillus* and oropharyngeal species predominate, to the anaerobic environment of the distal ileum and colon, where *Clostridium* and *Bacteroides* species far outnumber other bacterial groups. The aggregate microbial organisms in the intestine total 10–100 trillion. Greater than 85% of enteric bacteria belong to the *Firmicutes* and *Bacteroidetes* divisions, with only 5% or less *Proteobacteria*. Close host/microbial interactions have led to the concept that mammals are supraorganisms, i.e. a composite of mammalian and microbial cells, genomes and metabolomes. Evolution in a preexisting microbial environment helped shape the human genome. The majority of enteric bacteria cannot be cultivated by standard techniques. Recent breakthroughs in understanding the microbiota are a result of culture-independent sequencing analyses that take advantage of a unique property of the bacterial 16S ribosomal RNA gene structure, where species-specific hypervariable regions are flanked by highly conserved regions. PCR primers binding to the conserved regions are used to expand bacterial DNA, then rapidly evolving deep sequencing techniques are used to identify the hypervariable regions, and bacterial families are used to compare sequences with bioinformatic databases. Recent data support an integrated microbiome view in which bacterial function is more important than composition. As confirmed by metagenomic and metabolomic analyses, nutritional, metabolic and other key pathways are shared by overlapping bacterial species, such that the presence of certain bacterial groups can functionally compensate for the absence of other species, and different bacterial groups can synergistically interact. Key metabolic pathways of high relevance to mucosal homeostasis and inflammation include metabolism of nonabsorbed carbohydrates, primarily dietary fiber, that function as prebiotic substrates, to short-chain fatty acids (SCFAs; butyrate, propionate and acetate). These substances function as protective metabolites for colonic epithelial cells. In contrast, dietary and structural sulfites and sulfates are metabolized by other resident bacterial species to produce hydrogen sulfide, which injures epithelial cells.

Bacterial Regulation of Mucosal Homeostasis versus Inflammatory Bowel Diseases

Host genetic and environmental factors help determine the composition of intestinal microbiota (fig. 1) [4, 5]. Environmental contributions include colonization by maternal microbiota, with differences in the microbiome of infants who
are delivered vaginally versus Cesarean sections, diet, including breast versus formula feeding, infections and antibiotic exposure (see below). Microbiota community structure in normal individuals differs from that in patients with inflammatory bowel diseases (IBD). Normal resident bacterial communities are characterized by high diversity, with a predominance of Firmicutes and Bacteroidetes and low concentrations of Enterobacteriaceae. In contrast, the abnormal composition (dysbiosis) of patients with IBD is characterized by decreased bacterial diversity, expansion of Enterobacteriaceae, particularly γ-Proteobacteria such as *Escherichia coli*, with parallel contraction of certain *Clostridium* subsets, particularly clades IV and XIVa, that include *Faecalibacterium prausnitzii*. Many of the bacterial species that are decreased in IBD exhibit protective im-

**Fig. 1.** Conceptual framework for microbial regulation of mucosal homeostasis vs. IBD. Genetic and environmental factors determine intestinal bacterial community structure, which differs in normal individuals and patients with IBD. These bacterial populations and their metabolites reciprocally interact with genetically regulated intestinal mucosal and immune pathways to mediate mucosal homeostasis vs. chronic inflammation. Reproduced with permission from Sartor [4].
mucologic, mucosal barrier, and bacteriologic functions that mediate mucosal homeostasis (fig. 1; table 1). High bacterial diversity is responsible for colonization resistance against pathogens, best illustrated by the loss of protection against *Clostridium difficile* colonization following broad-spectrum antibiotic exposure. Resident enteric bacteria in general, and *Clostridium* and *Bacteroides* species subsets in particular, selectively induce regulatory T cells that secrete immunoregulatory cytokines, primarily TGFβ and IL-10 [6, 7]. In addition, *Clostridium* subsets also produce SCFAs that provide essential nutrients for colonic epithelial cells and also induce regulatory T cells [8]. Many bacterial groups activate innate immune defenses that are mediated by bacterial recognition receptors such as Toll-like receptors and NOD2 (through muramyl dipeptide, MDP) as well as the NFκB signaling pathway. Paneth cells are a key innate pathway that regulates mucosal bacterial populations and luminal/mucosal concentrations through production and secretion of antimicrobial peptides, including α-defensins. The net host response to normal resident microbiota by intestinal epithelial and immune cells is mucosal homeostasis and immunologic tolerance (fig. 1).

In contrast, abnormal dysbiotic microbiota activates dysregulated effector TH1 and TH17 cells in genetically susceptible hosts that mediate chronic intestinal inflammation that includes Crohn’s disease, ulcerative colitis (UC) and pouchitis in the absence of appropriate host regulatory responses (fig. 1) [2, 5]. We propose that Crohn’s disease is due to overly aggressive TH1/TH17 cell responses to a subset of luminal bacteria in the background of innate immune dysfunction. Susceptibility is determined by defects in genes that encode either immune responses, mucosal barrier function or enteric bacterial clearance. Finally, the onset and reactivation of disease are triggered by environmental stimuli that transiently break the mucosal barrier and initiate inflammation. This complicated hypothesis depends on the interaction of genetic, environmental, microbial and immune factors (fig. 2), which will be discussed in the following sections.

### Table 1. Protective effects of the normal microbiome

<table>
<thead>
<tr>
<th>Property</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonization resistance</td>
<td>prevent infection – <em>C. difficile</em></td>
</tr>
<tr>
<td>Educate the immune response</td>
<td>mucosal homeostasis – IL-10, inducible Treg</td>
</tr>
<tr>
<td>Host nutrition</td>
<td>SCFA metabolism by colonic epithelial cells, vitamin K synthesis</td>
</tr>
<tr>
<td>Activate innate immune defenses</td>
<td>TLR/NF-κB in epithelial cells, stimulation of antimicrobial peptides</td>
</tr>
<tr>
<td>Neuronal development</td>
<td>mediate pain threshold, CNS responses</td>
</tr>
</tbody>
</table>
Genetic Susceptibility

One hundred and sixty-three susceptibility loci have been associated with Crohn’s disease and/or UC [9]. Functional abnormalities can be broadly characterized as defective bacterial killing, barrier function and immunoregulation. Several innate genes in the autophagy (ATG16L1 and IRGM), microbial signaling (NOD2) and endoplasmic stress response (XBP-1) pathways are associated with Crohn’s disease. These genes regulate bacterial killing through either intracellular mechanisms (abnormal autophagosome formation or function) or by mediating defective Paneth cell function. These defects most likely lead to accumulation of mucosally associated bacteria and persistence of intracellular bacteria within phagocytic (possibly epithelial) cells (fig. 1). Gulati et al. [10] demonstrated by fecal transplant to germ-free (GF) mice that host genotype (mouse strain) determines composition of intestinal bacteria, but that deletion of the NOD2 gene in mice did not alter luminal microbiota compared with wild-type mice [11]. The latter result is
in contrast with human studies that implicate NOD2 in the regulation of mucosally associated microbiota in the ileum of Crohn’s disease patients [12].

**Environmental Triggers**

Multiple environmental agents have been implicated in the triggering of IBD or experimental enterocolitis. These include infections, nonsteroidal anti-inflammatory drugs (NSAIDs), diet, smoking, stress, and antibiotics (fig. 2). Others have covered many of these topics in detail. Highly relevant to the concept of high-diversity microbial communities protecting the mucosa, antibiotic use in childhood is a risk factor for developing Crohn’s disease, but not UC [13]. This large epidemiologic study in Denmark showed a relative risk of 3.41 for developing Crohn’s disease following childhood antibiotic exposure, with both time and dose response. It is unclear whether this represents a causal relationship or use of antibiotics for IBD symptoms prior to diagnosis. As discussed by Gary Wu on this issue, diet can profoundly affect bacterial community structure, metabolism and gene expression [14, 15]. Dietary iron and saturated fat potentiate experimental enterocolitis and induce a proliferation of proinflammatory bacterial species [16, 17]. Our preliminary data show similar effects with dietary sucrose and fructose.

**Intestinal Microbiota**

Enteric bacteria provide a constant stimulus that drives innate and adaptive effector immune responses that cause tissue injury. There is abundant, mostly correlative, clinical evidence that microbiota are involved in the pathogenesis of IBD, particularly Crohn’s disease and pouchitis (table 2) [2]. As mentioned earlier, bacterial concentrations are highest in the distal ileum and colon, and mu-

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**Table 2. Clinical evidence that enteric bacteria, viruses or fungi induce Crohn’s disease**

<table>
<thead>
<tr>
<th>Disease located in areas of highest bacterial populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased mucosal association and translocation</td>
</tr>
<tr>
<td>Abnormal composition commensals – dysbiosis</td>
</tr>
<tr>
<td>Certain Crohn’s disease-associated genes alter gut microbiota and bacterial killing</td>
</tr>
<tr>
<td>Infections can induce flares of IBD (C. difficile toxin, CMV)</td>
</tr>
<tr>
<td>Fecal stream diversion prevents disease relapse; disease recurs upon restoration of fecal flow</td>
</tr>
<tr>
<td>Manipulating bacterial populations treats certain subsets</td>
</tr>
<tr>
<td>Microbe-specific serologic and T cell responses</td>
</tr>
</tbody>
</table>
cosally-associated bacteria are dramatically increased in active IBD compared with the noninflamed intestine [18]. Frank et al. [19] were among the first to conclusively demonstrate that a subset of patients with Crohn’s disease and UC have abnormal mucosally associated bacterial community structures, with characteristic dysbiosis characterized by decreased *Clostridium* groups IV and XIVa, *Bacteroides* species and a compensatory proliferation of γ-proteobacteria. These abnormalities extend from the ileum to the rectum [20]. An important emerging concept in IBD pathogenesis is that of functional changes of the dysbiotic microbiome [5, 21]. Of note, many of the depleted species, including *F. prausnitzii*, efficiently produce SCFA [19]. As described earlier, SCFAs have important protective effects on mucosal barrier function and in immunosuppression. *F. prausnitzii* is of particular interest because low mucosal concentrations in resected ileal tissues predict postoperative Crohn’s disease recurrence, and this organism has immunoprotective functions in vitro and in several murine colitis models. An important subset of the γ-proteobacteria that proliferates in Crohn’s disease is *E. coli*. Functionally altered *E. coli* with a phenotype of increased epithelial adherence, epithelial invasion and persistence within macrophages, designated adherent/invasive *E. coli* are found in higher concentrations in the ileum of the subset of Crohn’s disease patients (approximately 30 vs. about 5% of controls) [22]. In addition, the majority of Crohn’s disease patients have serologic (antibody) reactivity to enteric bacterial or fungal species [23], and aggregate antimicrobial antibody concentrations have been reported to correlate with Crohn’s disease aggressiveness and complications [24].

Rodent models provide compelling evidence of a primary role of resident enteric bacteria in the pathogenesis of chronic, immune-mediated intestinal inflammation [2]. We and others have demonstrated that in the absence of intestinal bacteria, GF mice on multiple genetically susceptible backgrounds such as IL-10-deficient mice have no immune activation and no clinical, visible or histologic evidence of colitis [2, 25]. However, GF IL-10−/− mice colonized with specific pathogen-free enteric bacteria develop bacterial antigen-specific TH1 and TH17 cell activation and grossly intact colitis within one week of colonization. Exceptions to this rule are SAMP1/Yit mice, which have attenuated ileitis in bacteria-free conditions, and dextran sodium sulfate-treated mice, which exhibit potentiated colitis in GF conditions [26]. Gnotobiotic rodent studies indicate differential ability of various intestinal residential bacterial species to induce colitis. For example, HLA B27 transgenic mice have no colitis when GF, aggressive bacterial antigen-mediated colitis when colonized with complex resident intestinal bacteria, moderate colitis when monoassociated with *Bacteroides vulgatus*, but no inflammation when colonized with a resident *E. coli* strain and diminished colitis when *Lactobacillus rhamnosus* GG are mixed with complex resident microbiota
These studies demonstrate that all resident bacteria are not equal – some are aggressive, some are neutral, and yet others are protective. Parallel studies demonstrate host-specific responses since opposite results are seen in IL-10−/− mice where *B. vulgatus* induces no inflammation, while the same *E. coli* strain induces cecal-dominant colitis and *Enterococcus faecalis* causes distal colitis [28]. These gnotobiotic IL-10−/− studies are examples of how different bacterial species can induce different phenotypes of disease in the same host [28, 29].

Recent work has demonstrated that different strains of *E. coli* have functional differences that impact disease pathogenesis and have identified some of the virulence genes that mediate these functional properties. Various *E. coli* strains have differential abilities to induce chronic, bacterial antigen-specific enterocolitis in monoassociated IL-10−/− mice [30, 31]. Induction of colitis correlates with the ability of *E. coli* strains to adhere to the mucosa, persist within macrophages and translocate across the mucosal barrier. Mechanisms of these pathogenic functions are being elucidated, with an example of a relevant virulence gene being the chitin-binding domain of chiA, which binds to N-glycosylated chitinase 3-like-1 on intestinal epithelial cells [32]. Multiple other bacterial species, including Enterococci and *Bacteroides* exhibit fundamental variations that affect protective versus pathogenic properties.

### Future Directions

The relative balance of beneficial versus detrimental bacteria (table 3) strongly contributes to intestinal homeostasis versus inflammation, along with genetic and environmental factors. It is evident from the heterogeneous results in human disease and observations in animal models that this balance is unique in each individual and each person responds differently to various bacterial spe-

<table>
<thead>
<tr>
<th>Injurious (proinflammatory)</th>
<th>Protective (probiotic)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. vulgatus, B. thetaiotaomicron</em></td>
<td><em>Lactobacillus sp.</em></td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td><em>Bifidobacterium sp.</em></td>
</tr>
<tr>
<td><em>E. coli</em> – adherent/invasive</td>
<td><em>F. prausnitzii</em></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td><em>Roseburia species</em></td>
</tr>
<tr>
<td><em>Ruminococcus gnavus</em></td>
<td><em>Nonpathogenic E. coli</em></td>
</tr>
<tr>
<td>Segmented filamentous bacterium</td>
<td><em>Saccharomyces boulardii</em></td>
</tr>
<tr>
<td><em>Fusobacterium varium</em></td>
<td><em>B. thetaiotaomicron</em></td>
</tr>
<tr>
<td>Intestinal <em>Helicobacter</em> species</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Balance of beneficial vs. detrimental intestinal bacteria in IBD and mucosal homeostasis
cies. Functional changes of enteric microbiota must be considered in parallel with compositional changes. Identification of functionally relevant bacterial genes, virulence factors and metabolic profiles represents important areas of future investigation to define protective and detrimental activities of resident bacteria. Very recent studies of enteric viruses and fungi promise to expand our understanding of the host/microbe interactions that mediate this chronic dysregulated immune activation that causes IBD (fig. 1).

This dysregulated balance of protective and proinflammatory resident intestinal microbial components offers an attractive target for novel approaches to more effectively and physiologically treat IBD, with an effort to restore a more homeostatic profile of bacteria, fungi and viruses. In parallel, intestinal microbiota offers considerable potential for diagnostic tests that can identify clinically relevant subsets of patients with more predictable responses to therapeutic manipulation and clinical outcomes. Current therapeutic approaches that use standard antibiotics and probiotics have not been as successful as one would anticipate, given the strong influence of resident bacteria in IBD pathogenesis. Applying customized approaches to correcting an individual’s unique bacterial profile and augmenting levels and function of endogenous, resident protective bacterial species rather than standard probiotics that do not persist in the human intestine are areas of high therapeutic investigative priorities (table 4). Incorporating these novel microbial approaches should revolutionize treatment of IBD in parallel with the dramatic advances in immunotherapy provided by biologic agents.

**Disclosure Statement**

Dr. Sartor is a member of the Advisory Board of the North American Probiotic Council, sponsored by Danone and Yakult. He has been on nutrition Advisory boards from General Mills, Abbott and GSK and Advisory panels for Salix, Vertex and Merck.
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Abstract
Inflammatory bowel disease (IBD) is an abnormal inflammatory response within the gut to a trigger that has yet to be identified. The family history in many patients, especially those with Crohn’s disease, suggests a genetic predisposition. It has been hypothesized that the abnormal inflammatory response is due in part to genetic alterations in the normal homeostatic processes that regulate host interactions with the normal gut microbes. Genetic studies have identified increasing numbers of genetic risk factors that involve a diverse series of pathways such as receptors of innate immune response, defects in epithelial barrier function, immune- and cytokine-related genes and genes involved in autophagy. Studies further suggest that abnormal immune responses in IBD patients are directed against the intestinal microbiota, with activation of both innate and adaptive immune responses. Indeed, studies have shown bacterial-derived antigen as drivers of T cell immune responses. More recently, Th17, regulatory T cells and unconventional innate-like T cells have been implicated in the induction and regulation of intestinal inflammation. The seminal discoveries of pathogen recognition receptors including Toll-like receptors and nucleotide-binding oligomerization domain receptors have changed our understanding of how immune cells respond to microbes and the role this may play in IBD pathogenesis. Understanding these mechanism will lead to new strategies in the treatment and prevention of IBD.

Introduction
Inflammatory bowel disease (IBD) is thought to be due to an abnormal inflammatory response within the gut to a trigger that has yet to be identified. The strong family history in many patients, especially those with Crohn’s dis-
ease (CD), suggests a genetic predisposition. It has been hypothesized that the abnormal inflammatory response is due in part to genetic alterations in the normal homeostatic processes at play in the gut mucosa that normally serve to ensure a symbiotic relationship between the host and the normal gut microbes. Genetic studies in patient cohorts have identified increasing numbers of genetic risk factors that involve a diverse series of molecular and cellular pathways that may be altered in these individuals. These pathways include receptors of innate immune response, defects in epithelial barrier function, immune- and cytokine-related genes and genes involved in autophagy – a cellular pathway not previously considered in studies of IBD. Animal and human studies further suggest that abnormal immune responses in IBD patients are directed against the intestinal microbiota, with evidence of a loss of the normal homeostatic mechanisms leading to activation of both innate and adaptive immune responses. The adaptive immune response has long been the focus of study in understanding the pathogenesis of IBD. Th1 and Th2 cell subsets have been identified as mediators of immune inflammation in early studies and antigen specific responses key to their activation. Indeed, studies have shown bacterial-derived antigens as initiators and drivers of T cell immune responses. More recently, Th17, regulatory T cells (Treg) and unconventional innate-like T cells have been identified as important in the induction and regulation of intestinal inflammation, and efforts are in place to define their role in IBD. The seminal discoveries of pathogen recognition receptors including Toll-like receptors and nucleotide-binding oligomerization domain receptors have changed our understanding of how immune cells without antigen-specific receptors respond to microbes and secrete inflammatory cytokines in the intestine.

The Genetics of IBD

Epidemiological Evidence for a Genetic Contribution to IBD Pathogenesis

Epidemiological studies provide compelling evidence that genetic factors contribute to the pathogenesis of IBD. First-degree relatives of patients with IBD have a 3–20 times greater likelihood of developing the disease than the general population [1–3]. Siblings of CD patients have a relative risk of developing CD up to 35 times background population [3]. Finally, twin studies show that the heritability of IBD is high with CD and ulcerative colitis (UC) monozygotic-dizygotic concordance rates of 30 versus 4 and 16 versus 4%, respectively [4–6].
Progress in Characterizing the Human Genome

The progress in understanding the genetic basis of IBD has been possible by rapid changes in technology of genetic sequencing and by large-scale multi-centered collaborative studies of a very heterogeneous and polygenic disorder. The ‘completion’ of sequencing of the human genome in 2001 set the stage [7, 8]. Early drafts of the human genome showed previously unrecognized variability, with regions characterized by high gene density and others more sparsely populated. Further, the number of protein-coding genes (30–40,000) was much lower than expected and more complex than those described in other organisms [7]. Projects such as the HapMap and 1,000 Genomes Project, now provide accurate haplotype information on human DNA polymorphisms in multiple populations [9, 10]. The identification of millions of single nucleotide polymorphisms (SNPs), allowed for quantification of genetic variability across populations [11]. Despite these advances in understanding the human genome, the role of genetic factors in diseases such as diabetes, obesity and IBD demonstrates complex heritability, with numerous loci modulating disease risk. In addition, these diseases show incomplete penetrance, polygenicity and differential epigenetic regulation. Genetic heterogeneity, where multiple genes may cause similar phenotypes, and copy number variation, could lead to alterations in phenotype, further complicating genetic analyses [12, 13]. In spite of these complexities, major advances have been made in the genetic study of IBD.

Techniques for Analyzing the Human Genome

Early genetic studies used existing knowledge of either a disease or basic cellular process to identify candidate genes and then attempted to determine whether alterations to that gene were associated with disease pathogenesis. When candidate gene approach was not possible, positional cloning and linkage analysis were developed to identify loci involved in disease [12]. Recent studies more commonly use SNP markers, with substitutions (nucleotide switch e.g. from A to G) as the most common form of polymorphism measured in genotyping studies, and insertions and deletions less common. These studies assume that there are linkages between such markers and disease-specific polymorphisms [12]. Identifying transmission disequilibrium defined how often a specific marker allele is passed from a heterozygote parent to an affected offspring [14]. These approaches are both cost and labor intensive, and require a large number of families to provide statistical power. Current approaches to identifying polymorphisms or loci of interest involve the use of
case-control study designs or genome-wide association studies (GWAS), with large cohorts of cases and controls in conjunction with high-throughput technologies [15].

**SNP Genotyping Platforms and Analysis**

Several genotyping platforms have been developed which allow the high-throughput identification of loci of interest for genetic association studies. These can be used for both candidate SNP-based analyses and larger-scale GWAS. SNP markers are selected from HapMap datasets and must be detectable in a sufficient proportion of the population (detection in ≥1% of the population) to be measured in experiments [9] or the 1,000 Genomes Project [16]. Current GWA chips can provide information on up to 4.5 million SNP markers (Illumina), thus rapidly providing a huge amount of information.

**Key IBD Genetic Studies**

Major histocompatibility complex class II alleles were among the first candidate genes tested and shown to be associated with IBD [17]. A significant breakthrough utilizing linkage mapping studies was the discovery in 1996 of the IBD 1 locus, a pericentromeric region on chromosome 16 as a susceptibility locus for CD [18, 19]. Hugot et al. [20] further investigated the IBD 1 susceptibility locus for CD which had been mapped to chromosome 16. Using a positional cloning strategy, based on linkage analysis followed by linkage disequilibrium mapping, a frameshift variant and two missense variants of *NOD2* were identified as being associated with CD [20, 21]. The discovery of *NOD2* as an important genetic risk was a major advance in understanding CD pathophysiology as it highlighted the importance of innate immunity in CD.

The first GWAS in IBD was performed in 2005 with a modest-sized Japanese CD case-control study and a genome-wide panel of SNPs of relatively limited density. This study mapped the region containing tumor necrosis factor superfamily ligand member 15 as conferring the highest risk for CD among Pacific-Asian populations [22]. More recent GWAS in North American and European Caucasian populations have utilized larger case and control populations along with several hundred thousand SNP genotyping platforms [23–25]. Meta-analyses of these datasets have now identified loci with even smaller effects sizes, increasing to 163 the number of described IBD-associated loci [26–28].
Further important insight into IBD pathogenesis came from a whole-genome linkage study of consanguineous pedigrees with severe, infantile-onset, autosomal recessive mendelian CD, followed by sequencing candidate genes in these regions. By this approach, loss-of-function mutations in the interleukin (IL)-10 receptor subunit genes IL10RA and IL10RB and later the IL-10 cytokine were identified as causes of CD or CD-like disease. IL-10 and its receptors primarily function to downregulate inflammation [29].

It is beyond the scope of this article to review all published GWAS data; however, several important studies are highlighted. In 2006, a GWAS performed in Caucasian cases with ileal CD and controls found a highly significant association between CD and the IL23R gene on chromosome 1p31, a gene that encodes a subunit of the receptor for the proinflammatory cytokine IL-23. An uncommon coding variant (rs11209026) was shown to confer a strong protective effect against CD, and additional noncoding IL23R variants are independently associated [23].

In addition, a strong association was identified in the genomic region encoding ATG16L1, a component of a large protein complex essential for the process of autophagy. This was the first identification of an association between autophagy and CD pathogenesis. Autophagy describes the process whereby targeted cytoplasmic constituents are isolated from the rest of the cell within autophagosomes, which are then fused with lysosomes and degraded. This pathway is also recognized as an immune mechanism due to its role in the destruction of intracellular pathogens. The Wellcome Trust Case Control Consortium (WTCCC) performed a GWAS in the British population examining approximately 2,000 individuals for each of 7 major diseases and a shared set of approximately 3,000 controls. Case-control comparisons for CD identified 9 independent association signals for CD. Association signals were found at a number of previously identified loci including NOD2 and IL23R providing replication of these previous findings [30]. A further GWAS utilizing the WTCCC cohort identified an association between IRGM, another gene in the autophagy pathway and CD [25].

To identify loci that have even smaller effects on IBD risk, large datasets from individual GWAS have been combined in meta-analyses. A meta-analysis published in 2010 used data from 6 index CD GWAS providing a discovery cohort of more than 6,000 CD cases and 15,000 controls. Approximately 900,000 SNPs were tested for CD associations. This study identified 30 new CD susceptibility loci which in combination with previously confirmed loci increased to 71 the number of loci with genome-wide significance associated with CD [26]. Similarly, a meta-analysis including 6 UC GWAS with a large sample size (e.g. discovery cohort of 6,333 UC cases and 15,056 controls) identified 29 additional
UC risk loci, increasing the number of UC-associated loci to 47 and the total number of confirmed IBD risk loci to 99 [27].

The recently published International IBD Genetics Consortium Immunochip Study is the most comprehensive GWAS in IBD to date, compiling data from 7 CD and 8 UC GWAS in Caucasian cohorts with replication of these GWAS signals using independent CD, UC and healthy control cohorts and genotyping with the Illumina Immunochip (Illumina Inc., San Diego, Calif., USA). The Immunochip contains more than 200,000 autoimmunity disease-associated SNPs. The Immunochip Study evaluated a combined sample size total of more than 75,000 subjects with greater than 20,000 CD, 15,000 UC and 25,000 healthy controls [28]. As a result, 71 new IBD associations were discovered, resulting in a total of 163 IBD-associated loci, which meet genome-wide significance thresholds.

Insights into IBD Biology Derived from Genetic Studies

Rare cases of early-onset IBD may be single-gene, mendelian disorders, e.g. mutations in IL10RA or IL10RB; however, the genetic risk in the majority of IBD patients is mediated by relatively common alleles (allele frequencies greater than 5%) with modest or low effects sizes (odds ratios less than 1.5). A small number of lower frequency risk alleles with larger effects on IBD susceptibility have also been identified, including NOD2 and IL23R variants; however, none are sufficient alone to cause IBD. These findings suggest that most IBD cases are multifactorial in etiology, reflecting an interaction of multiple genetic risk alleles, microbial, immune and environmental factors [31].

It is important to point out that IBD genetic risk loci are not specific for IBD alone in that meta-analyses of GWAS and the recent Immunochip Study showed that some IBD risk loci are also associated with other immune-mediated disorders (66 with other immune-mediated diseases) [28, 32].

Challenges for Genetics

Of the estimated heritability of IBD derived from twin and family studies in IBD, less than 30% is explained by genetic variants discovered in GWAS [31]. This finding may be due to the possibility that heritability assessments have overestimated the heritability of IBD. There are however a number of other potential explanations for this ‘missing heritability’ which need to be explored in future studies. GWAS are not optimally designed to discover rare variants with large
effect sizes, and further linkage studies complemented by candidate gene approaches may uncover some of these variants. In addition, the issues of copy number variants, gene-gene interactions, gene-microbiota interactions and epigenetic phenomena may each contribute directly to or modulate the genetic risk of IBD.

Most of the identified 163 IBD-associated SNPs are in linkage with other proximal SNPs resulting in the implication of multiple genes per locus. Potentially, any SNP in a haplotype block may be a true causative polymorphism. Only a few loci thus far have been shown to be functional, i.e. an IBD-associated SNP can be implicated in the dysfunction of a particular gene. Determining which IBD-associated SNPs in coding regions result in functional consequences and which noncoding SNP variants regulate gene expression will be important future endeavors [32]. As knowledge of IBD genetics becomes further refined, follow-up studies to define the association of IBD-associated variants and disease behavior, risk of complications and response to medical therapy are required before genetic discovery can be translated into routine clinical practice.

**Immune Basis of IBD**

**Innate Immunity in IBD**

The innate immune response is an evolutionary conserved antigen nonspecific system of defense against microorganisms. Innate immune cells such as epithelial cells, stromal cells, dendritic cells (DCs), macrophages and innate lymphoid cells (ILCs) express pattern recognition receptors that can sense specific pathogen-associated molecular patterns expressed on microorganisms, and initiate the development of a rapid inflammatory response characterized by the secretion of cytokines and chemokines and the recruitment of immune cells. Innate myeloid cells can also activate the inflammasome, a multiprotein complex promoting the maturation of the inflammatory cytokines IL-1β and IL-18. In addition, DCs are antigen-presenting cells responsible for T cell activation, and RORγt+ ILCs have been shown recently to present antigen and regulate CD4+ T cell response to commensal bacteria [33].

**Epithelial Barrier Function**

The epithelial barrier provides anatomical containment of the microbiota and ensures a tolerant response to commensal microorganisms. Epithelial cells can sense intestinal microbiota through pattern recognition receptors and trigger
the first immune signals. In the small intestine, epithelial cells form a barrier covered by a discontinuous mucous layer secreted by goblet cells. Paneth cells, granule-containing specialized cells found in the epithelial crypts of the small intestine, secrete antimicrobial peptides with a bactericidal effect. In the large intestine, epithelial cells are covered by a thick, continuous, double mucus layer, which insulates the epithelium from the high microbial load. Mutations in genes involved in tight-junction structure or Paneth cell function have been described in IBD [34]. Animal studies have shown that a defective barrier can be associated with an increase in mucosal immune activation leading to an inflammatory environment in the gut [reviewed in reference 34].

**Autophagy**

Autophagy is a conserved pathway induced by cellular starvation, stress or infection. This process involves the formation of double membrane vesicles that surround and degrade cytoplasmic material, organelles and microbes that have invaded the cell. CD is associated with mutations in \( ATG16L1 \), \( IRGM \) and \( NOD2 \), which are all involved in autophagy; however, it remains unclear how alterations in this cellular process lead to a defective bacterial handling or antigen presentation by DCs and activation of mucosal inflammation [35, 36].

**Innate Lymphoid Cells and Innate-Like T Cells**

ILCs are emerging as fundamental effectors of innate immunity and tissue remodeling. Several subpopulations of ILCs have been identified and the heterogeneity within these populations continues to be an issue for discovery. The definitions for the different subsets are based on the profiling of their cytokine-producing ability and specific transcription factors they express, along with differences in cell surface molecules [reviewed in reference 37]. Although for some, their cytokine production profiles are similar to those of helper T cell subsets, they lack antigen-specific T cell receptors. How and if these ILCs are involved in the induction or perpetuation of IBD is still unknown. In the absence of adaptive immunity, these cells can trigger innate colitis in \( Rag1^{-/-} \) mice infected with \( Helicobacter hepaticus \) [38]. More recently, human studies have shown that ILCs secreting IL-17 [39] and IFN-\( \gamma \) [40] accumulate in the mucosa of CD patients indicating that these cells may play an important role in IBD.

Unconventional innate-like T cells include \( \gamma \delta \) T cells, invariant natural killer T cells and mucosal-associated invariant T cells. These cells are highly represented in mucosal sites, and they respond to IL-23 stimulation. How polymorphisms in the \( IL23R \) gene affect the function of these cells in IBD is still unknown.
Adaptive Immunity in IBD

Adaptive immunity involves antigen-specific responses mediated by T and B cells. These lymphoid populations produce and express antigen-specific receptors (T cell receptors and immunoglobulin, respectively). The ability to mount an antigen-specific response requires that these cells see and respond to foreign antigen and undergo antigen-induced expansion. This process takes time, and so adaptive immunity is seen as the induction of an immune response that is in place to protect against reexposure to a pathogen. The process of T and B cell education and elimination of self-reactive lymphocytes is an important mechanism in order to maintain a healthy and appropriate immune response. Much of this regulation involves lymphocytes that can turn off immune and inflammatory responses.

CD4+ T cell subsets are essential mediators of immune homeostasis and inflammation. Using a murine T cell transfer model, Powrie et al. [41] showed that transfer of naïve CD4+ T cell subsets to lymphopenic mice leads to the development of colitis. This animal model also led to the formal demonstration that colitis development requires antigen-specific T cell activation, while its prevention is dependent on Treg cell function.

Th1 and Th17 Cells

Human studies have described an increase in Th1-Th17 effector T cell activity in CD and Th2 effector T cells in UC, although this Th1-Th17/Th2 paradigm is being reconsidered [42, 43]. Intestinal Th1 cells respond to intracellular bacteria and viruses and develop in response to IL-12 secreted by antigen-presenting cells. Th1 cells express the transcription factor T-bet and secrete IFN-γ. In the murine T cell transfer model, treatment with anti-IFN-γ antibody can prevent the colitis induced by naive CD4+CD45RBhigh T cells [41]. However, anti-IFNγ antibody is ineffective in the treatment of humans with active CD [44].

Intestinal Th17 cells respond to extracellular pathogens and develop in response of TGF-β, IL-6 and IL-23. In IBD, IL23-induced Th17 cells are an important mediator of mucosal inflammation. Th17 cells express the transcription factor RORyt, and their function is mediated by different cytokines including IL-17A, IL-17F, IL-21, IL-22 and GM-CSF. Experimental studies have shown that IL-17 expression can induce inflammation and mucosal damage or intestinal protection. In the T cell transfer colitis, transfer of RORγ-null T cells failed to increase mucosal IL-17 cytokine levels and did not induce colitis [45], while in a Citrobacter rodentium infection model, IL-17A and IL-17F exert protective effects [46]. In the DSS model of colitis, IL-17F deficiency resulted in reduced colitis, whereas IL-17 knockout mice developed more severe disease, thus dem-
onstrating opposing activities of IL-17F and IL-17A [47]. The complexity of IL-17 biology has also been highlighted in clinical studies showing that inhibition of IL-17 can exacerbate CD [48].

IL-21 is an important factor for Th17 differentiation. However, in active human IBD, the majority of IL21-producing cells are IFN-γ+ Th1 cells and not IL-17A+ [49], thus raising the issue of the requirement of Th17-derived IL21 in disease pathogenesis.

IL-22 produced by Th17 cells appears to have an IL-23 dependency [50]. In CD, serum IL22 levels are increased in active disease and may correlate with disease-associated IL23R polymorphisms [51]. Interestingly, absence of IL-22-producing cells is observed in active UC [52].

Regulatory T Cells
Numerous studies using mouse models support a role for Treg cells in IBD. In the T cell colitis model, transfer of CD4+CD45RB<sup>low</sup> T cell subset containing a regulatory population prevents the development of colitis [41]. The majority of Treg cells express the transcription factor Foxp3, although some can be Foxp3−, a population termed Tr1 cells. Foxp3 is important in Treg development and function, as observed in humans carrying a mutation of Foxp3 who develop IPEX syndrome [53, 54]. Treg cells can exert their regulatory mechanism through secreted soluble cytokines such as IL-10, TGF-β<sub>1</sub>, IL-35 and cell-cell interaction mediated by surface proteins (CTLA-4, GITR, and OX40) [55, 56]. Treg isolated from the inflamed mucosa of IBD patients show normal suppressive function in vivo [57]; however, the failure of regulatory mechanisms in IBD may be due to a Smad7-dependent T effector resistance to suppression by TGF-β<sub>1</sub> [58], T effector resistance to apoptosis [59], Treg sensitivity to apoptosis [60], or by the plastic conversion of Treg cells into Th1 like and Th17 like as a result of the inflammatory intestinal milieu [61, 62].

Effect of Microbiota on T Cell Development and IBD
The human gut is home to more than a trillion bacteria, representing about 500–1,000 different species [63]. In a healthy individual, these bacteria are separated from the immune system by mucus and the epithelial barrier; however, this intense load of microbes can pose a burden on the immune system; an insult resulting in damage to the epithelium can lead to microbial translocation into the mucosa, which will induce an immune response. Without the presence of these bacteria, the immune system does not develop appropriately. In ‘germ-free’ or gnotobiotic mice, there is a reduction in the frequency and size of Peyer’s patches, decreased intestinal IgA production, decreased CD4<sup>+</sup> T cell numbers within the lamina propria and the intraepithelial compartment [64]. Therefore,
the microbiota is key to the development of a normal mucosal immune system. Indeed, Ivanov et al. [65] identified segmented filamentous bacteria as responsible for the induction of Th17 cells within the murine mucosa. Furthermore, work done by Mazmanian et al. [66] identified that a factor expressed on the surface of Bacteroides fragilis, a common gut microbe, has the ability to induce CD4+ T cells to express Foxp3 and convert into IL-10-producing Treg cells. Polysaccharide A, the factor capable of inducing these Foxp3+ Treg cells, has been shown to protect against colitis development and EAE. Most recently, work from Atarashi et al. [67] isolated and identified a cocktail of 17 Clostridia species from human feces capable of inducing and maintaining Treg development. The gut microbiota has an obvious role in inducing intestinal inflammation, but the extent to which the microbiota is involved in IBD pathogenesis is still unclear.

Manipulation of Intestinal T Cells in the Treatment of IBD

The understanding of how the adaptive immune system via Treg induction by antigen can regulate the development of colitis is important to developing new strategies for treatment of IBD. Indeed, feeding antigen to mice receiving effector T cell transfer can lead to the induction of Treg cells and prevention of colitis [68]. Building on the observation that feeding the monoclonal anti-CD3 antibody can prevent the induction of EAE, we have shown that feeding anti-CD3 antibody can prevent the induction of T cell-induced colitis [69]. Clinical trials are now evaluating approaches to induce Treg cell activity in patients with IBD. Indeed, in vitro-expanding Treg cells from humans were shown to provide some benefit in treating established mucosal inflammation [70].

Conclusions

In less than two decades, the field of IBD genetics has made huge advances in our understanding of the pathogenesis of IBD. The 163 IBD-associated loci identified thus far have identified and implicated numerous important molecular and cellular pathways which may contribute to IBD. The importance of host-microbe interactions in particular has been emphasized, and further study of the interaction of intestinal microbiota and host immune system is likely to further advance IBD knowledge. A focus on the definition of IBD risk variants associated with actual alterations in gene function will also be important, so that these alterations can be targeted by therapeutic interventions.

Numerous IBD risk variants are within critical immunological pathways, such as Paneth cell function, autophagy, bacterial sensing and production of cytokines. It has become evident that IBD is a multifactorial disease, involving
both innate and adaptive aspects of the immune system. Adding to the complexity is the enormous variation of the human microbiota and the effect it has on both immune development and disease pathogenesis. By studying patients with IBD and animal models of colitis, we can begin to piece together the complex network of immune pathways, signals and cells that lead to IBD development. Hopefully, this will lead to new strategies for treatment and prevention.

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Abstract
The intestinal mucosa is the largest body surface exposed to the environment. While there are common features when comparing immune responses along the intestinal mucosa, the small bowel and colon exhibit striking differences in their mechanisms driving immune regulation. The vitamin A (VA) metabolite all-trans retinoic acid (RA) signaling via RA nuclear receptors plays a key role in immune homeostasis in the small bowel, and recent work indicates that RA is required for establishing immune tolerance to dietary antigens in the upper intestinal tract by inducing α4β7+CCR9+ gut-tropic T REG. In contrast, microbiota-specific T REG in the colon do not appear to require RA, but can be regulated by short-chain fatty acids (SCFA), microbial metabolites that signal through the G protein-coupled receptor GPR43. Moreover, T REG do not need CCR9 to home to the colon, but utilize another G protein-coupled receptor, GPR15, which is upregulated by SCFA. Thus, the mechanisms governing intestinal tolerance to dietary antigens in the upper digestive tract differ from those controlling tolerance to the microbiota in the colon, with RA and SCFA playing key complementary roles in their respective compartments. In addition to VA and SCFA, recent studies have highlighted the roles of other dietary and microbial metabolites that influence immune cell homeostasis across the small and large bowel including dietary ligands for aryl hydrocarbon receptor and microbiota-modified bile acids. Understanding the complex and dynamic interplay between dietary metabolites and commensal microbiota within the intestinal microenvironment could therefore inform novel strategies for the treatment of food allergies and inflammatory bowel diseases.
**Introduction**

Early work in animal models showed that lack of vitamin A (VA) impairs protection against bacterial infections [1] and classical epidemiological studies documented that endemic VA deficiency was associated with significant mortality in children, in part due to intestinal and respiratory infections [2, 3]. However, the cellular and molecular mechanisms by which VA, and more specifically its metabolite all-\textit{trans} retinoic acid (RA), affects mucosal as well as systemic immune responses have only recently begun to be identified. Of note, RA can mediate seemingly antagonistic effects on the immune system by promoting pro-inflammatory as well as tolerogenic immune responses [4, 5] and the factors determining the final immune outcome are incompletely understood. Moreover, while recent data indicate that RA and canonical gut-homing receptors are critical for tolerance to dietary antigen in the upper intestinal tract [4, 6], they do not appear to be equally involved in tolerance to the microbiota in the colon. Emerging data in mice indicate that short-chain fatty acids (SCFA) and the SCFA receptor GPR43 play critical roles in this compartment. Here, we discuss our current understanding of the effects of RA on intestinal immune responses and tolerance, and emerging data suggesting a critical complementary role of SCFA in colonic immune regulation. In this context, we will also discuss the role of diet-derived aryl hydrocarbon receptor (AhR) agonists and microbiota-modified bile acids in intestinal homeostasis.

**Gut-Associated Dendritic Cells and All-\textit{Trans} Retinoic Acid in Gut-Homing Imprinting**

In addition to their key role in priming naïve T and B cells, dendritic cells (DC) can confer T and B cells with tissue-specific migratory capacity upon activation. Gut-associated DC from mesenteric lymph nodes (MLN) or Peyer’s patches are sufficient to induce the gut-homing receptors integrin $\alpha_4\beta_7$ and chemokine receptor CCR9 on mouse and human T cells and B cells upon activation [7–11], whereas DC from peripheral skin-draining lymph nodes predominantly induce the skin-homing receptors E- and P-selectin ligands on T cells, which appears to be a default pathway in the absence of gut-specific signals [12].

Mechanistically, VA plays a critical role in gut homing induction through gut-associated DC that efficiently metabolize VA into RA that is sufficient to induce gut-homing receptors on mouse and human T and B cells [10, 11, 13]. Gut-associated DC express high levels of the RA-synthesizing enzymes retinal dehydrogenases (RALDH), especially the RALDH2 isoform [10, 13–15] (fig. 1).
RA is produced by DC during T cell activation and acts on lymphocytes via interaction with nuclear RA receptors of the RAR family (RAR-RXR heterodimers) [13]. At the gene expression level, RA induces mRNA for $\alpha_4\beta_7$, but not the $\beta_7$-integrin chain [12, 16]. Consistently, the promoter of Itga4 (encoding the $\alpha_4$-chain) contains RA response elements (RARE) that bind RAR-RXR upon RA treatment [16]. RA also induces CCR9 gene expression, although it requires both RAR-RXR and NFATc2 binding to the Ccr9 promoter [17]. Interestingly, in addition to inducing $\alpha_4\beta_7$ and CCR9 expression, RA inhibits the induction of skin-homing receptors E- and P-selectin ligands and CCR4 on T cells [13].

In addition to DC, other gut-associated cells also express RALDH enzymes and can produce RA, including lamina propria (LP) macrophages [18], intestinal epithelial cells (IEC) [13, 19–21], and stromal cells [22, 23]. Cell-specific de-
letion of RALDH isoforms however will be needed to assess the relative physiological contribution of each cell subset in RA production in vivo.

Importantly, RA also plays a critical role in inducing gut-tropic lymphocytes in vivo. VA-deficient mice exhibit a marked reduction in the numbers of CD4 and CD8 T cells in the small intestine LP (SI-LP) and intraepithelial compartment [13], as well as a reduction in the numbers of IgA-antibody secreting cells (ASC) in the SI-LP [11]. The decrease in the numbers of gut T cells and IgA-ASC may therefore contribute to the increased susceptibility to intestinal infections in children with VA deficiency [2, 24, 25].

All-Trans Retinoic Acid Effects on T Helper and Regulatory T Cell Differentiation

T_{REG} and Th17 Cell Differentiation

A role for RA in potentiating TGF-β-dependent Foxp3+ T_{REG} induction has been demonstrated in vitro and in vivo [15, 26–32], and RA also induces gut-homing receptors on T_{REG} [33–37]. RA promotes T_{REG} induction directly in a cell-intrinsic manner by increasing the stability and suppressive capacity of T_{REG} [35, 38, 39] and by decreasing apoptosis of in vitro-generated murine [38] and human T_{REG} [39, 40]. Among the indirect effects of RA on T_{REG} differentiation is the counteraction of inhibitory signals provided by bystander effector/memory T cells, such as IL-4 [30, 31], IL-21, and IFN-γ [30]. RA also downregulates expression of the Th17 cell-associated genes IL-6R, IL23R, and IRF4 [29, 41], and enhances the expression of microRNA miR-10a, which prevents Th17 cell differentiation and increases T_{REG} stability [42].

In the context of the intestinal environment, CD103+ MLN-DC produce RA and potentiate T_{REG} induction [15]. In addition, CD103+ MLN-DC express the integrin α4β8, which is needed to convert latent LAP-TGF-β into active TGF-β, a process required to generate T_{REG} and suppress inflammation in vivo [43, 44]. However, macrophages that produce RA and IL-10 are the primary T_{REG}-inducing cell type in the SI-LP [18], and CX3CR1+ macrophages are critical for the expansion of T_{REG} in the SI-LP upon oral tolerance induction [6]. Thus, RA-producing CD103+ MLN-DC and SI-LP macrophages appear to fulfill complementary roles in T_{REG} induction and expansion/differentiation, respectively.

As discussed above, there are substantial data supporting a role for RA in promoting T_{REG} and inhibiting Th17 cell differentiation [26] (fig. 1). Consistent with this paradigm, RA has been shown to reduce Th17 cell numbers and inflammation in murine models of ileitis [45], experimental encephalomyelitis [29], rheumatoid
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arthritis [46], and graft-versus-host disease [47]. However, signaling via RARα was necessary to induce Th17 cells in vitro and protective Th17 responses in vivo in a murine model of intestinal toxoplasmosis [48]. Furthermore, RA induces α4β7 and CCR9 on Th17 cells, and VA-depleted mice exhibit a marked decrease in small bowel Th17 cells [49, 50]. Therefore, while RA appears to predominantly exert a negative effect on peripheral Th17 cell differentiation, it may contribute to the differentiation and/or homing of α4β7+ gut-associated Th17 cells. In this regard the role of α4β7 in the intestinal homing of proinflammatory T cells has been clinically translated by the success of therapies blocking this integrin in the context of IBD [51, 52]. Of note, these therapies might also affect the migration of regulatory T cells and/or tolerogenic DC/macrophages to the gut, hence potentially interfering with the establishment or maintenance of immune tolerance in this compartment [4, 6].

**Th1 and Th2 Cell Differentiation**

While several studies have addressed the role of RA in TREG and Th17 differentiation, less is known about RA effects on Th1 and Th2 differentiation. RA can promote the generation of Th2 cells in vitro by inducing expression of Th2-associated genes including Gata3, Maf, Stat6, and Il4, and by inhibiting the expression of the master Th1 transcription factor Tbet [53–55]. However, recent work showed that RA signaling via RARα is required for protective Th1 responses in vivo and for optimal Th1 differentiation in vitro [48]. Moreover, when combined with IL-15, RA can have an adjuvant role in Th1-driven pathology in a model of celiac disease [5]. Therefore, the effect of RA on Th1/Th2 cell differentiation in vivo appears to be immune context-dependent.

**Complementary Roles of Nutrients and Microbial Metabolites in Intestinal Immune Homeostasis**

**All-Trans Retinoic Acid and Tolerance to Dietary Antigens**

Tolerance to dietary antigens, such as food, xenobiotics, and soluble proteins, mainly originates in the upper part of the small bowel [56]. This type of tolerance, often referred to as oral tolerance (OT), occurs in mice and humans [57, 58], and requires the presence of MLN [59]. Recent work showed that OT critically depends on RA [4, 60] and on the expression of gut-homing receptors α4β7 [4, 6] and CCR9 [4, 61] on TREG, although it should be noted that the requirement of CCR9 in OT appears to be restricted to low doses of antigen [61].
Thus, although OT originates in the MLN, it requires a subsequent step in the SI-LP for $T_{REG}$ expansion [6] and differentiation into IL-10-producing $T_{REG}$ [4]. IL-10+ $T_{REG}$ then leave the intestinal mucosa via the lymphatics and travel via the blood to suppress inflammation in peripheral tissues [4, 57] or in the gut itself [6, 60], hence preventing damaging inflammatory or allergic responses to dietary antigens (fig. 2).
SCFA (acetate, propionate, and butyrate) are the main fermentation products of anaerobic bacteria in the gut [62]. In addition to their roles as fuel for colon intestinal epithelial cells (IEC), it has become increasingly clear that SCFA are powerful mediators of the effects of microbiota on intestinal immunity [63, 64]. SCFA act on leukocytes and IEC via G protein-coupled receptors (GPCR), including GPR41 and GPR43, and by inhibiting histone deacetylases (HDAC) [62, 65, 66]. Interestingly, part of the HDAC inhibitory effects of SCFA appears to require GPR43 signaling [64]. Moreover, butyrate and niacin (vitamin B₃) can also inhibit HDAC in DC and macrophages by signaling via GPR109a [67].

Recent evidence indicates that colon T_{REG} play a key role in establishing and maintaining tolerance to the microbiota [68–70]. While α₄β₇ and α₄β₁ are partially required for T_{REG} homing to the colon [71], CCR9 is not involved in this process. Alternatively, another GPCR, GPR15, appears to be required for the migration of T_{REG} into this compartment [71]. Whether GPR15 is also required for homing of pro-inflammatory cells to the colon remains to be determined.

Recent work points to a critical role of SCFA and the receptor GPR43 in preventing intestinal inflammation [63] and in the generation of IL-10-producing T_{REG} in the colon, but not in the small bowel [64]. Of note, SCFA not only promote T_{REG} differentiation and proliferation in the colon, but also upregulate GPR15 [64], thus promoting T_{REG} homing to this compartment [71] (fig. 2). Among the bacterial species able to produce the SCFA butyrate in humans are Clostridium cluster XIVa [72] and XVII [64], Roseburia sp, Coprococcus sp, and F. prausnitzii [73] (the latter also involved in biliary acid metabolization, as described below). Interestingly, SCFA-producing bacteria are decreased in IBD patients, and children with IBD exhibit lower fecal SCFA levels [74, 75], suggesting that lack of SCFA-producing bacteria might contribute to IBD pathogenesis, and some studies indicate that treatment of ulcerative colitis patients with butyrate enemas can suppress colonic inflammation [76].

In sum, while tolerance to dietary antigens in the upper intestinal tract requires RA and the expression of gut-homing receptors α₄β₇ and CCR9 on T_{REG}, tolerance to the commensal microbiota in the colon appears to rely on SCFA and on the expression of GPR43 and GPR15 on T_{REG}. Future studies will need to assess whether these paradigms also apply to human intestinal homeostasis.
Bile Acids and Intestinal Immune Homeostasis

In addition to the generation of SCFA from dietary nutrients, the commensal microbiota can influence intestinal immune responses through their role in host lipid and bile acid metabolism. Bile acids are amphipathic cholesterol metabolites produced in the liver and delivered to the SI where they facilitate the absorption of dietary fat and fat-soluble vitamins and act as potent signaling molecules. The microbiota perform two key functions in bile acid metabolism: the conversion of primary bile acids (e.g. cholic acid and chenodeoxycholic acid) to secondary bile acids (e.g. deoxycholic acid and lithocholic acid) and the deconjugation of bile acids to their unconjugated forms for excretion [85]. Notably, while bacteria can functionally modify bile acid species, bile acids can reciprocally regulate bacterial growth. In this regard, it has been hypothesized that bacteria expressing bile acid-deconjugating enzymes may autoregulate intestinal bacterial populations by generating unconjugated bile acids that directly inhibit bacterial growth [77]. Conversely, a recent study has established a link between dietary saturated fat intake and the generation of taurine-conjugated bile acids that can promote the outgrowth of a pathological bacterial species, resulting in increased susceptibility to intestinal inflammation and suggesting a complex interplay between diet, commensal intestinal bacteria, bile acid metabolism, and inflammation [79].

A role for dysregulated bile acid metabolism in IBD has been implicated by the correlation of changes in bile acid species with dysbiosis and severity of disease in human Crohn’s and ulcerative colitis patients [80]. In that study, dysbiosis was characterized by decreased ratios of *F. prausnitzii* to *E. coli* and was correlated with low levels of secondary bile acids, high levels of sulphated bile acids and active inflammation in IBD patients. Further supporting a role for bile acids in regulating intestinal immune inflammation, mice with genetic deletions in either the nuclear bile acid receptor, NR1H4, or the surface bile acid receptor TGR5, exhibit increased susceptibility to intestinal inflammation in multiple preclinical models of IBD [81–83]. NR1H4 (also known as farnesoid X receptor) is a nuclear hormone receptor that signals as a heterodimer with RXR and is highly expressed in the liver, intestine, kidneys and adrenal glands, while TGR5 is a membrane-bound GPCR with highest expression in the gallbladder and lower expression in brown adipose tissue, liver, intestine and central nervous system [84]. Consistent with an anti-inflammatory function for these bile acid receptors, agonism of either receptor through their respective endogenous or synthetic ligands has been shown to decrease proinflammatory cytokine production by epithelial cells and innate immune cells, in part through inhibition of NFκB activation through a cAMP-PKA-CREB pathway (TGR5) or stabiliza-
tion of the nuclear receptor corepressor 1 on NFκB response elements (NR1H4) [85]. In addition, agonism of the nuclear bile acid receptor NR1H4 has been shown to limit translocation of bacteria and enhance barrier integrity in the SI in a bile duct ligation model of intestinal inflammation [78]. Taken together, furthering our understanding of the relationship between dietary fat, changes in commensal bacterial populations, and bile acid metabolism could lead to novel therapeutic targets in IBD.

Immune Regulation through Aryl Hydrocarbon Receptor

AhR is a ubiquitously expressed cytosolic receptor that when bound by ligand translocates to the nucleus where it interacts with its dimerization partner, ARNT, to activate transcription of target genes [86]. Although historically known for its role in mediating signaling downstream of environmental toxins such as dioxin, recent studies aimed at understanding the physiological role of AhR and its endogenous ligands have identified a critical role for AhR in the regulation of intestinal immune homeostasis. Of the known natural AhR ligands, relevant to this discussion are various indole derivatives generated by intestinal bacteria through tryptophan catabolism, including the recently identified indole-3-aldehyde generated by lactobacilli [87], and indole-3-carbinol, a dietary ligand derived from cruciferous vegetables such as broccoli, cabbage and cauliflower [88].

To date, the most pronounced functions of AhR signaling within the immune system have been demonstrated in T cell and innate lymphoid cell (ILC) populations, particularly those belonging to the group 3 ILC [88, 89]. Endogenous AhR signals appear to be required for the expansion and function of adult ILC and intraepithelial lymphocyte (IEL) populations. After weaning, AhR-deficient mice have significantly reduced numbers of intestinal ILC and IEL populations, while conversely treatment of wild-type mice with the potent AhR ligands 6-formylindolo(3,2-b)carbazole (Ficz) or indole-3-carbinol enhances both the number and function of ILC and IEL in the large and small intestine [88, 89]. This role of AhR in ILC homeostasis is likely to be cell autonomous and independent of either T and B cells or potential effects of AhR deficiency on the microbiota, as demonstrated by the persistence of altered ILC populations in RAG-deficient bone marrow chimeras and in antibiotic-treated AhR-deficient mice [89].

AhR signaling has also been shown to regulate Th17 and T\textsubscript{REG} cell differentiation both in vitro and in vivo. However, as with RA, the effects of AhR on the balance between Th17 and T\textsubscript{REG} cell biology are complex, and studies have implicated AhR in both the promotion and inhibition of Th17 cell differentiation, although IL-22 expression appears to be consistently decreased in the absence
of AhR and enhanced with AhR signaling. AhR is highly expressed in Th17 cells, and AhR signaling has been shown to promote IL-17 production and contribute to development of experimental autoimmune encephalitis (EAE) [90]. However, other studies have suggested that the outcome of AhR signaling is ligand dependent and showed that stimulation with the environmental toxin 2,3,7,8-tetrachlorodibenzo-p-dioxin induced T_{REG} cells that suppressed EAE, while stimulation with Ficz led to increased Th17 cell differentiation and promoted EAE [91]. Thus, discrepancies in the role for AhR signaling in Th17 or T_{REG} cell biology appear to be highly context dependent. For example, stimulation of AhR through Ficz has also been shown to promote IL-10 production and Tr1 cells in the context of TGFβ and IL-27 [92], and in humans, activation of AhR in naïve T cells in combination with TGFβ stimulation promotes the generation of suppressive Tr1 and Foxp3^+ iT_{REG} cells in vitro, an effect mediated in part through increased expression of Smad1 and the Ikaros family transcription factor Aiolos [87]. Highlighting the complexity of the relationships between AhR, ILC, Th17 cells, and the intestinal microbiota, in the absence of AhR decreased numbers of group 3 ILC and IL-22 production were associated with an outgrowth of segmented filamentous bacteria (SFB) [93]. This expansion of SFB promoted Th17 cell expansion and increased susceptibility of AhR-deficient mice to both spontaneous and experimentally induced colitis [93].

Given the recently identified roles for AhR in regulating intestinal immune homeostasis, it is not surprising that altered AhR signaling has been linked with changes in susceptibility to intestinal inflammation. AhR-deficient mice, for example, exhibit increased susceptibility to multiple murine models of intestinal inflammation including enteric infection with C. rodentium and the TNBS, DSS, T cell transfer and oxazalone models of colitis [94]. Additionally, mice given L. bulgaricus OLL1181 or mice in which there is an outgrowth of L. reuteri (IDO^{−/−} mice, mice fed an enhanced tryptophan diet) exhibited an AhR-dependent increased resistance to DSS colitis [95] and C. albicans infection [87], respectively, supporting a role for bacterially-derived AhR ligands in limiting intestinal inflammation and promoting intestinal immunity. Thus, understanding the homeostatic roles of endogenous dietary and microbial-derived ligands for AhR may provide new opportunities for therapeutic intervention in IBD.

Conclusions

Emerging evidence suggests that distinct mechanisms may exist for induction of intestinal immune tolerance when comparing the upper intestinal tract and the colon. While RA is required to establish tolerance to dietary antigens, SCFA ap-
pear to be critical for inducing tolerance to the commensal microbiota. Addi-
tional dietary nutrients and microbial-derived metabolites such as endogenous
AhR ligands and bile acids also have potent effects on intestinal immune homeo-
stasis that appear to manifest in both the small and large intestine, although fur-
ther studies will be required to determine whether these molecules also exhibit
preferential activity based on distribution of ligands and receptors within the
digestive tract. A clearer understanding of the mechanisms regulating immune
tolerance in the upper and lower intestinal compartments will be critical to de-
vise rational immunotherapies aimed to ameliorate abnormal immune respons-
es to dietary antigens (e.g. in food allergies or celiac disease) or versus the mi-
icrobiota (e.g. in inflammatory bowel diseases).

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Manipulating the Gut Microbiome as a Therapy for IBD

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Abstract
The human gut contains a vast number of microorganisms known collectively as the gut microbiota. Despite its importance in maintaining the health of the host, growing evidence suggests the gut microbiota may also be an important factor in the pathogenesis of various diseases, a number of which have shown a rapid increase in incidence over the past few decades. In some of these diseases, such as inflammatory bowel disease (IBD), the microbiota is dysbiotic with an abnormal community structure and decrease in diversity. If the dysbiotic microbiota plays a role in disease pathogenesis, interventions that modify its composition to make it more similar to the microbiota observed in health, might be a strategy to treat certain disease processes. Indeed, the high-level efficacy of fecal microbiota transplantation in the treatment of refractory Clostridia difficile infection supports this notion as proof-of-principle. The composition of the microbiota can be influenced by many factors including age, genetics, host environment, and diet. With respect to the later, diet has an impact upon both the composition and function of the microbiota. There are epidemiologic data associating diet with the development of IBD as well as evidence that diet can influence both the form and function of the microbiome in a manner that impacts upon the development of intestinal inflammation. Based on this evidence, studies are now underway to examine the effect of defined formula diets, an effective therapeutic modality in Crohn’s disease, on both the gut microbiome and its metabolome as a therapeutic probe with the hope of better defining the ‘healthy’ diet in patients with IBD.

Introduction

Human microbiomes are very distinctive amongst various body sites and are composed of not only bacteria but also other microorganisms including other prokaryotic organisms such as Archaea, microeukaryotes such as fungi, and viruses, principally bacteriophage – the latter being amongst the most abundant biologic entities in the biosphere. In this review, the term microbiota will be used to denote the compilation of bacterial microorganisms within a specific environment, whereas the microbiome refers to not only the bacterial taxa but also their collective genomes. The human gut microbiota is a densely populated bacterial community with approximately $10^{11}$ organisms per gram of fecal weight composed of more than 1,000 species, most of which are obligate anerobes [1, 2], with a collective genome size 150-fold greater than that of its human host [1]. Although there are over 50 bacterial phyla on Earth, human-associated bacteria largely belong to one of four phyla, Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes. Mammalian hosts have coevolved to exist with our gut microbiota in a mutualistic relationship, where we provide a uniquely suited environment in return for physiological benefits provided to us by our gut microbiota [3]. Examples of the latter include the fermentation of indigestible carbohydrates to produce short-chain fatty acids that are utilized by the host, biotransformation of conjugated bile acids, synthesis of certain vitamins, degradation of dietary oxalates, the hydrolysis of urea by urease activity that participates in host nitrogen balance, and education of the mucosal immune system [3].

The Association between the Gut Microbiota and Human Disease

Despite the importance of the gut microbiota in maintaining the health of the host, growing evidence suggests that it may also be an important factor in the pathogenesis of a variety of diseases, particularly those that have shown a rapid increase in incidence over the past few decades. These include both type 1 and type 2 diabetes mellitus, atherosclerosis, asthma, colon cancer, and inflammatory bowel disease (IBD), to name a few [4]. Advanced genomic technology, principally DNA sequencing and SNP mapping used for genome-wide association studies combined with biocomputational algorithms, have revealed the genetic underpinnings of these complex disease processes. In most circumstances, the contribution of host genetics to the risk of disease development is significantly less than 50%, implicating the importance of environmental influences [5]. The observation that these diseases have shown a steadily increasing incidence over the past several decades, the geographic distribution of disease clus-
tering in industrialized nations, and immigration studies revealing the adoption of disease risk of the host country within one or two generations all emphasize further the importance of environment in the pathogenesis of these diseases.

Interestingly, inflammation has been strongly associated with many of these ‘Westernized’ disease processes. In addition to IBD and asthma, which are principally diseases due to unrestrained immune processes, T1DM has been associated with type 1 interferon production and altered T cell signaling, suggesting an autoimmune response and insulin resistance, the hallmark of T2DM, and is associated with an inflammatory response in adipose tissue [6]. Even obesity and atherosclerosis have been associated with chronic inflammation with elevations of serologic markers such as C-reactive protein.

Although a causal relationship has been demonstrated primarily in animal models and a functional effect in human disease is currently lacking, the role that the gut microbiota plays in the establishment of host immunity together with its effects on the inflammatory response suggests that continued investigation may lead to direct evidence for the role of the microbiota in at least some of these disease processes. Indeed, early-life exposure to the gut microbiota and its effects on the development of immunologically based disease have recently been demonstrated in an animal model [7].

**Determinants of Gut Microbiota Composition, Dysbiosis and IBD**

Using our current understanding of disease pathogenesis in IBD as a paradigm, functional genomics has revealed a complex interaction between host innate and adaptive immunity that provides protection against microbial invasion, yet demonstrates tolerance to colonization with the microbiota at mucosal surfaces (recently reviewed in Khor et al. [8]). In the case of IBD, the loss of mucosal tolerance, together with a defect in protective host innate immunity to a dysbiotic microbiota, leads to an unrestrained mucosal immune response, the hallmark of this disease process. Indeed, of all the chronic disease states currently associated with the gut microbiota, the evidence for a causative role in the pathogenesis of IBD is the strongest.

Dysbiosis of the gut microbiota, an alteration of the microbial community structure associated with disease, has been consistently observed in patients with IBD. Although the dysbiosis may simply be a result of the inflammatory process [9], it may play a role in the pathogenesis of disease, where there is an increase in potentially harmful and a reduction in more protective bacterial species [10]. Functional evidence for a role of the dysbiotic microbiota in the pathogenesis of disease is supported by animal models, where colitis, or predisposition to dis-
ease, can be transferred to wild-type mice using genetically defined disease mice as gut microbiota donors [11, 12].

The notion of an alteration in the composition of the gut microbiota as a possible etiologic factor in the predisposition to immunologically mediated disease has been proposed as one of the environmental factors that may play in a role in the increasing incidence of the diseases associated with the gut microbiota mentioned previously [13]. Indeed, many aspects of our environment have been changed dramatically over the past few decades concurrent with the increasing incidence of these disease processes. Elements of the modern lifestyle that have been postulated to result in changes in the gut microbiota include improved sanitation, vaccinations, increased antibiotic use, decline in parasite infections, caesarean section, decline in *Helicobacter pylori*, smaller family size, refrigeration, less crowded living conditions, sedentary lifestyles, food processing, and dietary changes. The impact of host genetics on the gut microbiota may also play a role, but evidence is largely based on studies in model organisms such as rodents and has been reviewed recently [14]. The impact of host genetics on the gut microbiota in healthy human populations, based on current evidence [15], may be relatively modest, but further studies are needed.

**Short- versus Long-Term Diet and the Gut Microbiota**

The impact of diet on the composition of the gut microbiota begins early in life. Colonization of the gut begins at birth and, following an initial chaotic community structure during the first year of life, the human gut microbiota becomes more stable and adult-like [16] concurrent with the introduction of solid foods into the diet [17]. However, it is not until 2–3 years of age that the gut microbiota in childhood strongly resembles that of the adult [18]. Several studies have explored the impact of diet on the newborn gut microbiota and have compared breastfeeding with formula feeding. A consistent finding has been the higher proportion of *Bifidobacteria* in breastfed infants as compared to formula-fed infants [19–22].

Although studies have examined the impact of diet on the gut microbiome of various mammalian species in a cross-sectional fashion [23] as well as in a mouse intervention study [24], until very recently, there have not been any studies broadly examining the association between diet and the composition of the adult human gut microbiota. We recently reported two separate experiments, whereby the association between diet and the human gut microbiota was evaluated in a cross-sectional and short-term interventional study [25]. Dietary questionnaires, used to assess dietary consumption and 16S rRNA gene sequencing to
determine the composition of the gut microbiota in 98 healthy human subjects, revealed a statistically significant association between overall diet and the composition of the gut microbiota. Spearman correlation coefficient associations between bacterial taxa and micronutrients revealed that major nutrient categories clustered independently with relative proportions of bacterial taxa, where ‘fat’ and ‘fiber’ were inversely correlated as were ‘amino acids’ and ‘carbohydrates’ [26]. These observations are also consistent with a study comparing the gut microbiota of children from a village in the West African country of Burkina Faso with those in Europe [26], as well as a more recent study comparing the gut microbiome of residents in the agrarian Malawi and Amerindian societies with residents in the US [18], where the inverse relationship between Bacteroides and Prevotella genera were also noted. Although the abundance of Prevotella could be considered a discriminative taxon associated with the residence within an agrarian society, the fact that these associations were also observed in residents of the US [25] supports the notion that the observed inversely related proportions of Prevotella and Bacteroides may be, in part, due to diet.

A second study to evaluate the impact of a short-term dietary intervention with either a low fat/high fiber or high fat/low fiber on the human gut microbiota revealed that the gut microbiota responds to a dietary change within 24 h [25]. Although the short-term diet-induced alteration in the gut microbiota was highly statistically significant, the effect was modest relative to inherent intersubject variability in gut microbiota composition. Furthermore, we did not find any evidence that short-term diet reduced this intersubject variability. In addition, broad taxonomic alterations, often observed in mice to be associated alterations in fat/fiber [27, 28] were not observed in this short-term dietary intervention in humans, supporting the notion that long-term dietary alterations may be needed to broadly alter the composition and/or richness (number of distinct bacterial taxa) of the gut microbiota in humans [25, 29, 30]. Clearly, further investigation into this important topic is needed.

**Diet and the Structure versus Function of the Gut Microbiome: Impact on the Metabolome and the Identification of Therapeutic Targets**

Although diet can have an effect on the composition and/or richness of the gut microbiota, perhaps more important is its impact on the microbial metabolome. Indeed, diet may serve as a substrate that can be used by the gut microbiota for the production of small molecules that, after first pass metabolism through the liver, can have an important impact on host physiology [31]. An example of this would be the delivery of indigestible carbohydrates to the gut microbiota through
dietary intake leading to the production by bacterial fermentation of short-chain fatty acids that play a role in immune function [32, 33], intestinal hormone production and lipogenesis [34]. Another example would be the role that gut microbiota may play in augmenting the development of atherosclerosis through the production of certain metabolites of dietary lipid phosphatidylcholine that are associated with the risk for the development of cardiovascular disease (CVD). Using a targeted approach to identify plasma metabolites which predict CVD in patients, Wang et al. [35] and Tang et al. [36] identified a novel pathway linking dietary lipid intake, intestinal microbiota and atherosclerosis. Foods rich in phosphatidylcholine are a major source of choline. Catabolism of choline by the intestinal microbiota results in the formation of the gas TMA (trimethylamine) that is metabolized by the liver to form TMA oxide (TMAO), a small molecule that is strongly associated with the increased risk for coronary vascular disease in humans. TMAO also augments the development of atherosclerosis in animal models, thus providing the first link between dietary lipid intake, the intestinal microbiota, and the risk for the development of atherosclerosis [35]. A similar pathway has been identified for conversion of dietary carnitine, which is high in red meat, and its conversion into TMAO [35, 36].

Recently, the bacterial gene family responsible for the conversion of choline into TMA, known as choline TMA-lyases, has been described [38], where investigators have shown that the greatest abundance of nonpathogenic bacterial taxa with this gene representation is located in three of the four major phyla of the human gut microbiome. With this knowledge, several possible avenues can be envisioned by which this information can now be used to develop technologies that may directly impact upon human health: (1) quantify the risk for heart disease attributed to the consumption of choline by characterizing the abundance of bacteria in the gut that have a choline TMA-lyase gene; (2) design an approach to reduce or extinguish TMA-lyase-expressing bacteria in the gut; (3) develop drugs to inhibit TMA-lyase activity in bacteria, and (4) develop ‘designer foods’ or ‘medical foods’ to reduce the production of TMA by bacteria from the diet. These concepts are of considerable importance to the field as scientists search for opportunities, whereby the knowledge gained from our understanding of the gut microbiome can be used to prevent and/or treat human disease.

**Diet, the Gut Microbiota and IBD**

Certain nongenetic factors associated with the development of IBD may be due, in part, to their effects on the gut microbiota. Environmental factors that may alter the composition of the gut microbiota include diet, the use of antibiotics,
and geographic location. Population-based studies suggest that IBD is unevenly distributed throughout the world with the highest disease rates occurring in industrialized nations [39, 40]. One theory, the hygiene hypothesis, suggests that humans living in more industrialized countries are exposed to fewer microbes or less complex microbial communities at an early age leading to the development of an immune system less able to tolerate exposure to the microbial-laden environment in later life resulting in inappropriate immune activation. Consistent with this notion is the possible role of diet in light of the differences in access to clean water and availability of food refrigeration in underdeveloped parts of the world. Alternatively, a Westernized diet rich in animal fat and protein while low in fiber, may alter the gut microbiome in a way that increases the risk for the development of IBD.

Regardless of the mechanism, there are reasonable data to support a role for diet in IBD pathogenesis. Several investigators have examined the association of dietary patterns and the incidence of IBD [41, 42]. For example, the authors of a systematic review concluded that high dietary intake of total fats, polyunsaturated fatty acids, omega-6 fatty acids, and meat were associated with an increased risk of CD and UC; high fiber and fruit intakes were associated with a decreased CD risk, and high vegetable intake was associated with a decreased UC risk [42]. These studies support a potential role for dietary patterns in the pathogenesis of IBD. As proof of principle, the consumption of milk fat has been shown to alter host bile acid composition, thereby promoting the expansion of the sulphite-reducing pathobiont Bilophila wadsworthia, resulting in an exacerbation of colitis in IL-10 KO mice [43]. Together with the recent data characterizing the impact of diet on the gut microbiome [25], it is tempting to speculate that the alteration of gut microbiota community structure through the consumption of agrarian versus a Westernized diet may play a role in either reducing or increasing, respectively, the risk for the development of IBD.

In Crohn’s disease, exclusive enteral nutrition (EEN) with elemental, semi-elemental, and defined formula diets has been widely studied for induction of remission and is considered first-line therapy in certain parts of the world [44, 45]. These diets are also efficacious in maintaining remission [46]. The most common protocol involves the administration of a defined formula at 100% of caloric needs for 4–12 weeks in order to induce remission [47]. A smaller percentage of calories provided by the defined formula may be required in order to maintain remission, allowing additional flexibility in the diet [46]. Despite the efficacy of this therapeutic modality, the mechanisms by which EEN reduces inflammation in patients with Crohn’s disease are unknown. Current studies are underway to determine the effect of EEN on the composition of the gut microbiota in the hope of identifying microbial taxa and/or metabolites that are either
beneficial or deleterious in Crohn’s disease pathogenesis. Conceptually, of fundamental importance to these studies is to understand how the consumption of these defined formulas is different from dietary intake of whole foods.

**Conclusions**

In this review, we highlight diseases for which the gut microbiota has been implicated in disease pathogenesis, focusing on those associated with the consumption of a Westernized diet. Although studies now suggest that diet has an impact on the human gut microbiota, there is clearly much to be learned. The impact of diet on the composition of the microbiota may ultimately be less important to host physiology than its impact on the microbial metabolome, where the production of a multitude of small molecules may have important consequences for human health and disease [30]. Additionally, associations between the gut microbiota and human disease, including the impact of diet, do not prove cause-and-effect relationships. Indeed, most data supporting a functional effect of an altered microbiota on host physiology are based primarily on murine models. Although such studies provide fundamentally important information about disease mechanisms demonstrating proof of principle, the degree to which they reflect human pathophysiology awaits further investigation.

**Disclosure Statement**

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**References**


Manipulating the Gut Microbiome as a Therapy for IBD


Antibiotics, Probiotics and Prebiotics in IBD

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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 nitroimadole postoperatively in Crohn’s disease (CD) to prevent disease recurrence. Otherwise, the data are limited with 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.

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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, fluroquinolones, 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 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. delbrueckii* 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 mesalazine 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 \textit{L. acidophilus} La-5 and \textit{B. animalis} subsp. \textit{lactis} BB-12 or placebo (n = 32) [22]. No other concomitant medications including mesalazine 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 mesalazine would be of benefit. A large 1-year study (n = 157) evaluated two probiotics (\textit{L. salivarius} subsp. \textit{salivarius} UCC118 or a \textit{B. infantis} 35624) versus placebo in UC administered 1 month after a steroid-induced remission. Subjects were allowed mesalazine 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 mesalazine randomized to 100 ml of bifidobacteria-fermented milk (containing a \textit{Bifidobacterium} and \textit{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.

\textit{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. bifidum*...
Antibiotics, Probiotics and Prebiotics in IBD

*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 dispersions 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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Manipulating the Gut Microbiome as a Therapy for IBD


Abstract
Fecal microbiota transplantation (FMT) is a targeted microbiome-based therapy that has garnered a great deal of attention from the scientific, clinical, and lay communities. An increasing number of studies have demonstrated that FMT is a highly effective therapy for recurrent/refractory \textit{Clostridium difficile} infection and appears safe in the short term. Uncontrolled reports suggest the possibility of benefit in a select group of IBD patients, but there is quite limited information so far. FMT for IBD raises significant issues and concerns that warrant further systematic investigation. In this review, we discuss the background and rationale for this innovative therapy, the current knowledge for FMT in IBD, the logistical issues, and the important issues regarding ethics, social acceptability, and regulation. © 2014 Nestec Ltd., Vevey/S. Karger AG, Basel

Although we have only begun to understand the complex microbiology of the human body, there is little doubt that the microbiome plays a fundamental role in health and disease. Since its inception, the Human Microbiome Project (HMP) has supported research aimed at characterizing the bacteria in and on our bodies and has fostered thousands of other projects with a similar goal [1]. This large-scale initiative has not only revolutionized our understanding of disease pathogenesis, but it has fundamentally changed the way we think about developing novel therapies and treating disease. Numerous microbiome-based approaches to treating diseases are being investigated, but none has garnered as much attention or interest as fecal microbiota transplantation (FMT). FMT is neither a new concept nor a new treatment, with reports dating back to 4th century China [2]. Despite its long history, we have little to no data about which
diseases FMT will be most effective in treating, optimal delivery methods and doses, how to identify and screen healthy donors, and the long-term implications and safety of FMT.

Much of our current understanding of FMT is drawn from its use in treating recurrent or relapsing *Clostridium difficile* infection (CDI). For recurrent/relapsing CDI, FMT appears to be incredibly effective and safe regardless of the delivery method, dose, or donor [3–6]. The therapeutic potential of FMT has been proposed for a number of other diseases including IBD [7–9]. Unfortunately, to date, there have been no published controlled trials of FMT in IBD, and the current basis for this innovative therapy is based on animal studies [10, 11], studies describing the variations in the commensal intestinal microbiota in patients with IBD [12–16], and a few small case reports and series of FMT in IBD [8, 9, 17–19]. This paucity of data has not dampened enthusiasm; patients, physicians, scientists, and the media continue to demonstrate a great deal of interest in FMT for IBD [20–23]. Although this enthusiasm is important in driving this innovative research forward, we must temper our enthusiasm and acknowledge that currently FMT is an experimental and unproven therapy for IBD. In this regard, FMT is analogous to any other unproven therapy and must be evaluated in the same controlled systematic approach. Furthermore, we believe that because of the availability of the ‘treatment’ (stool), FMT for IBD raises unique issues and concerns that require further investigation. It is clear that there is a gap in our knowledge and a need for more research on this promising and innovative therapy. In this review, we will discuss the background and rationale for this innovative therapy, the current knowledge for FMT in IBD, the logistical issues, and the important issues regarding ethics, social acceptability, and regulation.

**Background and Rationale**

FMT is the delivery of an entire microbial community from a healthy individual to an individual with disease. Dysbiosis, a theory first outlined over a century ago by Nobel Laureate Élie Metchnikoff, is a disruption in the otherwise normal balance of protective and pathogenic commensal bacteria [24]. The rationale is that FMT restores balance and corrects the underlying dysbiosis associated with the disease state. FMT involves the following:

- Identification of a donor who submits to extensive screening for infectious diseases.
- Collection and preparation of the fecal matter.
- Delivery of the preparation to the patient.
In addition to the numerous logistical, screening, and safety issues that warrant the investigation outlined in this review, an equal number of questions exist regarding the definition of a healthy donor and the potential implications of microbiota transplantation on other health related issues, such as allergy, obesity, and metabolism.

A growing body of (uncontrolled) literature has demonstrated that FMT has unquestionable efficacy for the treatment of recurrent or relapsing CDI, with cure rates of over 90% and a favorable side effect profile, with no reports of serious adverse events in children or adults [3, 5, 25, 26]. FMT also appears to be safe and effective in patients with severe fulminant CDI [27, 28]. Microbiological investigations to explore the mechanisms underlying this response have demonstrated that patients with CDI have dysbiosis that is corrected and sustained by treatment with FMT [5, 29, 30]. Having established that FMT treats dysbiosis, we must ask if FMT can treat other diseases associated with dysbiosis. IBD is a group of diseases with clinical features that overlap with CDI, but have a much more complex basis. Current etiological theories of IBD suggest that disease results through a complicated interaction between genes, immune dysregulation, and environmental and microbial factors [31]. With the widespread use of novel molecular techniques, we have increasing evidence that microbial factors such as dysbiosis are central to the development of IBD [12, 15, 16, 32]. As such, many theorize that repopulation of the commensal bacteria through FMT may help correct dysbiosis and downregulate the overwhelming inflammatory cascade in IBD [33–36].

**Current Evidence for Fecal Microbiota Transplantation in IBD**

Unfortunately, current evidence for the utility of FMT in IBD is limited to a handful of abstracts, reports, and case series (table 1) [9, 18, 19, 37–39]. The descriptions include variable amounts of data about the patients, donors, and delivery methods. Others have summarized the experiences through useful systematic reviews [40, 41]. The majority of the reports have been favorable; however, a few patients had no response or worsening of their IBD [37, 39, 42, 43]. It is unclear if there are specific biological or clinical factors that will predict a response or ‘intolerance’ to FMT, reaffirming the need for methodical systematic investigations of FMT in different patient populations with IBD.

The first reported use of FMT in IBD was by Bennet and Brinkman in 1989 [17]. Bennet, both a physician and a patient with medically refractory ulcerative colitis (UC), treated himself with large-volume retention enemas of donor flora.
Flexible sigmoidoscopy at 3 months after transplant demonstrated chronic but no active inflammation, and, at 6 months after FMT, he was symptom-free and off all medications.

The most convincing evidence for FMT in IBD is based on several case series from Borody’s group in Australia [8, 9, 18, 37, 38]. In 2006, Borody and colleagues reported the results of daily FMT enemas for 5 days in 6 adults with severely active UC. Clinical improvement was noted as early as 1 week after FMT, and complete symptomatic remission was achieved in all patients by month 4. All subjects displayed no clinical, endoscopic, or histological evidence of active UC 1–13 years following treatment [9].

In March 2013, Kunde et al. [19] reported the first series of pediatric patients with IBD treated with FMT. They described 10 youths, aged 7–21 years, with mildly to moderately active UC treated with FMT enemas daily for 5 days. Al-

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Disease</th>
<th>Patients</th>
<th>Delivery mode and frequency</th>
<th>FMT donor</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bennet et al.</td>
<td>1989</td>
<td>severe UC</td>
<td>1</td>
<td>large-volume enemas for 1 week</td>
<td>?</td>
<td>clinical remission, no active inflammation at 6 months</td>
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<tr>
<td>Borody et al.</td>
<td>1989</td>
<td>active UC</td>
<td>1</td>
<td>not described</td>
<td>?</td>
<td>clinical, endoscopic, histological remission at 3 months</td>
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<td></td>
<td></td>
<td>small-bowel Crohn’s disease plus stenosis</td>
<td>1</td>
<td></td>
<td></td>
<td>clinical remission at 4 months</td>
</tr>
<tr>
<td>Borody et al.</td>
<td>2001</td>
<td>quiescent UC</td>
<td>3</td>
<td>enema daily for 5 days</td>
<td>?</td>
<td>clinical and endoscopic remission at 8–16 months</td>
</tr>
<tr>
<td>Borody et al.</td>
<td>2003</td>
<td>severe UC</td>
<td>6</td>
<td>enema daily for 5 days</td>
<td>family or close relation</td>
<td>clinical and histological remission at 1–13 years</td>
</tr>
<tr>
<td>Borody et al.</td>
<td>2012</td>
<td>active UC</td>
<td>62</td>
<td>not described</td>
<td>?</td>
<td>68% complete clinical remission, 24% clinical response, 8% no response; 12/21 ‘profound’ mucosal healing</td>
</tr>
<tr>
<td>Vermeire et al.</td>
<td>2012</td>
<td>medically refractory Crohn’s colitis and ileocolitis</td>
<td>4</td>
<td>nasojejunal infusion, three doses over 36 h</td>
<td>?</td>
<td>no clinical or histological response at 8 weeks</td>
</tr>
<tr>
<td>Kunde et al.</td>
<td>2013</td>
<td>mild-to-moderate UC (pediatric)</td>
<td>10</td>
<td>enema daily for 5 days</td>
<td>family or close relation</td>
<td>3/9: clinical remission at 1 week; 6/9: clinical response at 1 month; 1/10: unable to retain enema</td>
</tr>
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</table>

Some subjects may have been included in more than one report. ? = Donor not specified; IBS = irritable bowel syndrome.
though this small pilot study only followed the subjects for 1 month, 9 out of 10 subjects tolerated FMT, 3 out of 9 were in clinical remission at 1 week, and 6 out of 9 had a sustained clinical response at 1 month. These series highlight the fact that larger studies with long-term follow-up and evaluation of endoscopic and histological healing will be essential before FMT can be accepted as a therapy for IBD.

More recent reports of FMT in IBD have focused primarily on patients with IBD and recurrent CDI. The reason for this shift is 4-fold: first, FMT is becoming increasingly accepted as a treatment for recurrent/relapsing CDI; second, increasing oversight and regulation have limited the use of FMT; third, the incidence and virulence of CDI in patients with IBD has risen sharply in the past decade with a dramatic impact on morbidity and mortality [44]; fourth, CDI is more difficult to treat in patients with IBD. Results of FMT treatment of CDI in patients with IBD have been uniformly successful in the clearance of CDI. Unfortunately, the results in treating the underlying IBD have been mixed, with some patients going into remission, some having no change in their disease activity, and a few patients have had worsening of their disease [42]. Future studies that characterize the microbiota before and after FMT will help clarify if it is the bacterial load delivered, the specific bacteria in FMT, the underlying inflammation and immune activation, or other host factors that lead to worsening of disease.

Logistical Issues

Many protocols for FMT exist and have been proposed with variations in donor selection, screening tests, dose, pretreatment and patient preparation, and delivery methods. The Fecal Microbiota Transplantation Workgroup has outlined the most comprehensive and useful FMT guidelines to date; however, these were based on the recommendations for the use of FMT in CDI, not IBD [45]. Given the long list of immunosuppressive medications used by patients, as well as the other immune, genetic, and microbial factors in patients with IBD, a thorough evaluation will be essential before FMT protocols can be standardized for patients with IBD. Both those in favor of and those opposed to FMT agree that standardization of protocols is critical [2, 45, 46].

Although we cannot ensure uniformity of the product between donors, guidelines will help ensure that adequate safety measures have been taken. Figure 1 is a comprehensive checklist of the currently recommended donor history screening questions, and the recommend donor stool and serum screening tests. Given the novelty of this therapy in clinical practice, it is unclear if all of these
Eligibility screening questionnaire

- Social screening: high-risk sexual behaviors, illicit drug use, recent tattoos or body piercing, travel history, history of incarceration
- Past medical history: exclude those with GI disorders, metabolic syndrome, malignancy
- Past surgical history: exclude those with major GI surgery, polyps
- Antibiotic and probiotic exposure
- Exclude acute illness or fever
- Risk factors for exposure to transmissible diseases including variant Creutzfeldt-Jakob disease
- History of transplant, skin graft, or recent transfusion
- Assessment of decisional capacity and willingness to donate without coercion

Serum screening tests (within 4 weeks of donation unless otherwise indicated)

- HIV 1 and 2 EIA (within 2 weeks of donation)
- Hepatitis A IgM, hepatitis B surface Ag and core Ab, hepatitis C Ab
- Treponema pallidum for syphilis with RPR or VDRL and FTA-Abs

Not required but may be indicated or recommended:

- Cytomegalovirus IgG and IgM
- Epstein-Barr Virus IgG and IgM
- Human T lymphotropic virus types I and II antibodies

Stools screening tests

- Culture for enteric pathogens: *Escherichia coli*, *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *Vibrio*, and *Listeria*
- *Clostridium difficile* toxins A&B PCR
- Fecal *Giardia* and *Cryptosporidium* Ag by DFA
- Microscopic examination for ova and parasites
- Microscopic examination for *Cyclospora*, *Microsporidium*, and *Isospora*

Pre-donation confirmation questionnaire

- Have you developed diarrhea since the initial screening?
- Have you been ill since the initial screening?
- Have you used antibiotics since the initial screening?
- Have you traveled abroad since the initial screening?
- Has your sexual activity changed since the initial screening?
- Are you currently ill or febrile?

<table>
<thead>
<tr>
<th>Eligibility screening questionnaire</th>
<th>Serum screening tests (within 4 weeks of donation unless otherwise indicated)</th>
<th>Stools screening tests</th>
<th>Pre-donation confirmation questionnaire</th>
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<tbody>
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<td>Social screening: high-risk sexual behaviors, illicit drug use, recent tattoos or body piercing, travel history, history of incarceration</td>
<td>HIV 1 and 2 EIA (within 2 weeks of donation)</td>
<td><em>Escherichia coli</em>, <em>Salmonella</em>, <em>Shigella</em>, <em>Yersinia</em>, <em>Campylobacter</em>, <em>Vibrio</em>, and <em>Listeria</em></td>
<td>Have you developed diarrhea since the initial screening?</td>
</tr>
<tr>
<td>Past medical history: exclude those with GI disorders, metabolic syndrome, malignancy</td>
<td>Hepatitis A IgM, hepatitis B surface Ag and core Ab, hepatitis C Ab</td>
<td><em>Clostridium difficile</em> toxins A&amp;B PCR</td>
<td>Have you been ill since the initial screening?</td>
</tr>
<tr>
<td>Past surgical history: exclude those with major GI surgery, polyps</td>
<td>Treponema pallidum for syphilis with RPR or VDRL and FTA-Abs</td>
<td>Fecal <em>Giardia</em> and <em>Cryptosporidium</em> Ag by DFA</td>
<td>Have you used antibiotics since the initial screening?</td>
</tr>
<tr>
<td>Antibiotic and probiotic exposure</td>
<td>Not required but may be indicated or recommended:</td>
<td>Microscopic examination for ova and parasites</td>
<td>Have you traveled abroad since the initial screening?</td>
</tr>
<tr>
<td>Exclude acute illness or fever</td>
<td>Cytomegalovirus IgG and IgM</td>
<td>Microscopic examination for <em>Cyclospora</em>, <em>Microsporidium</em>, and <em>Isospora</em></td>
<td>Has your sexual activity changed since the initial screening?</td>
</tr>
<tr>
<td>Risk factors for exposure to transmissible diseases including variant Creutzfeldt-Jakob disease</td>
<td>Epstein-Barr Virus IgG and IgM</td>
<td></td>
<td>Are you currently ill or febrile?</td>
</tr>
<tr>
<td>History of transplant, skin graft, or recent transfusion</td>
<td>Human T lymphotropic virus types I and II antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assessment of decisional capacity and willingness to donate without coercion</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Fig. 1.** Checklist for fecal microbiota transplantation donors for patients with IBD. Ab = Antibody; Ag = antigen; DFA = direct fluorescent assay; EIA = enzyme immunoassay; FTA-Abs = fluorescent treponemal Ab-absorption; GI = gastrointestinal; Ig = immunoglobulin; NAT = nucleic acid test; PCR = polymerase chain reaction; RPR = rapid plasma regain test; VDRL = Venereal Disease Research Laboratory test.

Screening tests are appropriate or cost-effective. Patients have raised concerns about the impact of donor diet, exposure to toxins, and other environmental factors that are not easily measured by currently available screening tests [20]. Although we cannot screen for ‘everything’, FMT protocols will need to be continuously evaluated and updated to ensure the safest standards possible. As we gain a better understanding of the role the microbiome plays in IBD, future screening protocols may vary based on patient factors such as disease type, severity, immune status, concurrent medications, and genetic profiles.

In addition to these screening tests, potential fecal donors are asked to complete detailed screening questionnaires, with as many as 50 questions ranging
from whether or not the donor has had high-risk sexual behavior, has tattoos and piercings, to stooling patterns and antibiotics exposure [5, 45]. None has been specifically designed for donors of patients with IBD, but these questionnaires may also need to be tailored to specific patient populations. For example, in recalcitrant CDI, the majority of donors have been a spouse or family member; however, given the role of genetics in IBD, nonrelated donors may be more appropriate.

Although we can extensively screen and interrogate donors, there is no way to guarantee the safety or potential long-term impacts of FMT until it is studied in a prospective controlled manner. Some groups have moved to using anonymous banked donor stool, which will help control some of the confounding factors and variability [4]. It is also likely that as our understanding and characterization of the human microbiome evolves, we may be able to ‘match’ donors and patients based on microbial fingerprinting, further assuring us of both the safety and expected therapeutic response.

**Patient Interest in Fecal Microbiota Transplantation**

Despite the unconventional nature of this therapy, there is a demand from patients requesting this procedure. A Google search for ‘fecal transplant’ reveals over 700,000 news articles, scholarly articles, and blog posts [47]. Included in these are numerous sites and posts that promote FMT ‘retreats’ as well as do-it-yourself instructions [48, 49]. The safety and legality of such programs is questionable especially given the recent move by the US Food and Drug Administration (FDA) to require an Investigational New Drug (IND) for all patients undergoing FMT. While being a more ‘natural’ treatment option is one of the aspects patients like the best [20], consultation with a gastroenterologist who will perform the procedure is essential to ensure proper screening, monitoring, and safety.

Given our interest in ethical and social issues related to innovative therapies, we sought to better characterize how patients and families perceive FMT. We began with a qualitative study that looked at six focus groups for adult patients with UC and for parents of children with UC or indeterminate colitis. The participants, who consisted of 15 adult patients and 7 parents, were asked about perceptions of and interest in FMT. Despite the lack of evidence about the safety or efficacy of FMT, most participants felt that with adequate donor screening, FMT was ‘natural’ and safe compared with conventional therapies. All but one of the participants expressed interest in the treatment, and despite the ‘yuck factor’, many wished it were already an option [20].
To further evaluate patient interest in FMT, we developed a follow-up survey study of 95 adult patients with UC who were given a brief description of FMT using lay terminology, followed by 38 questions about FMT, their disease activity, clinical effectiveness, and satisfaction with current treatments. Only 11% were unwilling to consider FMT. However, most interesting was the fact that despite reporting satisfactory to excellent management of their disease, almost half (46%) were willing to undergo FMT and 43% were willing to consider it. Primary concerns about FMT included adequate screening of fecal matter for infections, cleanliness, and potential to worsen UC; however, 21% reported no specific concerns [50]. These findings reaffirm patient interest in this innovative therapy and the need for additional investigations.

Physician Perspectives

While there is increasing patient interest in FMT, not all physicians are as enthusiastic. Critics have cited a number of concerns including: the ‘yuck’ factor, logistical issues, adequate donor screening, the lack of standardized preparation and delivery, and safety measures [22, 23, 46]. Others have noted that physicians may have concerns due to the fact that donor screening and the transplant itself may not be covered by insurance [21]. Furthermore, there are some that have cited the potential problems due to variability in fecal matter between donors, the absence of information about the specific microbial content of each preparation, transfer of disease-associated microbes, and the lack of mechanistic data to support its efficacy [51, 52].

El-Matary et al. [46], in their critique of FMT, also raised major concerns about patients performing FMT at home without medical supervision due the availability of stool. We agree that this is a significant concern; however, delaying clinical trials of FMT will not prevent patients from undertaking this risk. In reality, it may have the opposite effect and encourage patients to ‘treat’ themselves if they do feel that doctors are not adequately investigating this potential therapy.

Although there have been no studies to date investigating physicians’ attitudes and concerns about FMT, the growth in the number of groups publishing on the subject suggests a fair number of providers believe that additional studies are justified and essential to advance our understanding of FMT for IBD. In fact, it is important to note that even the strongest proponents of FMT agree that standardization of protocols and more rigorous investigations are necessary before it is accepted as a therapy for IBD and other gastrointestinal diseases [53–57]. Regardless of the therapeutic potential of FMT for IBD, physicians and sci-
entists agree that the microbiome is an important therapeutic target and in the coming years will lead to the development of new drugs and approaches to treating disease [58–60].

Ethical, Legal, and Social Issues

As with any innovative experimental therapy, there are numerous ethical, legal, and social issues (ELSI) that need to be addressed. Recognizing the need for research on this topic, the HMP has specifically called for research into microbiome-related ELSI [1]. After its inception, McGuire et al. [61] outlined specific ethical, legal, and social considerations, as they related to the HMP, including: informed consent, respect for autonomy, informing subjects of research-related results, data sharing, privacy protection, invasiveness of sampling and minimizing risk, diversity of subjects, and justice. Unfortunately, to date, there has been little research progress on these key identified issues with the exception of two qualitative studies describing perspectives of HMP investigators and healthy HMP participants on informed consent, data sharing, return of results [62], commercialization, and regulation of probiotics and supplements [63]. Of note, this is in stark contrast to the thousands of ELSI publications, workshops, and references produced by a similar large-scale National Institutes of Health initiative, the Human Genome Project [64]. It is unclear why the HMP is lagging in its ELSI research, but it is evident this will continue to be an important area of research in the future.

There has also been very little published on ELSI and FMT. Three studies have evaluated patient perspectives and attitudes towards FMT, two by our group on FMT in IBD [20, 50] and one by Zipursky et al. [65] on FMT in recurrent CDI. All of the studies confirm patient interest in FMT despite the ‘yuck’ factor and highlight the fact that patients are interested and invested in exploring this potential treatment.

Beyond stigma and patient interest, there are still numerous issues that necessitate additional ethical, legal, and social investigations. In figure 2, we outlined some of the most important and pressing ELSI related to FMT. This list is by no means exhaustive, and as our scientific and clinical knowledge about FMT grows, the ELSI will continue to evolve.

Among the most pressing ELSI are the regulatory and commercialization issues. The dearth of concrete evidence for FMT in IBD has been compounded by safety concerns and regulatory issues. In the US, the FDA now requires an IND application to be submitted for any use of FMT, either for research or treatment purposes [66]. Others have argued that FMT has not been more ag-
Progressively studied because it is not profitable [67]. Nonetheless, a few groups in the US, Canada, and Europe have begun to enroll IBD patients in pilot studies of FMT. Hopefully, these results will soon be forthcoming so that we can begin to answer the important clinical, microbiological, and ethical questions related to FMT.

### Clinical issues affecting doctors, healthcare providers, patients, and the general public
- How do we prepare healthcare professionals for FMT and other microbiome-based therapies?
- How do we prepare the public to make informed choices about FMT?
- How do we as a society balance current scientific limitations and social risk with long-term benefits?
- Who will be responsible for maintaining FMT donor banks and the associated costs?

### Uncertainties associated with potential impact of FMT in vulnerable populations
- Should critically or terminally ill or immunocompromised patients be considered candidates for FMT?
- Should FMT be performed on patients when there are no other treatment options available?
- Should there be age limits for children and the elderly?
- Should pregnant or nursing women be allowed to be FMT donors?
- Should pregnant or nursing women be eligible for treatment with FMT?

### Privacy, protection, and confidentiality
- How do we ensure protection of donor privacy and confidentiality?
- How do we inform donors of a positive screening test result?
- Should minors be allowed to be FMT donors?
- Who owns and controls microbiome information obtained through FMT research?

### Psychological impact and stigmatization
- How will FMT affect an individual and society's perceptions of that individual?
- How will FMT donation affect donors and society's perceptions of FMT donors?
- How does personal microbiome information affect an individual and society's perceptions of that individual?

### Conceptual and philosophical implications and concepts of health and disease
- How will a diagnosis of dybiosis impact an individual and society's perceptions of that individual?
- How will FMT alter perceptions of health and disease?

### Fairness and justice
- Who will be eligible for FMT?
- Who will cover FMT donor screening costs?
- Who will cover the costs of FMT?
- Should unrelated donors be compensated?
- How will treatment with FMT impact insurability?

### Legal issues and regulation
- Who should be allowed to perform FMT?
- Who should regulate FMT?
- Who should be responsible for monitoring FMT programs and safety?

### Commercialization of FMT including property rights and accessibility of data and materials
- Can fecal microbiota profiles be patented?
- Can FMT delivery methods be patented?
- Will patenting of fecal microbiota profiles limit their accessibility and development into useful products?

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**Fig. 2.** ELSI related to FMT. Adapted from The Human Genome Project Information on Ethical, Legal, and Social Issues (US Department of Energy Genome Programs: http://genomics.energy.gov).
Conclusion

FMT is a novel but unproven approach to the treatment of IBD. In addition to the issues raised in this review, we still have the important question of how FMT works and how it will impact our treatment and understanding of IBD. Successful therapeutic innovation must strike the balance between advancing science and patient care on one side, and methodical rigorous research and patient protection on the other. We look forward to completing such research in the near future.

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References


Abstract
Inflammatory bowel disorders (IBD) are characterized by chronic and recurrent inflammatory reactions of the intestinal mucosa resulting in progressing ulcerating lesions. Research over the past decade clearly identified in patients with Crohn’s disease (CD) a marked dysregulation of the intestinal microbiome (dysbiosis) as one trigger factor in these inflammatory processes, particularly in patients with a high genetic risk. When treating patients with CD, most drugs aim to control the inflammatory process (either by inhibiting inflammatory pathways or by reducing the activity of immune cells). Given the importance of the disturbed interaction between the microbiota and the host immune system, there might be a different therapeutic approach in targeting directly the intestinal microflora. There are good data to believe that the use of exclusive enteral nutrition (EEN) is one such option. Historically, enteral nutrition (EN) was used as supplemental nutritional therapy in CD patients with planned resection surgery. This treatment option showed unexpected and very powerful anti-inflammatory effects, and it was rapidly introduced as induction therapy for active CD. Several clinical trials and case series confirmed the efficacy of EN to induce remission in approximately 80% of patients equaling the potential of steroids. It is well established that EN has this strong anti-inflammatory potential only when given on an exclusive basis, without any additional food. This raises major compliance issues, probably one of the reasons why it is less used in adult patients. A recent study demonstrated that EEN has a specific effect on the intestinal microbiota, which is markedly different from steroid-induced remission, while all patients obtained complete clinical remission. These observations give a first basis for the understanding of the impact of EEN on dysbiosis in patients with CD.
Introduction

Inflammatory bowel disorders (IBD) are chronic recurrent inflammatory diseases affecting the intestinal mucosa. The inflammatory reaction is characterized by a polymorphic infiltrate causing alterations of the epithelial cell turnover and barrier with subsequent erosions and ulcerations of the intestinal mucosa. These inflammatory lesions cause symptoms such as diarrhea, intestinal bleeding and abdominal pain, as well as systemic symptoms, such as fever in some patients. The precise causes triggering this chronic inflammatory reaction are not yet completely understood; research over the past decade clearly identified in patients with Crohn’s disease (CD) a dysregulation of the intestinal microbiome (dysbiosis) as one key trigger factor [1–3]. However, up to now, it is not clear if this dysbiosis is primary and thus causative of the inflammatory reaction or if it occurs secondary once the inflammation is installed. Large prospective cohort studies in North America and Europe are underway to address this point. The disturbed microbial-immune interaction leading to chronic intestinal inflammation is more likely to occur in individuals with a particular genetic risk. Up to now, over 150 susceptibility genes were identified which confer a particular risk to develop CD or ulcerative colitis [4–7]. Therefore, we consider the distinct phenotypes of IBD as multifactorial disorders with a complex genetic background and not yet identified environmental trigger factors affecting the homeostatic interaction between intestinal microbiome and the host’s immune system.

Treatment strategies for patients with CD are based on a rationale aiming to control the inflammatory process (either by inhibiting inflammatory pathways or by reducing the activity of immune cells). Over the last decade, several new drugs, mainly biologics were tested and subsequently introduced in treatment strategies [8–10]. These drugs are highly efficient in blocking the immune system and thereby in inducing remission in patients. Their safety profile being good, there are still important side effects that can occur, even if they are rare. Mainly infectious and aberrant immune reactions have been reported; however, some cases of tumors and cancers were also attributed to the (prolonged) use of immunosuppressants [11, 12]. Given the importance of the disturbed interaction between the microbiota and the host immune system, there might be a possibility for a different therapeutic approach in targeting directly the intestinal microflora. There are good arguments to believe that the use of exclusive enteral nutrition (EEN) is one such option. The perfect safety profile of this treatment approach is an additional advantage to use it, especially in vulnerable patients, such as children.
Use of Nutritional Therapy for Crohn’s Disease

Historically, enteral nutrition (EN) was used as supplemental nutritional therapy in adult patients with CD complicated by marked malnutrition as well as in undernourished and severely growth-retarded children with CD. The idea was to improve the nutritional status of patients prior to resection surgery. This treatment option showed unexpected and very powerful anti-inflammatory effects, and some patients did not require resection surgery anymore. Thus, EN was rapidly introduced as induction therapy for active CD. It is important to use EN appropriately to obtain its anti-inflammatory effects [13]. There is evidence that supplementary EN is not efficacious enough to induce remission in CD, while when given on an exclusive basis, without any additional food, a high anti-inflammatory effect can be obtained [14] (see below).

Efficacy of Exclusive Enteral Nutrition as Treatment Option for Crohn’s Disease

It is now well established that EEN has a strong anti-inflammatory effect with reduction of systemic and mucosal inflammatory parameters within few days [15]. So far, no controlled RCT compared EEN to placebo in children with CD. But several studies compared EEN with steroids leading to two pediatric meta-analyses as well as a Cochrane review combining pediatric and adult data and analyzing the efficacy of EEN as induction therapy for CD [16–18]. In the RCTs that compared EEN to steroids and used remission rates as outcome parameter, an overall combined remission rate of approximately 75% for EEN at the end of exclusive treatment has be shown. There was no marked difference to the remission rates obtained with steroid medication (fig. 1) in these two meta-analyses based on a total of 11 pediatric RCTs [19–28]. A further pediatric RCT was recently published [29], and several small open-labelled studies looked on the anti-inflammatory potential of EEN. It is somewhat challenging to summarize efficacy of EEN based on these open-labelled studies since there are major differences in the way EEN is performed with regard to the duration of exclusive EN, feed type, as well as the way efficacy is measured [30, 31]. Two recent large single-center studies, each based on more than 100 pediatric CD patients, further support the results of the RCTs, and both show a remission rate of approximately 80% [32, 33]. It is interesting to pin point that in the pediatric experience EEN has the same potential to induce remission as steroids, contrasting with adult data. These excellent results for EEN occur in IBD centers that regularly use EEN as a treatment option; however, in centers that rarely or almost never use EEN,
remission rates differ significantly, indicating that the successful use requires some experience.

Many different liquid nutritional products were tested in the treatment of CD, and as demonstrated by randomized controlled trials in children and in adults, the protein source in the feed does not affect its efficacy [27–29, 34]. Efficacy does not depend on the protein nature; polymeric as well as elemental feeds have the potential to equally induce remission in CD patients. However, acceptability and cost of EN differ markedly between elemental diet and polymeric feeds. Elemental feeds are less often tolerated by mouth, and patients most often require a nasogastric tube for treatment that is less well accepted by patients. In contrast, in patients receiving polymeric feeds [32], oral use is possible, making it the first choice for patients. We recently demonstrated that there is no significant difference in the potential to induce remission between 8 weeks of oral or continuous nasogastric feeds [32]. This is in keeping with studies that have given polymeric feeds only by nasogastric feeds: results were similar between children treated with oral or a combination of oral and nasogastric feeding. Therefore, in our center, we always offer patients oral feeds with a polymeric formula, while EEN via nasogastric tube remains reserved for patients unable to achieve the desired caloric intake or who refuse oral feed due to taste or texture. Elemental feeds should only be reserved for patient intolerant to cow’s milk proteins. It is of importance to offer an appropriate energy amount. Since most patients have weight loss and often growth retardation, the estimated energy requirements are above recommended intake; we most often
use at least 120% of normal caloric requirement adapted to the age and the estimated catch-up growth.

The most important detail in the use of EN as induction therapy is the fact to use it on an exclusive basis, without any additional foods. In their RCT (exclusive EN vs. partial EN with normal diet over 6 weeks using an elemental formula), Johnson et al. [14] showed clear superiority of full EEN over partial EN in remission rates at 6 weeks [10/24 (42%) vs. 4/26 (15%)]. It is important to point out that in this study the induction of remission by EEN is markedly lower than most other published studies, and that there was a high dropout rate in both arms [14]. As highlighted in this study, compliance is a major issue, and probably one of the reasons why EN is less used in adult patients. Compliance is a priori not better in children, but since they have most often marked growth retardation and EEN allows efficiently gaining catch-up growth, their motivation to follow a complete cycle of EEN is in most studies excellent. In our personal experience, adherence to EEN is close to 90%, probably due to the way we monitor patients on EEN. They have a regular home visit by a dietician or nurse and patients are always seen within 4 weeks from starting EEN.

The duration of EEN treatment in clinical studies varies considerably (2–12 weeks), but the majority of clinical centers routinely use cycles of 6–8 weeks. As already mentioned, inflammatory parameters drop within days of starting EEN [15, 35, 36], but weight gain and catch-up growth require a longer treatment period. In addition, there is some evidence that use of at least 8 weeks of EEN allows to induce mucosal healing. Six different studies analyzed the potential of EEN to induce mucosal healing, with healing rates from 19 to 75% [15, 26, 32, 36–38]. There are clear differences between these studies in terms of definition of mucosal healing which make them difficult to compare. The rate of mucosal healing was markedly higher in patients on EEN compared to steroid-induced remission [26, 37]. One RCT included mucosal healing as outcome parameter, indicating a clear superiority of 10 weeks’ EEN compared to steroids, with mucosal healing rates of 74 versus 33% for EEN and steroids, respectively [26].

Recent studies measuring fecal calprotectin during EEN noted reduction in values continues up to 8 weeks. At the end point, many patients still have not achieved normal values, despite being in clinical remission with normalization of CRP, providing proxy evidence that longer courses may be more advantageous [29, 39].

Who Are Optimal Candidates for Exclusive Enteral Nutrition?

Initially, EEN was thought to work only in CD patients with small bowel disease, with some studies showing differential healing rates between ileal and colonic lesions [15, 40]. However, when cumulating all available stud-
ies, there is evidence supporting EEN as induction therapy for all CD patients with luminal disease regardless of site, which is further supported by the Cochrane meta-analysis. Indeed some recent studies specifically looking at patients with isolated colonic disease confirmed that remission rates are not different in this subgroup of patients from children with small bowel involvement [17, 32, 33]. There are no clear data regarding isolated perianal (and also oral) lesions; in some patients, these lesions tend to improve, but no study analyzed this adequately. It is important to mention that so far no study showed improvement of fistula with EEN. It seems that in CD luminal inflammatory lesions respond to EEN, while penetrating lesions do not. There are no data to support the use of EEN in patients with ulcerative colitis.

**Mode of Action**

While there is no doubt about the efficacy and the potential of EEN to treat CD, many questions arise regarding its mode of action. It was discussed that the reduced allergenic load, nucleotide-free diet, no addition of food additives, and anti-inflammatory lipid composition explain its efficacy. These might all be relevant factors in the anti-inflammatory reaction by the immune system which manifests within few days. A new hypothesis was developed recently in that EEN has a specific effect on the intestinal microbiota potentially correcting the observed dysbiosis in CD patients. First studies analyzed changes in the microbiome during and after EEN [41–43]. A recent study compared the composition of the intestinal flora of a CD patient with EEN-induced remission with that of a CD patient with steroid-induced remission [43]. This study showed that the microbiota is significantly different in patients with EEN-induced remission from that in patients with steroid-induced remission, while all patients were in complete clinical remission. These observations give a first basis for the understanding of the impact of EEN on dysbiosis in patients with CD.

In conclusion, EEN is a very safe and potent strategy to induce remission in patients with luminal active CD. Clinical trials showed that it works in pediatric and adult CD patients, but not in ulcerative colitis. The acceptability and compliance with the exclusivity principle of EN is the major obstacle of this strategy. As it became clear that oral use is as efficacious as the enteral route via nasogastric tube, the use of EEN might increase in the future. Growth retardation is a major indication for the use of EEN; therefore, it is more common in pediatric IBD centers, but there is no reason not to use EEN for treating adult patients.
with luminal CD. It constitutes a real alternative to immunosuppressive therapy for the treatment of CD due to its excellent safety profile and probably also its mode on action on top of the inflammatory cascade.

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The Future of Nutrition in IBD


Effects of Exclusive Enteral Nutrition on Bone Mass, Linear Growth and Body Composition in Children with Crohn’s Disease

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Abstract
Inflammatory bowel disease, especially Crohn’s disease, is linked to significant growth stunting, sarcopenia (loss of skeletal muscle mass), deterioration of bone architecture and reduction in bone mass. Exclusive enteral nutrition (EEN) has been shown to correct nutritional deficiencies, provide adequate calories for growth, and alleviate intestinal inflammation in Crohn’s disease with a favorable adverse effect profile. In this chapter, we report a summary of the effects of EEN on linear growth, skeletal health and lean body mass in children with Crohn’s disease.

Introduction
Children with inflammatory bowel disease (IBD), especially Crohn’s disease, are particularly vulnerable to disease and treatment factors that can affect physical development. Most children with IBD are diagnosed during puberty or right before puberty, which is usually a time of acceleration of physical growth. Normally during puberty children achieve peak height velocity and accumulate a significant amount of muscle mass and bone mass [1, 2]. During active growth, the length and shape of long bones are determined by the combined activity of cells in the growth plate, osteoclasts that expand the medullary cavity and osteoblasts that appose bone matrix in the periosteal surface, which is later mineralized. This process is called bone modeling [3]. In addition, the significant expan-
sion of skeletal muscle mass produces large mechanical forces that induce bone apposition during growth [4]. Endocrine pathways such as sex steroids and insulin-like growth factor 1 are also critically important in promoting linear growth and inducing gains in lean body mass and bone mass. Proper nutrition, absorption and nutrient utilization are required to fuel the large metabolic demands generated by physical growth and development during childhood.

IBD (especially Crohn’s disease) can affect these developmental processes due to disease factors such as inflammatory cells and cytokines, poor caloric intake and malabsorption, decreased physical activity, delayed puberty, end-organ resistance to anabolic growth factors and also medications to treat IBD (i.e. glucocorticoids) [5–10]. Therefore, significant reductions in linear growth and alterations in bone mass, bone architecture and body composition are frequently present in children with IBD, even at diagnosis [5, 7, 8, 11]. While conventional induction of remission with a glucocorticoid followed by maintenance mesalamine or an immunomodulator does not accelerate linear growth in children with Crohn’s disease [12], exclusive enteral nutrition (EEN) has shown promise in restoring normal pubertal development, linear growth and body composition [13, 14].

**Exclusive Enteral Nutrition, Growth and Skeletal Development**

EEN is an effective therapy to induce and maintain remission in Crohn’s disease [15, 16]. EEN consists of ingesting a commercially available formula exclusively for 6–8 weeks instead of food. Remission rates with EEN range from 50 to 85% in pediatric Crohn’s disease, depending on the study. Patients with small bowel involvement may derive more benefit from EEN than patients with primary colonic Crohn’s disease [17]. Typically, an immunomodulator is started shortly after commencing EEN with the goal of maintaining remission induced by EEN in patients with Crohn’s disease.

EEN can be administered continuously overnight or orally (intermittent boluses). Both dosing schemas are equally efficacious to induce remission in pediatric Crohn’s disease, although continuous administration of EEN results in better weight gain [18]. While elemental formulas were used initially to provide EEN, subsequent studies have shown that they do not offer any advantage over polymeric formulas in terms of their anti-inflammatory effect in Crohn’s disease, and polymeric formulas are cheaper and more palatable [13, 19]. Despite the better palatability of polymeric formulas, most children on EEN opt to administer the formula via nasogastric tube and in some cases a gastrostomy tube to bypass the need to ingest the formula for many weeks.
The many advantages for induction of remission in pediatric Crohn’s disease of EEN over glucocorticoids notwithstanding, the use of EEN is limited in the United States compared to Australia and Europe, where it is considered first-line therapy of pediatric Crohn’s disease. The monotony of EEN, the likely need for a feeding tube, and physician and patient attitudes are barriers to the more widespread use of EEN in the United States [20]. Alternate schemas of enteral nutrition (EN) that allow one small regular meal a day in addition to formula are being studied to improve acceptance of EN as a treatment modality for pediatric Crohn’s disease [21]. The adverse effects of EEN include nocturnal awakening to urinate, sensation of morning satiety, nausea, vomiting, diarrhea, and complications secondary to using feeding tubes.

The mechanisms by which EEN alleviates Crohn’s disease are not yet known, but probably involve a combination of factors, including nutritional rehabilitation, modification of the intestinal microbiome, with consequent provision of short-chain fatty acids, a preferred fuel of intestinal epithelial cells [22] and a decrease in the antigenic load delivered to the intestinal mucosa [13]. As a consequence, EEN reduces inflammation, heals the intestinal mucosa, improves nutrition, and allows glucocorticoid withdrawal [23].

EN, even when not exclusive, may have anti-inflammatory benefits to patients with Crohn’s disease. For example, logistic regression analysis in a cohort of 74 adults with Crohn’s disease in Japan who were on maintenance therapy with infliximab and supplemental nutrition revealed that concomitant use of EN ≥600 kcal/day was an independent factor associated with sustained response to infliximab [24]. In another retrospective cohort study in 102 adults with Crohn’s disease, using EN together with maintenance infliximab was associated with improved remission rates compared to infliximab alone [25]. While these findings need to be confirmed in prospective studies, they suggest that supplementation with EN may be an adjuvant to treatment with infliximab in patients with Crohn’s disease.

EEN has a number of beneficial effects on body composition. EEN is associated with significant weight gain [26] and in some studies it appeared to accelerate linear growth in children with Crohn’s disease [27], although not in others [26]. With respect to bone health, EEN for 8 weeks was linked to an increase in serum bone alkaline phosphatase, a biomarker of bone formation, and a decrease in serum collagen C-telopeptide, a marker of bone resorption in newly diagnosed children with Crohn’s disease, suggesting that EEN is anabolic to bone [28]. Whether this increase in bone anabolic activity translates into sustained gains in bone mass over time is less well established. EEN also results in significant short-term gains in lean body mass [29], which probably reflects, at least in part, muscle mass accrual [29]. This is important because sarcopenia as-
associated with pediatric Crohn’s disease reduces bone mass and alters bone architecture [30, 31]. It follows that correcting sarcopenia in Crohn’s disease by EEN may be anabolic to bone. In this regard, Werkstetter et al. [14] recently reported improvements in bone geometry and muscle mass in 10 children with newly diagnosed Crohn’s disease treated with EEN. In this study, they induced remission with EEN (8/10 patients achieved remission) and maintained patients on an immunomodulator. They used peripheral quantitative computed tomography of the forearm to measure bone mass, bone architecture and muscle mass. They observed improved trabecular bone density and increased cortical bone turnover at 12 weeks, with no further improvements. Interestingly, muscle mass also improved, but only in the first 12 weeks of the study [14]. Taken together, these data suggest that the benefits of EEN may not be sustained once EEN is stopped, making an argument to study intermittent administration of EEN (or supplemental EN) to improve body composition outcomes in Crohn’s disease.

**Conclusion**

EEN induces remission in children with Crohn’s disease and has many beneficial effects on body composition, at least while EEN is being delivered. Additional studies are required to improve acceptance of EEN in North America among patients with Crohn’s disease and their physicians, and to achieve sustained effects of EEN on growth and bone and muscle mass in children with Crohn’s disease.

**Disclosure Statement**

The author declares that no financial or other conflict of interest exists in relation to the content of the chapter.

**References**


Abstract
Crohn’s disease (CD) is a complex inherited disorder of unknown pathogenesis. Recent evidence suggests that CD may involve genetic or environmental factors that impair the normal innate immune system’s ability to contain bacteria to the lumen. Multiple dietary components may impact on the resident flora, diminish or damage the mucous layer, increase intestinal permeability or increase the ability of pathobionts to adhere to epithelial cells or translocate across the epithelial barrier. This chapter reviews the possible effects of different dietary components present in the Western diet to affect bacterial clearance mechanisms, and offers a hypothetical model for an acquired bacterial clearance defect in CD.

Introduction
Crohn’s disease (CD) is caused by a combination of environmental and genetic factors. Dysregulation of the innate immune response to luminal microbial or nutritional antigens seems to play a major role in the pathogenesis of CD [1–6].

The subsequent proinflammatory response involves primarily T helper (TH)1 and TH17 cells, characterized by the secretion of tumor necrosis factor-α, interferon-γ, interleukin (IL)-12, and IL-23 in response to exposure to bacterial products [2, 5, 6]. Bacteria play a major role in the pathogenesis of CD, as they are found in granulomas, and are adherent to the intestinal mucosa [2, 5, 6]. Microbial biofilms may also trigger an adaptive immune response.
The microbiome of patients with CD is now known to differ from that of healthy subjects (manifested as reduction in diversity, a reduction in Firmicutes, with increased Enterobacteriaceae) [7–10]. Disease-specific inflammatory bacteria isolated from ileal tissue from patients with CD, such as adherent-invasive Escherichia coli (AIEC), are shown to replicate in macrophages and epithelial cells under certain circumstances and are abundant in patients with CD [11, 12].

Although progress has been achieved in determining the genetic and immune basis for susceptibility to the disease, understanding the contribution of potential environmental risk factors has been difficult. Exposure to infections as risk factors has been the focus of interest of many studies. Similar to other autoimmune diseases, some epidemiological studies have suggested a role for the ‘hygiene hypothesis’, whereby exposure to infections in childhood confers protection against disease [13–15].

An alternative environmental factor, which has not been adequately explored, is the effect of diet on CD. Diet has a dramatic effect on the composition of the intestinal microbiome and gut immune status [16–18]. Understanding the environmental component is important as it may have implications for the treatment of children with CD, and secondly it could provide the foundation for disease prevention. There are multiple lines of evidence from epidemiologic, clinical and animal studies demonstrating an impact of dietary exclusion on treatment, or dietary exposure on the pathogenesis of intestinal inflammation.

Evidence for Individual Dietary Components and Patterns and Susceptibility to IBD

CD is most prevalent in Westernized countries with a clear North to South gradient [10]; countries with the highest prevalence also have increased exposure to increased Western diet, which includes more protein, animal fat, dairy products and industrialized food. Our daily diet consists of multiple traditional nutrients which can be easily grouped such as: carbohydrates, protein, fat, vitamins and minerals. These components have been evaluated primarily in retrospective case-control or epidemiologic studies. However, Western diet often includes increased exposure to combinations of protein fat and sugars, with increased consumption of one component (for instance fats) often leading to increased consumption of another component (i.e. sugars), and evaluation is hampered by assessment of food frequency questionnaires at diagnosis. Thus, individualization may be misleading, and may reflect dietary patterns.
Protein

Epidemiological studies have demonstrated that consumption of high animal protein is associated with a significantly higher risk of CD (animal protein intake IBD, nutritional risk of developing IBD) [19, 20]. In a French prospective study of 67,581 middle-aged women by Jantchou et al. [20] with a mean follow-up of 10.4 years, 77 cases of IBD were identified. High animal protein intake was associated with risk of IBD. In particular, a high consumption of meat and fish but not of eggs or dairy products was associated with IBD risk (hazard ratio 1.87, 95% CI: 1.00–3.49, p value = 0.02). However, animal protein consumption is usually associated with animal fat consumption, so it is unclear if this component can be adequately evaluated in isolation.

Carbohydrates

Carbohydrates can be divided into 3 main groups: monosaccharides and disaccharides (sugars), oligosaccharides (e.g. fructo-oligosaccharides, maltodextrin), and polysaccharides (e.g. starch, cellulose). Several studies have shown that an increase in monosaccharides and disaccharides is associated with a risk for CD [21–24].

Tragnone et al. [23] and Persson et al. [24] evaluated the relative risk (RR) based on sugar intake. Persson et al. [24] found that the RR of CD was increased in individuals with a high intake of sucrose (>55 g/day; RR 2.6, 95% CI: 1.4–5.0). Tragnone et al. [23] found a significantly higher RR associated with sugar intake in CD as well (RR 3.5, 95% CI: 1.5–8.1). The RR for patients with CD to have increased intake of sucrose was 2.6 (95% CI: 1.4–65.0) [25]. In a recently published Danish cohort, high consumption of sugar was associated with an increased risk for CD (OR 2.9, 95% CI: 1.0–8.5) [26].

Fat

Fats consist of many subgroups such as saturated fat, monounsaturated fat, polyunsaturated fats (PUFA), and trans fats. In epidemiological studies, increased consumption of monounsaturated fatty acids was associated with a higher risk of developing CD [27]. In a case-control study, D’Souza et al. [28] administered a semiquantitative questionnaire acquiring information on 151 foods, including variety and portions. The frequency of consumption assessed ranged from ‘never’ or ‘less than once per month’ to ‘6 per day’. In girls, high
intake of meats, fatty foods, and desserts was positively associated with CD (OR for the third vs. first tertile 4.7, 95% CI: 1.6–14.2, p = 0.006). A dietary pattern, characterized by high levels of vegetables, fruits, olive oil, fish, grains, and nuts, and was inversely associated with CD in both genders [28]. On the other hand, three different studies [23, 29, 30] have shown that there is no influence of the type of fat consumed on the risk for CD, but it should be noted that these studies are dated.

Fiber, Fruit and Vegetables

Fiber can be divided into two groups; fermentable and nonfermentable. Fermentable fibers are fermented by the flora in the gut, and short-chain fatty acids (SCFA) are produced as an end product. SCFA are considered anti-inflammatory metabolites that lower intestinal permeability (IP) [25]. Fiber is a component that has been shown to decrease the risk for CD in adults. Exclusive enteral nutrition (EEN) has been shown to alter SCFA production [31], and in particular to increase butyrate. High-fiber diets (>22.1 g/day) had a lower risk than low-fiber diets <13.8 g/day [32–34]. This is a very high amount of fiber and very difficult for the average person to consume – especially children. For example, a banana that is considered to be rich in fiber contains on average ~1.9 g of fiber and an apple with the peel has 3.5 g fiber; an average slice of whole-wheat bread contains around 2 g of fiber. A Danish study demonstrated that whole-meal bread that was rich in fiber may have a protective effect (OR 0.4, 0.2–0.9). The main problem is that patients tend to reduce their fiber consumption after the first flare, which may lead to a low-fiber diet. Many studies show that fruit consumption has a protective effect and thereby lowers the risk for CD [27]. With regard to vegetables, a recently published Danish cohort demonstrated that daily consumption of vegetables lowered the risk for CD (OR 0.39, CI: 0.1–1.0) [25–27].

Western Diet and Susceptibility to Develop Crohn’s Disease (Animal Models)

A Western diet is typically high in animal protein, total fat, n-6 PUFA and sugar. This trend has been the focus of animal studies that have aspired to find a connection between the Western diet and CD etiology. In a recently published study by Martinez et al. [35], CEABAC10 mice were compared with wild-type mice; both groups were fed a Western diet rich in fats and simple sugars (13.1%
protein, 60.6% lipids, 26.3% carbohydrates) or standard chow for 12 weeks. One group was exposed to AIEC. After 12 weeks of diet, there was a decrease in the diversity of bacteria in the colonic mucosa of both groups. The Western diet promoted the growth of *Bacteroides*, *Prevotella* and mucin-degrading bacteria in both AIEC+ and AIEC– groups, and increased IP. CEABAC 10 mice treated with the Western diet had higher levels of cytokines such as tumor necrosis factor-α. Fecal counts for AIEC were much higher in the CEABAC 10 group consuming the high-fat high-sugar diet. The increase in IP as a result of Western high-fat diet was put to the test in a study conducted by Suzuki and Hara [36]. They compared mutant obese mice with lean mice with 2 different diets – standard chow and high fat. After 3 weeks of a high-fat diet, there was a rise in IP; the high-fat groups had higher IP compared to controls (p < 0.05); IP increased by week 9, and was even higher by week 15. This may suggest that the effect of dietary exposure to fats is dose dependent. All these findings in cell and rodent models seem to indicate that Western/high-fat diet causes an increase in IP that can lead to increase in bacterial penetration of the epithelium [36]. In a study performed by Devkota et al. [37], three different diets were given to IL10-/- mice – low fat (LF), PUFA and milk-derived fat (MF); the 2 high-fat diets were isocaloric. The mice exposed to PUFA and the mice on the MF diet had a lower diversity of the microbiome compared to the LF mice. Higher counts of *Bacteroidetes* and low counts of *Firmicutes* were found in the PUFA and MF groups. The most interesting finding was the presence of *Bilophila wadsworthia* solely in the MF diet group. An increase in colitis was found in the MF group. The incidence of colitis was similar in the mice exposed to PUFA and LF. *B. wadsworthia* is a proinflammatory sulfite-reducing bacterium that is not found in healthy subjects; it grows in the presence of taurine-conjugated bile acids rich in organic sulfur; milk fats induce the production of taurine-conjugated bile acids which create very strong micelles, and that creates an optimal environment in the intestine for *B. wadsworthia* to grow as it is sulfur dependent [37].

**Additional Dietary Factors That Alter Intestinal Permeability and Microbiome**

The traditional approach to dietary components evaluates protein, fat, carbohydrates vitamins and minerals. However, Western dietary exposure includes increased exposure to emulsifiers and food preservatives.

Swidsinski et al. [38] evaluated the effect of carboxymethylcellulose (CMC), a preservative commonly found in bread, ice cream and dairy products. As reported by the food industry, the amount of CMC (E466) used in the industry is
growing every year. IL10-deficient mice were given 2% CMC solution, and a control group of mice were given water. The results showed that the mice given CMC had bacterial overgrowth especially between the villi and bacteria were adherent to the mucosa. The bacteria count in the CMC group increased from 0.001 to 6 × 10^8 ml in the jejunum and 2 to 30 × 10^8 in the ileum (p < 0.001). The water-treated mice had counts of 0.001 to 0.0001 × 10^8 in the jejunum and 0.001 to 4 × 10^8 in the ileum. Leukocytes were found within the lumen of the mice treated with CMC and not found in the water-treated mice [38].

Roberts et al. [39] evaluated the effect of an emulsifier polysorbate 80 (E433) as well as fiber on translocation of AIEC across follicular epithelium and Caco 2 cells. AIEC isolated from CD patients translocated across the M cells at a higher rate than through Caco2 cells (15.8-fold). Translocation was reduced by non-starch polysaccharides (NSP) from plantain and broccoli (p < 0.01); this association was dose dependent. On the other hand, NSP from leeks and apple did not prevent the translocation. Polysorbate 80 was found to significantly increase the translocation in M cells (p < 0.05), FAE (p < 0.01) and Caco2 cells (p < 0.05) in a dose-dependent manner [39].

Another food additive that alters the intestinal function is carrageenan (E407); it is a sulfated polysaccharide that is extracted from red seaweed. It is used in the food industry as a stabilizer of dairy and meat products [40]. In a study by Choi et al. [40], carrageenan reduced transepithelial resistance (TEER) in cell cultures; this was associated with discontinuous and irregular zonula occludens expression that increased IP [40].

Gliadin is present in wheat and many other cereals. Lammers et al. [40] showed that gliadin increases intestinal permeability by binding to CXCR3 on the enterocytes and releasing zonulin, leading to decreased TEER [41].

**Dietary Intervention and Remission in Crohn’s Disease**

While multiple dietary components seem to induce inflammation in animal models, the most important link between diet and CD comes from dietary interventions in children with active CD. EEN is a well-documented method of treatment. It involves placing children on a strict a standardized restricted diet composed of a single polymeric formula as the sole source of nutrition over 6–8 weeks. Use of this treatment method early in the disease (usually during the first year of disease) results in clinical remission in 50–80% of children by week 8 with no additional pharmacological treatment. We now have evidence that in mild to moderate uncomplicated disease, EEN is as effective as steroids for induction of remission, and it improves mucosal healing [42]. In a prospective study, Bu-
chanan et al. [43] reviewed 114 children who were treated by EEN over 8 weeks (51.2% were fed orally and 48.8% were tube fed). 80% of the children achieved clinical remission by week 8 with a significant reduction in ESR and CRP; the patients in remission also significantly improved the z score for weight and BMI. In their study in adults, Yamamoto et al. [44] examined the efficacy of an elemental formula for maintenance of remission after surgically induced remission. Forty patients after ileo- or ileocolonic resection were allocated into two groups; one received EEN for one year via overnight tube feeding, the other 20 patients were allowed free diet. Only 5% of the EEN group versus 35% of the free diet group had a clinical recurrence of the disease in the first year of follow-up. After one year, endoscopic follow-up was performed; 30% of the EEN versus 75% of the free diet group had an endoscopic recurrence [44]. Rubio et al. [45] reviewed 106 pediatric patients who were treated by EEN either orally or by tube feeding over 8 weeks. They demonstrated that the route of feeding was not significant as 75% of the patients on oral feeding versus 85% of those on tube feeding achieved clinical remission (p = 0.157) [45].

One of the pertinent questions regarding EEN that continue to crop up is the need for exclusivity. Is it the content of the formula that is beneficial, or is it the exclusion of normal diet that leads to remission? In a study performed by Johnson et al. [46], 50 children with active CD were randomized into two groups; one group was treated with EEN and the other was given partial enteral nutrition (PEN) with free diet. The remission rate was lower in the PEN + free diet group (15 vs. 40% in the EEN group, p = 0.035). Moreover, only the EEN group showed improvement in inflammatory markers. This study was the first to prove the principle of exclusivity. While the underlying mechanism of exclusivity is still unknown, it reinforces the concept that diet may be an important environmental trigger that should be further studied.

Fitting the Pieces to the Puzzle

An attractive but speculative hypothesis for the observed increase in the prevalence of IBD, and CD especially may be that a persistent change in dietary intake leads to the breakdown of the epithelial barrier. This might allow adherence, translocation, and penetration of bacteria that under normal conditions would be nonpathogenic in susceptible individuals (e.g. genetically determined Paneth cell dysfunction or defective autophagy). Persistent exposure to adherent or penetrating bacteria may then trigger the adaptive immune system, resulting in inflammation, further breakdown of the epithelial barrier, and increasing migration and sensitization to these bacteria; this could induce a vicious cycle that
we have termed the ‘bacterial penetration cycle’. Exclusive enteral nutrition, especially in early stages of small intestinal disease, might act by decreasing exposure to offending agents which induce dysbiosis, reduce the mucous layer, increase IP or reduce bacterial clearance, leading to a decline in penetrating or resident bacteria, with epithelial restitution [47]. Alternatively, EEN or dietary interventions might act by adding or subtracting dietary components, which affect microbial composition, likewise allowing termination of this vicious disease-forming cycle before a critical threshold is reached. In any case, the hypothesis that one dietary component is responsible for the rising incidence of CD is simplistic.

Our knowledge at present is far from complete, but multiple signals from epidemiological, animal model and interventional studies indicate that diet may have a profound effect on either the pathogenesis or management of CD. The challenges in identifying which components are important, how they affect pathogenesis, and how dietary manipulation may affect disease management are still important. True progress will require that more resources be focused on research into environmental and dietary components that may affect the pathogenesis of the disease.

Disclosure Statement

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The Future of Nutrition in IBD


Current State of the Art of Medical Foods

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Abstract

Inflammatory bowel disease (IBD) is one of the five most prevalent gastrointestinal disease burdens in the US, with an overall health care cost of more than USD 1.7 billion. It commonly requires a lifetime of care, and accounts for more than 700,000 physician visits, 100,000 hospitalizations, and disability in 119,000 patients each year. IBD is a multifactorial disease and comprises genetic susceptibility, uncontrolled immune responses, and environmental factors which play a role in the pathogenesis and course of the disease. IBD patients are lifelong on medication, either for induction or maintenance therapy. Current treatment option (corticosteroids, immune suppressants, biologics), administered in mono- or combination therapy, are still unsatisfactory. Due to the nature of disease, 20–40% of patients relapse within the first 12 months. Although modern treatment algorithms have diminished the risk of surgery, the treatments harbor significant side effects, which impacts patients’ quality of life. The role of nutrition in IBD has gathered high interest, especially in pediatric Crohn’s disease, where studies have shown that exclusive enteral nutrition can induce remission in mild-to-moderate disease comparable to corticosteroids. Thus, gastroenterologists and patients become increasingly aware that specific nutritional interventions offered in addition to the standard of care are an appealing option for a safe long-term disease management. Such specific nutritional solutions should be based on scientific/clinical evidence and specifically designed to address the patients’ distinct nutritional requirements. As per definition, these nutrition products fall under the regulatory framework of a Medical Food (Foods for Special Medical Purposes in Europe).

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Introduction

Definition of a Medical Food

The infant formula Lofenalac for the dietary management of phenylketonuria, formerly classified as a drug, is the first example of a revised view of the FDA
who opted to regulate such products as special foods that supplied a nutritional need arising from a physiological or pathological condition (1972). In 1988, the Congress defined the term ‘medical food’, thereby creating a legal category for special foods like Lofenalac.

Finally, in 1993 the FDA, by regulation, amplified the statutory definition of a ‘medical food’ such that a food is a medical food only if:

- it is a specially formulated and processed product (as opposed to a naturally occurring foodstuff used in its natural state) for the partial or exclusive feeding of a patient by means of oral intake or enteral feeding tube
- it is intended for the dietary management of a patient who, because of therapeutic or chronic medical needs, has limited or impaired capacity to ingest, digest, absorb, or metabolize or excrete ordinary foodstuffs or certain nutrients contained therein or metabolites, or who has other medically determined nutrient requirements, whose dietary management cannot be achieved only by modification of the normal diet, by other foods for particular nutritional uses, or by a combination of the two
- it provides nutritional support specifically modified for the management of the unique nutrient needs that result from the specific disease or condition, as determined by medical evaluation
- it is intended to be used under medical supervision, and
- it is intended only for a patient receiving active and ongoing medical supervision wherein the patient requires medical care on a recurring basis for, among other things, instructions on the use of the medical food (table 1).
How Do Regulators Define ‘Distinct Nutritional Requirements’?

As per the definition of the FDA, a distinct nutritional requirement of a patient is ‘the nutritional needs of a healthy person adjusted for the distinct nutritional needs of a patient due to the effects of a disease on absorption, metabolism, and excretion’ or simplified ‘nutritional needs different from those of healthy people’ and that result from a disease condition that causes a limitation in the ability of a patient to ingest or digest conventional food [1].

Although this definition is rather vague, the FDA reserves the view that with respect to any ‘dietary management of disease’ claim, the disease itself must be shown to create a distinctive nutritional need which cannot be addressed by normal diet alone and, secondly, must be substantiated by convincing scientific evidence.

In recent letters, the FDA has expressed that it is ‘not aware of any distinct nutritional requirement for patients with inflammatory bowel conditions or inflammatory bowel disease’. In contrast, in the August 2013 update of the Draft Guidance for Industry: Frequently Asked Questions about Medical Foods, ed 2, there was no specific statement that inflammatory bowel disease (IBD) is a condition or disease for which a medical food should not be developed.

Is There Evidence of Distinct Nutritional Requirements in IBD Patients?

Malnutrition is common in pediatric and adult patients with IBD, especially in those with Crohn’s disease (CD). It typically presents as protein-energy malnutrition, general weight loss, and/or vitamin/mineral deficiencies. In general, poor dietary intake secondary to postprandial abdominal pain and diarrhea is the most common cause of malnutrition in IBD [2].

Protein-energy malnutrition most often occurs with active, severe IBD. The suboptimal nutritional intake is associated with anorexia or food aversions, combined with an increased nutrient utilization due to the hypermetabolism as a result of immune activation, inflammation and tissue repair processes [3]. The degree of malnutrition depends on the duration, severity and extent of the disease, as well as loss of function due to bowel resection or fibrosis. This is more commonly observed in CD patients, especially with longstanding disease. IBD patients have also been reported to have fat and muscle mass depletion [4]. Micronutrient deficiencies also occur with mild disease or in patients in remission [5]. Additionally, patients may avoid certain nutrient-rich foods due to a misperception that the food may exacerbate
symptoms, even when the disease is in remission. Avoidance of these foods can lead to nutrient deficiency (i.e. dairy: calcium, vitamin D) [2]. A recent study of patients with inactive disease found that avoidance of major food groups remained common, with ∼33% avoiding grains, ∼33% avoiding dairy, and 18% avoiding vegetables entirely [6]. A number of studies have documented inadequate nutrient intake and nutrient deficiencies in IBD patients [7–13].

Nutrient malabsorption and the resulting deficiencies are due in part to chronic, longstanding bowel mucosal inflammation and diarrhea, which in turn leads to losses of protein, blood, minerals, electrolytes, and trace elements. Systemic proinflammatory cytokines (interleukins IL-1, IL-6), and tumor necrosis factor-α (TNF-α) contribute to nutrient deficiencies in IBD. The

<table>
<thead>
<tr>
<th>VITAMINS</th>
<th>CD</th>
<th>UC</th>
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<tbody>
<tr>
<td><strong>B1 (thiamine)</strong></td>
<td>32</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>B9 (folate)</strong></td>
<td>40–78% IBD patients with inadequate intake 0–26% of CD patients with deficiency</td>
<td></td>
</tr>
<tr>
<td><strong>B12</strong></td>
<td>11–22 100% of patients with ileal resection &gt;60 cm 48% of resections 20–40 cm</td>
<td></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>&gt;50</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>A</strong></td>
<td>35–90% of IBD patients with inadequate daily intake 0–44% with low serum levels</td>
<td></td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>22–70</td>
<td>0–45</td>
</tr>
<tr>
<td><strong>E</strong></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>K</strong></td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<th>MINERALS</th>
<th>CD</th>
<th>UC</th>
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<tr>
<td><strong>Calcium</strong></td>
<td>80–86% of IBD patients with inadequate intake</td>
<td></td>
</tr>
<tr>
<td><strong>Magnesium</strong></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Iron</strong></td>
<td>36–90% of IBD patients with iron deficiency anemia</td>
<td></td>
</tr>
<tr>
<td><strong>Zinc</strong></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Selenium</strong></td>
<td>N/A</td>
<td>N/A</td>
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Figures indicate percentages.
inflammatory process increases protein catabolism and alters normal protein synthesis. When TNF-α levels are increased, protein synthesis is diverted from nutritional proteins to inflammatory proteins. Cytokines also stimulate osteoclast activity, causing increased bone resorption [2].

There are several medications used to treat IBD that can interfere with normal micronutrient absorption. Glucocorticoids may inhibit calcium, phosphorus and zinc absorption, and may also lead to impaired metabolism of vitamins C and D. Sulfasalazine is a folate antagonist, while cholestyramine can interfere with absorption of fat-soluble vitamins [5].

Of particular relevance is the impact of these micronutrient deficiencies on clinical outcomes, specifically anemia and bone loss. Anemia can be the result of deficiencies of iron, folic acid, or vitamin B₁₂. Chronic inflamma-
It is the most common systemic complication of IBD, with reported rates of 40–70% in historical cohorts of hospitalized IBD patients [14, 15]. More recent studies of outpatient IBD patients, using population-based datasets in Switzerland and Scandinavia, found the prevalence of anemia to be 19–25% [16, 17]. Osteopenia and osteoporosis occur more frequently in IBD patients and at an earlier age of onset than in the general population. The exact prevalence of decreased bone mass density (BMD) varies in the IBD literature as a result of heterogeneous study populations and various definitions of bone disease. Osteopenia (BMD between −1 to −2.5 SDs below average for healthy individuals) and osteoporosis (greater than −2.5 SDs below) have been reported in 22–55 and 3–58% of CD patients, respectively, and 32–67 and 4–50% of UC patients [18, 19]. The rate of fracture has also been demonstrated in large epidemiological studies to be 40–60% higher than among controls [20, 21].

Table 2 provides a comprehensive overview of the prevalence of micronutrient deficiencies in IBD patients.

### Evidence of an Increased Need of Macronutrients in Catabolic Disease Condition such as IBD

There is accumulating evidence in the understanding of the functional properties of specific proteins, peptides and amino acids (or their metabolites) beyond the sole nutritional value. This relates to the quality (source) of proteins, peptide sequence or specificity of amino acids. In addition, the daily amounts needed may vary from the healthy to the disease state, and may not be covered by normal dietary intake.

In this chapter, selected preclinical research is going to exemplify the rationale.

Intestinal mucins forming the mucus gel and protecting the intestinal epithelium have been suggested of crucial importance in the epithelial wound healing process after mucosal injury in IBD [22].

The body’s capacity to maintain adequate mucin synthesis is directly related to the bioavailability of specific amino acids, such as threonine, serine and proline, which account for up to 28, 14 and 13%, respectively, of the total amino acid composition of the mucin molecule [23] as compared to only 3–7% incorporation into most other proteins (fig. 1). Moreover, the rate of mucin synthesis has been demonstrated to be directly related to the availability of dietary threonine in healthy rats [24] and piglets [25, 26].
The impact of various disease states on specific dietary requirement is generally underestimated: in a rat model of sepsis, threonine utilization for the synthesis of intestinal mucins was 70% greater than in healthy conditions (table 3) [26]. Similarly, in an experimental model of colitis, intestinal mucin production was not stimulated in pair-fed animals (normal diet ad libitum) [27–29]. Only the increased dietary supply of threonine, serine, proline and cysteine was effective in promoting colonic mucin synthesis in a dose-dependent manner, identifying threonine as the rate-limiting amino acid for mucin, but not total protein synthesis in the intestinal mucosa. In addition, the reappearance of mucin-2-containing goblet cells in the epithelium of ulcerated areas suggested the initiation of the healing processes of the intestinal epithelium (fig. 2, 3) [30].

The impact of various disease states on specific dietary requirement is generally underestimated: in a rat model of sepsis, threonine utilization for the synthesis of intestinal mucins was 70% greater than in healthy conditions (table 3) [26]. Similarly, in an experimental model of colitis, intestinal mucin production was not stimulated in pair-fed animals (normal diet ad libitum) [27–29]. Only the increased dietary supply of threonine, serine, proline and cysteine was effective in promoting colonic mucin synthesis in a dose-dependent manner, identifying threonine as the rate-limiting amino acid for mucin, but not total protein synthesis in the intestinal mucosa. In addition, the reappearance of mucin-2-containing goblet cells in the epithelium of ulcerated areas suggested the initiation of the healing processes of the intestinal epithelium (fig. 2, 3) [30].

**Table 3.** Increased threonine utilization for intestinal mucin synthesis in conditions of systemic inflammation (sepsis)

<table>
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<tr>
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<th>Pair-fed</th>
<th>Sepsis</th>
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<tr>
<td>Valine, μmol/day</td>
<td>0.113±0.01</td>
<td>0.112±0.14</td>
</tr>
<tr>
<td>Threonine, μmol/day</td>
<td>0.72±0.08</td>
<td>1.23±0.11*</td>
</tr>
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</table>

* p < 0.05 vs. corresponding pair-fed, † p < 0.05 vs. corresponding valine, n = 6 (sepsis) or 7 (pair-fed). From Faure et al. [26], reprinted with permission.

**Fig. 1.** Illustrative overview on the importance of mucin.
The role of proline as a key amino acid involved in wound healing in general is also likely to be important. Proline is a precursor of both amino acids hydroxyproline and arginine. Hydroxyproline is major constituent of collagen, while arginine is involved via nitric oxide synthesis in the irrigation of wounded tissue. Indeed, proline supplementation has been shown to stimulate collagen synthesis in cultured fibroblasts [Nestlé, unpubl. data].

**Fig. 2.** Colonic inflammation (dextran sulfate sodium, DSS, in rats). Specific stimulation of colonic mucin synthesis. A normal nutritional support does not cover the high amino acid (AA) needs associated with IBD. † p < 0.05, DSS vs. controls; * p < 0.05 vs. DSS. Modified from Faure et al. [30].

**Fig. 3.** Colonic inflammation (DSS in rats). Recovery of a colonic lining with MUC2-containing goblet cells. Supplementation with threonine, serine, proline and cysteine increases mucin synthesis and tissue repair. Modified from Faure et al. [30].
Increased gastrointestinal threonine uptake and mucin synthesis in intestinal inflammation were also shown in enterally fed minipigs (table 4) [31].

Taken together, these results suggest, that under acute or chronic inflammatory conditions, the requirements of specific nutrients (here: distinct amino acids) could not be covered by normal dietary intake, but warranted a targeted nutritional solution.

These examples, amongst others, suggest that the current understanding of ‘distinct nutritional requirements’ in IBD patients has to be revised and the recent experimental insights may challenge our today’s interpretation of these ‘distinct nutritional needs’, underestimating the role of nutrition as part of the therapeutic plan.

In this respect, effective nutritional approaches to fulfill the specific and increased requirements of these patients to improve overall clinical outcomes would be required to support the maintenance of remission, as suggested by Akobeng and Thomas [32] and Arslan et al. [33].

As health authorities might not be fully aware of the most recent advancements in nutritional research, it will be our responsibility as clinicians, basic scientists and nutritionists to provide the data to generate convincing evidence for ‘distinct nutritional needs’ in IBD patients. Research in molecular nutrition and high-performing analytical platforms will contribute to a better understanding of molecular mechanisms and targets of nutrients and will pave the future for an integrated patient care where medical food is recognized as a credible, scientific and clinically validated part of the long-term therapeutic plan to improve patient’s quality of life and disease outcome.

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Disclosure Statement

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References

Abstract
Inflammatory bowel diseases (IBD) are chronic progressive diseases. Current therapeutic strategies have not significantly altered the natural history of IBD. In the wake of other chronic diseases, such as rheumatoid arthritis, the goal of therapy has now shifted from mere control of symptoms to altering natural history to prevent bowel damage and disability. A ‘treat to target’ approach using endoscopic healing as a first definition of the target is now proposed together with tight control of inflammation based on monitoring of symptoms and biomarkers. In order to reach the target, optimization of current therapies using better understanding of pharmacokinetics is needed as well as development of predictors of disease progression to avoid under- and overtreating. Advances in the understanding of the roles of the adaptive and innate immune systems, as well as the intestinal epithelium and endothelium have resulted in the development of multiple new biologics. There is great optimism that an integrated ‘omics’ approach incorporating genetic, microbiota with clinical and environmental data will help in choosing for each patient a personalized approach targeting the mechanisms driving the disease. Finally, in this era of increasing complexity of care, education of patients to involve them in a well-informed decision-making process is mandatory.

Introduction
Inflammatory bowel diseases (IBD) are chronic disabling, progressive and destructive diseases. The goals of IBD therapy are progressively evolving from prevention of death in the 1950s to eventually prevention of the occurrence of the disease. For decades, IBD therapy was based on a so-called step-up approach where for instance in Crohn’s disease (CD) the first flare was to be treated by...
steroids (such as budesonide or prednisolone), followed by azathioprine or methotrexate in cases of new flares requiring frequent steroid therapy, then monoclonal antibodies directed against tumor necrosis factor (TNF) as a final option before surgery. Current therapeutic strategies have not significantly altered the natural history of IBD. In a Danish cohort, even though mortality from ulcerative colitis (UC) decreased from 1982 to 2010, largely because of reduced mortalities from gastrointestinal disorders and colorectal cancer, people with CD had 50% greater mortality than the general population, and this value did not change over this time period [1]. In a study from Norway, 10 years after disease onset, IBD patients had an increased relative risk (RR) for disability pension as compared with the background population, the youngest patients having the highest RR [2]. The risks for surgery after diagnosis with CD and UC have decreased significantly over the past 6 decades but remain significant. In a recent systematic review based on all population-based studies, the risks of surgery 1, 5, and 10 years after diagnosis of CD were 16.3% (95% confidence interval, CI: 11.4–23.2%), 33.3% (95% CI: 26.3–42.1%), and 46.6% (95% CI: 37.7–57.7%), respectively. The risks for surgery 1, 5, and 10 years after diagnosis of UC were 4.9% (95% CI: 3.8–6.3%), 11.6% (95% CI: 9.3–14.4%), and 15.6% (95% CI: 12.5–19.6%), respectively [3]. These outcomes clearly highlight the importance of defining new therapeutic targets and strategies and the extent of unmet needs in IBD.

**New Targets in IBD**

Changes in the therapeutic paradigm of CD are resulting from its recognition as a progressive disease [4]. CD was traditionally characterized by periods of clinical remission alternating with periods of relapse defined by recurrent clinical symptoms. However, only 10% of patients experience prolonged remission of symptoms, meaning that in most cases disease activity is relapsing or continuous and, importantly, asymptomatic patients often have evidence of active inflammation on endoscopy. Persistent inflammation is believed to lead to progressive bowel damage over time, which manifests with the development of strictures, fistulae, and abscesses. These disease complications frequently lead to loss of function and need for surgical resection, which in turn lead to disability (fig. 1). As in other chronic destructive diseases such as rheumatoid arthritis, treatment paradigm is currently shifting in CD from mere control of symptoms towards the improvement of long-term disease outcomes, i.e. reduced structural damage and disability [5, 6]. A new instrument, the Leman score, has thus been developed to measure the cumulative structural bowel damage caused by CD over
The introduction of novel therapies and the development of new approaches to treatment in several chronic diseases such as rheumatoid arthritis led to better outcomes for patients. Prominent among these is a ‘treat to target’ strategy that is based on regular assessment of disease activity using objective outcome measures and the subsequent adjustment of treatments. This approach is currently explored in CD. However, there is currently no accepted, well-defined, comprehensive treatment goal that entails the treatment of both clinical symptoms and biologic inflammation. As a starting point, a working definition of sustained deep remission (that includes long-term biological remission and symptom control) with defined patient outcomes (including no disease progression) has been proposed [7–9]. The concept of sustained deep remission represents a goal for CD management that may still evolve. Clinical trials are needed to evaluate whether treatment algorithms that tailor therapy to achieve deep remission in patients with CD can prevent disease progression and disability.

Assessment of objective measures of inflammation is an increasingly important part of the management of IBD. Thorough assessment of disease activity and extent at presentation is needed to ensure a correct diagnosis of IBD (vs.

Fig. 1. Progression of digestive damage and inflammatory activity in a theoretical patient with CD. CDAI = Crohn’s disease activity index; CDEIS = Crohn’s disease endoscopic index of severity. Reproduced from Pariente et al. [6].
non-IBD) and of CD (vs. UC), avoid delay in diagnosis, identify complications, help assess prognosis and take appropriate therapeutic decision. During follow-up, clinical decision-making is increasingly being driven by the findings of continued monitoring (for objective evidence of inflammation), with the aim of optimizing treatment for tight disease control. Despite the potential benefits of longitudinal monitoring in CD, there are several unanswered questions around implementing this model in practice: Which monitoring tools should be used? When should they be used? How should the monitoring strategy differ in different patient scenarios? Given the uncertainties around these issues, practice recommendations were recently developed based on the best published evidence available and/or expert opinion [10]. A number of ongoing clinical studies will provide further data and assess the impact on clinical outcomes of a ‘tight control’ approach to treatment based around objective parameters of inflammation beyond mere control of symptoms.

Complementary to the treat to target concept is early intervention: losing time in high-risk patients will lead to less chance to reach the target and increased risk of further progression and bowel damage. In this regard, targeting CD early might be the only way to change the disease course [11, 12]. In 2012, an international definition of early CD was proposed and defined by disease duration ≤ 18 months and no previous use of disease-modifying agents [13]. However, early introduction of disease-modifying anti-IBD drugs cannot be recommended in all patients with CD. First, scientific evidence from prospective studies is still lacking. An open-label RCT comparing early combined immunosuppression [three infusions of infliximab (5 mg/kg body weight) at weeks 0, 2 and 6 with azathioprine, and, if necessary, corticosteroids] or conventional treatment (corticosteroids, followed, in sequence, by azathioprine and infliximab) in CD of <4 years’ duration showed a higher remission rate within the first year in the top-down group than in the control groups, but this difference disappeared at week 78 [14]. More recently, the RAPID (Effect of Early Prescription of Immunosuppressants on First Three-Year Course of Crohn’s Disease) study from the Groupe d’Etudes des Thérapeutiques et Affections Inflammatoires Digestives (GETAID) compared two therapeutic approaches in patients with CD who had an established diagnosis of the disease for <6 months. The patients were treated either with early use of immunosuppressants or with a step-up approach. No difference was observed between the two groups regarding the number of trimesters without disease activity, with steroids, infliximab, surgery, or requirement for hospitalization [15]. Second, it is necessary to identify patients with CD who are at a high risk of having a complicated and/or severe disease course and who could benefit from a more aggressive therapeutic strategy in order to avoid overtreatment.
Unmet Needs in IBD

Optimization

Although new biologics are highly effective for induction and maintenance of clinical remission, not all patients respond, and a high proportion of patients lose response over time. There is thus a strong need for optimization. Little is known about their exposure-response relationship and the factors that may affect their disposition. Understanding these factors is essential to further improving the therapeutic efficacy of these drugs [12]. Monitoring serum drug concentrations and antibodies (immunogenicity) may lead to more appropriate therapeutic management in patients with loss of response. The pharmacokinetics of biologics appears to be strongly influenced by several factors related to patient and disease characteristics such as age, BMI, albumin level, CRP and combination therapy with immunomodulators. Evaluation of the covariates that influence drug disposition may help to carefully select those patients that are more likely to benefit from receiving higher doses due to an accelerated clearance. Several descriptive studies and post hoc analyses from clinical trials have reported consistent associations between serum anti-TNFs and antibody levels and clinical responses. Algorithms have been proposed in which interventions are based on a combined assessment of IFX bioavailability and immunogenicity at the time of therapeutic failure [16]. The first prospective study showing the cost-effectiveness of interventions defined by this kind of algorithm has recently been published [17]. The number of approved biologics for the treatment of IBD is expected to increase in the forthcoming years. Therefore, a better understanding of the factors that impact the pharmacokinetics and pharmacodynamics of biologics is crucial to ensure more efficient dosing regimens which in turn may enhance the therapeutic success of these therapies.

Prediction

At present, three possible treatment strategies could be considered in CD: (1) classical step care; (2) immunosuppressives in combination with a tapering course of steroids (accelerated step care), and (3) TNF antagonists (either as monotherapy or in combination with immunosuppressants) [11]. An essential prerequisite of any treatment paradigm is recognition that overtreatment of low-risk patients will result in a poor therapeutic index. Consequently, it is essential to identify patients who are suitable for early treatment because they are
at high risk of disease progression. Several clinical markers (disease location, extent and behavior, age, mucosal aspect, tobacco used), and possibly serologic and genetic markers have been considered as predictors of disease outcome [reviewed in 11]. However, few of these markers have been tested prospectively, and the definition of disease ‘outcome’ has been inconsistent throughout these studies. Still, algorithms based on existing knowledge have been proposed [11]. Prospective trials to evaluate (a) the relevance of markers to predict long-term outcomes such as bowel damage and disability and (b) the benefit of early intervention in patients with CD who are at high risk of disease progression are urgently needed.

Personalization

Advances in the understanding of the roles of the adaptive and innate immune systems as well as the intestinal epithelium and endothelium have resulted in the development of multiple biologics that all represent an alternative to the use of current therapies in patients with refractory CD or UC. However, there is a huge potential for variation in inflammatory response from patient to patient and within a single patient over time and then in response to different treatments. Even when all classical aspects of a disease phenotype are present, the mechanism responsible for the pathology observed may differ from one person to another. Genome-wide association studies have revealed a wealth of genes potentially involved in CD and UC, but no single gene or set of genes is prognostic. A new view of the genotype-phenotype relationship in which different sets of loci are responsible for mechanistically distinct subtypes of diseases has been proposed [18]. It is possible that the future of IBD therapy would include computing gene and protein function for a more targeted, personalized patient-based approach.

Education

Disease-related knowledge is associated with quality of life, coping skills and medication adherence. Several surveys have shown that IBD patients are ill-informed about their disease and its associated risks. Improvement of patient education is necessary to appropriately involve patients in the decision-making process. There are limitations in education linked to poor access to specialized centers. Studies from Scandinavia have shown that these limitations may be overcome by personalized self-management training of patients. A web-guided
approach was shown to be feasible, safe and cost effective [19]. It empowers patients with UC without increasing their morbidity and depression. Such initiatives should be further tested.

**Conclusion**

The treatment of IBD is rapidly evolving. Together with the development of new drugs, new therapeutic goals and new strategies have emerged in order to alter the progressive natural course of IBD. As in other chronic diseases, a treat to target approach with ‘tight control’ based on monitoring is now proposed. However, it must be recognized that these concepts have not been validated, and prospective studies to evaluate their long-term impact on new end points such as bowel damage and patients’ functional status are needed.

**Disclosure Statement**

J.-F. Colombel has served as consultant, advisory board member or speaker for Abbvie, Amgen, Bristol Meyers Squibb, Celltrion, Ferring, Genentech, Giuliani SPA, Given Imaging, Merck & Co., Millennium Pharmaceuticals Inc., Nutrition Science Partners Ltd., Pfizer Inc., Prometheus Laboratories, Sanofi, Schering Plough Corporation, Takeda, Teva Pharmaceuticals, UCB Pharma, Vertex, Dr. August Wolff GmbH & Co.

**References**

This 79th Nestlé Nutrition Institute Workshop was dedicated to various aspects of inflammatory bowel diseases (IBD) focusing on nutrition, gut microbiota and immunity as potential therapeutic targets for IBD. During this 1.5-day meeting, 15 expert presentations with extensive panel discussions led to a comprehensive view of IBD epidemiology, pathogenesis, and treatment. Importantly, epidemiologic observations showing a rapidly increasing incidence of IBD for both adults and children emphasize the complex interplay between environmental changes with subsequent activation of the mucosal immune system. Particularly important may be the impact of a Westernized diet on the intestinal microbiota composition and function, a critical factor in the pathogenesis of IBD. There are clear associations between patterns of food consumption, and intestinal inflammation is associated with the development of dysbiosis, an alteration of gut microbiota composition associated with a disease process. But is this dysbiosis primary, thus causing inflammation, secondary, just reflecting the inflammatory state, or both? – a question intensively discussed during this NNIW. Perhaps an ongoing Canadian cohort study will bring some clarity to this issue by focusing on the evaluation of healthy family members of IBD patients with a high risk for disease development. Current evidence strongly supports the notion that these environmental and microbial changes play a role in disease pathogenesis in genetically determined susceptible individuals who harbor one or a few of over 160 genetic susceptibility loci identified to date. Another key issue raised during this NNIW is the observation that nutritional manipulation is a pillar in the treatment of Crohn’s disease (CD); but is this through withdrawal of potentially ‘harmful’ ingredients of usual diet or are there biologically active or anti-inflammatory molecules in enteral nutrition that play a role in inducing
and potentially maintaining remission in patients with CD? The answer to this question will be fundamentally important in advancing our understanding of IBD disease pathogenesis and for the development of the ‘healthy diet’ in patients with IBD. In this regard, new dietary intervention plans to treat CD were discussed. Ultimately, the complex interplay of genes, immune reactions and environmental factors of food ingredients or additives to microbiota (including bacteria, fungi, parasites or viruses) may all be potential targets for the treatment of patients with IBD. In the future, there might be a treatment strategy for targeting the interaction between the gut microbiota and the mucosal immune system by acting on several levels, the microbial dysbiosis, as well as the exaggerated immune response using food or pre-/probiotics in adjunction to potent immunosuppressors and biologics. The conclusions of this NNIW can be summarized in the question: ‘can we treat or prevent IBD through lifestyle/environmental changes? If yes, which are the most relevant?’

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