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REVIEW

Intestinal microbiota, fecal microbiota transplantation, and inflammatory bowel disease

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ABSTRACT

Inflammatory bowel disease (IBD) is a complex set of diseases that lead to chronic inflammation in the gastrointestinal tract. Although the etiology of IBD is not fully understood, it is well-known that the intestinal microbiota is associated with the development and maintenance of IBD. Manipulation of the gut microbiota, therefore, may represent a target for IBD therapy. Fecal microbiota transplantation (FMT), where fecal microbiota from a healthy donor is transplanted into a patient's GI tract, is already a successful therapy for *Clostridium difficile* infection. FMT is currently being explored as a potential therapy for IBD as well. In this review, the associations between the gut microbiota and IBD and the emerging data on FMT for IBD will be discussed.

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Introduction

Inflammatory bowel disease (IBD) affects over 1 million individuals in the United States alone, and the incidence of these diseases in both the US and in developed countries worldwide continues to grow.[1,2] The current paradigm of the pathophysiology of IBD is an inappropriate immune response to the microbiota in a genetically susceptible individual.[3,4] IBD is grouped into predominantly phenotypic patterns based on the location of inflammation: in Crohn's disease (CD), the inflammation can be in any part of the intestine, while in ulcerative colitis (UC) the inflammation is limited to the colon. In IBD, an abnormal intestinal microbiota (dysbiosis) is clearly associated with certain disease phenotypes, and may be a causal or synergistic factor in perpetuating chronic inflammation. Thus, manipulating the intestinal microbiota represents a potential treatment of IBD.[2] One form of manipulating the microbiota is through fecal microbiota transplantation (FMT), where fecal microbiota from a healthy donor are transplanted into the distal GI tract of a patient. FMT has already emerged as a successful therapy for *Clostridium difficile* infection,[5-7] and is currently being explored as a potential treatment of IBD.[8-10] This review will outline the associations of IBD and the gut microbiota, and then discuss the current data on fecal microbiota transplantation in IBD.

Associations between the intestinal microbiota and IBD

Microbiota and early intestinal immune system development

Evidence in both human and mouse studies strongly suggests that early intestinal immune system development is highly dependent on the intestinal microbiota,[11] which in turn may impact susceptibility to IBD. It is well-established that germ-free animals have an under-developed intestinal immune system compared with conventionally raised animals, characterized by smaller and fewer Peyer's patches, mesenteric lymph nodes, and isolated lymphoid follicles.[12,13] Germ-free mice also lack certain helper T cell subtypes in their intestinal tracts.[14] Although many of these changes can be ameliorated by the later introduction of specific pathogen-free microbiota,[15] transcriptional profiles in the jejunum and colon of these mice remain altered compared to conventionally-raised mice,[16] suggesting that there is a critical period during which exposure to microbes informs appropriate immune and mucosal development.

Appropriate exposure to a healthy microbiota early in life appears to be important in the resistance of

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chemical induced colitis later in life. In germ-free mice, there is an accumulation of invariant natural killer T (iNKT) cells in the lamina propria of the colon, with subsequent worsening of colitis upon exposure to oxazolone compared with conventionally-raised animals.[17] Although introduction of a conventional microbiota to neonatal germ-free mice protected animals against both the accumulation of iNKT cells and worsened colitis, these effects were not seen following conventionalization of adult animals. At least in this model, early exposure to microbiota is critical to normal immune development and protection against colitis.

Recent work has demonstrated that maternal microbial exposure may also play a role in immune system development and protection against inflammation.[18] Transient colonization (gestational only) of otherwise germ-free pregnant mice induced innate immune development in subsequent germ-free offspring, which in turn decreased the inflammatory response of pups to microbial molecules. These findings suggest that microbial manipulation *in utero* may already set the stage for inflammation later in life.

Studies in humans have also suggested that early-life manipulation of the gut microbiota, primarily through the use of antibiotics, is associated with altered susceptibility to IBD. Case-control analyses demonstrate that early exposure to antibiotics increases the risk of IBD later in life.[19-21] Interestingly this risk appears to be the highest for the first year in life. Similarly, another study noted that early-childhood infectious diseases, including gastroenteritis and respiratory infections, were protective against the development of IBD.[22] While the associations are compelling, it remains unclear if these microbial changes are causal for IBD, or rather markers for an underlying immune dysfunction that eventually manifests as IBD. However, overall, it appears that early-life microbial exposures in mice and humans influence the development of the intestinal immune system in such a way to promote or protect against the development of IBD.

NOD2 – bridging the immune system and commensal bacteria

One of the clearest human genetic associations with IBD is the nucleotide-binding oligomerization domain-containing protein 2 (NOD2), an intracellular immune receptor for components of the bacterial cell wall.[23,24] *NOD2* polymorphisms confer an increased risk for the development of Crohn's disease.[23,25,26] Mice lacking *Nod2*, as well as humans with *NOD2* mutations, have an altered microbiota.[27-30] In both humans and animal models, a lack of functional NOD2 results in an increased abundance of the Bacteroidetes phylum, one of the major bacterial phyla found in the gut.[28] Interestingly, *Nod2*-deficient mice do not spontaneously develop colitis..[31] However, compared with wild type mice, *Nod2*-deficient mice have increased susceptibility to dextran sodium sulfate (DSS)-induced colitis, which is both transmissible to wild-type animals via cohousing and ameliorated by fecal transplantation from wild-type donors.[32] This suggests that the risk of colitis from *NOD2* mutations is from the resulting dysbiosis and can be ameliorated or worsened by altering the microbiota.

Alterations in other key immune pathways may also explain why individuals with *NOD2* mutations are predisposed to, but do not always develop, IBD. NOD2 is important in the clearance of intracellular pathogens through autophagy via interaction with ATG16L1.[33,34] Notably in Crohn's disease with *NOD2* variants, this effect is absent, resulting in failure to induce autophagy for intracellular *Salmonella enterica* ser. Typhimurium, adherent-invasive *Escherichia coli*, and *Shigella flexneri*.[25] Again, it appears that the underlying mutation alone does not cause inflammation, but rather the resulting changes to the microbiota; in this case through failure to clear potentially pathogenic microbes

The intestinal microbiota is required for mouse models of colitis

The majority of mouse models of colitis require a microbiota to develop intestinal inflammation.[35] Although no mouse model can fully replicate the complex pathophysiology of human IBD, several models that spontaneously develop inflammation when housed under typical conditions show no evidence of inflammation when raised under germ-free conditions.[36-38] These findings support the role of the intestinal microbiota in the development of inflammation and IBD.

The importance of the intestinal microbiota in inflammation in animal models is demonstrated via transfer of inflammation-associated microbial

populations into germ-free mice. In some mouse models of intestinal inflammation, the transfer of fecal pellets from conventionally raised or specific pathogen free animals with an IBD-like phenotype into germ-free animals recapitulates both the phenotype and the dysbiotic microbiota.[39,40] In certain murine models (including *Nod2*-deficient mice as above,[32] *T-bet* and *Rag-2* deficient (TRUC) mice,[41] and *Nlrp*-6 deficient mice)[42] colitis can be reproduced in conventionally-raised wild-type animals by cohousing, an effect presumably mediated by the microbiota. Similarly, *Casp3/11*-deficient mice, which are protected against DSS-induced colitis, lose this protection when cohoused with wild-type mice,[43] while cohousing *Il10*-deficient mice with *Apoe*-deficient mice worsens colitis in IL-10 knockout animals.[44] This provides evidence not only of the significance of the microbiota in intestinal inflammation, but also suggests that this inflammation is transmissible.

Specific bacteria are associated with IBD

Certain bacteria are implicated in the development of intestinal inflammation in animal models. For example, *Proteus mirabilis* and *Klebsiella pneumoniae* correlate with colitis in *T-bet*$^{-/-}$ *x Rag2*$^{-/-}$ mice, a mouse model of ulcerative colitis.[45] Additionally, these bacteria may be associated with maternal transmission of disease, potentially indicating a causal link. Recent work found that *Bilophila wadsworthia*, a typically low-abundance commensal organism, is associated with colitis in *Il10*$^{-/-}$ mice.[46] In this study, an increase in dietary milk fat increased the proportion of taurine-conjugated bile acids in the colon, subsequently increasing organic sulfur compounds available to sulfite-reducing microbes including *B. wadsworthia*, which resulted in an expansion of this organism. *B. wadsworthia* can activate dendritic cells to promote a Th1-mediated colitis, likely explaining why its expansion induced colitis in a susceptible host, and also linking diet to colonic inflammation. Another recent study identified *Atopobium parvulum* as a driver of colitis via altered metabolism of H_2S, and subsequent amelioration of colitis with the H_2S scavenger bismuth.[47] Finally, supernatant from *Fusobacterium varium* cultures, isolated from the colonic mucosa of UC patients, can induce colonic ulcer formation in mice.[48] These findings indicate that individual commensal organisms have the capability of inducing colitis in certain mouse models, although the translation to human IBD is less apparent.

Despite an extensive search, no single specific pathogen appears to cause IBD.[49] Certainly some infections cause a phenotype similar to IBD (e.g. intestinal tuberculosis, *Campylobacter jejuni, Yersinia enterocolitica* and others),[50,51] raising the possibility that some subsets of IBD are actually unrecognized intestinal infections. Some potential examples include, *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and adherent-invasive *E. coli* (AIEC), both of which are associated with Crohn's disease. In one study, peripheral blood from patients with active Crohn's disease had MAP DNA prevalence of 68%, while those with Crohn's in any stage had *E. coli* 80% of the time.[52] In subjects whose sera tested positive for antigens against MAP, early uncontrolled trials suggested antibiotics against MAP could induce symptomatic improvement.[53-55] A large randomized controlled in trial from Australia found an early clinical benefit of antibiotics for MAP in addition to steroids compared to placebo (week 16, 66% v. 50%, $p = 0.02$).[56] Unfortunately there were no changes in inflammatory parameters, endoscopic endpoints, or maintenance of remission As of this writing, a Phase III trial is underway to further explore the use of antibiotics against MAP in Crohn's disease (NCT01951326).

Helminthic infections and IBD

Non-bacterial microbes, notably parasites, may also have a role to play in the development of IBD. Early epidemiological data linked the decreased incidence of parasitic infections with an increase in Crohn's disease, positing that the mechanism behind this observation may be a shift toward T helper type 1 (Th1) cytokine production.[57] Several studies in mice have demonstrated amelioration or prevention of colitis following exposure to a variety of helminths, including *Heligmosomoides polygyrus, Trichinella spiralis*, and the parasite *Schistosoma mansoni*.[58-61] These organisms may either directly alter the immune response of the host, or may act via modulation of the gut microbiota.[62] For example, infection with *H. polygyrus* in mice appears to alter the intestinal microbiota.[63]

While some limited data in humans suggests that treatment with *Trichuris suis* may be a potential

therapy for IBD,[64,65] phase 2 clinical trials in Crohn's disease did not meet early end points and were stopped (NCT01576471 and NCT01279577).[66] A study of *Trichuris suis* ova in ulcerative colitis failed to recruit sufficient subjects to draw meaningful conclusions (NCT01433471) and further trials in ulcerative colitis were halted (NCT01953354). *Necator americanus*, a hookworm, has also been studied in IBD.[67] Of 9 patients who received hookwork treatment, 7 improved. Hookwork colonization seems to increase the diversity of the gut microbiota,[68] although this effect has not always been reproducible.[69] Certainly the idea of using a single agent to orchestrate a more diverse microbiota is appealing, as in general diversity is associated with health, however much more research is needed before this is trialed in human diseases.

Global shifts in the gut microbiota and IBD

In addition to associations with specific organisms, substantial work has found that the overall composition of the gut microbiota is highly altered in IBD. Typically, microbial diversity is substantially diminished in patients with IBD compared with healthy individuals.[70-72] Furthermore, in contrast to healthy individuals, the fecal microbiota of patients with both Crohn's disease and ulcerative colitis contains a significantly lower proportion of the Bacteroidetes and Firmicutes phyla (particularly *Clostridium*), which are normally dominant in the human fecal microbiota,[12] and a significantly higher proportion of the Proteobacteria phylum.[70,71,73,74] Notably, these differences are present even in patients with Crohn's disease who are treatment-naïve, suggesting that intestinal dysbiosis in these patients is not a consequence of therapy, but rather a potential early change in IBD.[72]

Evidence from post-surgical patients who have been treated for Crohn's disease also suggests a causal relationship between gut microbiota composition and IBD. For example, diversion of the fecal stream via diverting loop ileostomy can prevent Crohn's disease recurrence, an effect that is abrogated upon restoration of bowel continuity.[75] One study noted that Crohn's patients with mucosal-associated microbiota that were more similar to healthy individuals were more likely to remain in remission following surgery.[76] Specific functions of the microbiota may also be associated with improved outcomes following resection for CD. Notably, the presence of mucosal bacteria associated with saccharolytic metabolism, including *Bacteroides, Prevotella*, and *Parabacteroides* species, has been correlated with increased remission compared with the presence of bacteria associated with fermentation and lactic acid production, such as *Enterococcus* and *Veillonella*.[77] These studies demonstrate the importance of the global gut microbiota composition and function in the development and severity of IBD.

Clinical use of FMT for IBD

Therapeutic manipulation of the gut microbiota

Given these well-described changes in the gut microbiota in patients with IBD, it is unsurprising that several therapeutic strategies have focused on manipulation of the gut microbiota. Indeed, there is a large body of literature on the use of antibiotics and probiotics in IBD. While a full review of antibiotics and probiotics is beyond the scope of this article (especially given other excellent recent reviews),[78,79] several points are highlighted to demonstrate the importance of microbiota manipulation in IBD. The use of antibiotics has variable success for the treating IBD, although in one pooled analysis there was a trend toward benefit, particularly in fistulizing or post-surgical Crohn's disease.[80] In these 2 settings, it may be that secondary bacterial overgrowth (in an area of a sinus tract or anastomosis) may perpetuate chronic inflammation, in which case antibiotic therapy may ameliorate symptoms. Some studies suggest that specific antibiotics improve disease severity,[81-83] although these studies are not always reproducible and often use symptomatic endpoints without measurement of inflammatory parameters. It is possible that decreasing the overall bacterial burden via antibiotics decreases symptoms such as diarrhea or bloating, which subsequently reduces disease activity scores, without necessarily improving the mucosal inflammation. Additionally, as IBD is typically characterized by reduced microbial diversity, it is counterintuitive to think that further reduction in bacterial diversity through antibiotics will reverse the underlying inflammatory process, especially given the associations between early-life antibiotic use and IBD as reviewed above.

An additional method of microbiota manipulation in IBD is the introduction of specific bacteria, or

probiotics, in an attempt to control the growth of pathological organisms or shift the global composition of toward a healthier state. *E. coli* Nissle 1917 is a well-studied probiotic which has been shown to be as effective as mesalazine at maintaining remission in ulcerative colitis.[84,85] Other individual probiotics with demonstrated efficacy in IBD include *Lactobacillus* GG, bifidobacteria strains, and the yeast *Saccharomyces boulardii*.[86-88] One of the most promising probiotic supplements, VSL#3, is a set of 8 bacterial strains that significantly reduces disease severity and induces remission in patients with UC compared to the placebo.[89,90] Additionally, VSL#3 can prevent pouchitis following total proctocolectomy and J-pouch formation.[91] However, engraftment of probiotics is often poor as demonstrated by lack of detectable probiotic strains 2 weeks following cessation of intake.[92]

In contrast to both antibiotics and probiotics, fecal microbiota transplantation (FMT) may represent a more robust method of manipulating the gut microbiota as a therapy for patients with IBD. This procedure involves the transfer of processed feces from a donor into the GI tract of a recipient, and has been successfully used to treat infection with *C. difficile* for nearly 60 y.[93] Unlike antibiotics, FMT increases the diversity of fecal bacterial populations in recipients,[94,95] likely contributing to its success in *C. difficile* infection. Furthermore, unlike probiotics, evidence suggests that FMT results in long-term engraftment in recipients with *C. difficile* infection,[96] The scale and content of FMT also varies considerably from probiotic therapy, as donor fecal material contains approximately 10^{11} bacterial cells per gram of stool, in addition to viruses, fungi, and archaea.[97] All together, these factors suggest that FMT may be a more promising therapy for IBD than either antibiotics or probiotics. Below we discuss current evidence available on the use of FMT for IBD.

Ulcerative colitis

Early reports of FMT for UC suggested a reversal of disease in selected patients. The first report of FMT use in UC, in 1989, was of a single patient with no endoscopic or histopathology follow-up.[98] The authors later noted in a review that the initial patient treated remained endoscopically and histologically disease free for over 20 y.[99] Subsequently, a series of 6 patients who received daily enema administration of donor fecal material for 5 d resulted in cessation of all UC-related medications, and over 1–13 y of follow-up had no clinical, endoscopic, or histologic evidence of UC.[100] Notably all patients had at least left sided ulcerative colitis and at least 5 y duration of disease. Later follow-up from this same center reported substantial success, with over 90% of a cohort of 62 patients achieving complete or partial remission.[101] Further case series in children with UC demonstrated that FMT was safe and potentially effective in improving disease status.[102,103]

Several recent case series in adults have shown mixed results for FMT as a treatment of UC. Generally, a few subjects improved, although none reached remission, and the benefit appeared to be short-term.[104-108] Interestingly, one study of 12 patients found that the clinical benefit of FMT was associated with a higher proportion of butyrate-producing bacteria in their feces following transplant, suggesting a possible mechanism behind those procedures that are successful.[109] Two essential issues with these small case series are single FMT infusions and selection bias in recruitment; patients can be very motivated for an FMT trial to the point of compromising research protocols, as a failed trial of FMT in UC noted.[110]

Given the mixed success of these small studies, 2 recent randomized trials evaluated the clinical efficacy of FMT in UC. A group from McMaster University used weekly retention enemas with donor fecal material or placebo for 6 weeks.[111] Unfortunately, the Data and Safety Monitoring Board discontinued the trial based on futility to reach the primary end point at a planned interim analysis. At that time, 4 of 27 subjects (14.8%) in the FMT arm and 2 of 26 (7.7%) in the placebo arm were in clinical remission. Patients already enrolled in the trial were allowed to complete the study and ultimately 9 of 38 (24%) in the FMT arm were in remission versus 2 of 37 (5%) in the placebo (risk difference: 17%, 95% CI: 2 – 33%). Other end points such as symptomatic improvement or changes in quality of life were similar between the 2 groups. Interestingly in this trial, nearly 40% of the patients in remission following FMT received material from a single donor. Additionally, those with UC for less than one year were more likely to achieve remission. Following FMT all subjects demonstrated increased diversity and similarity to the donors. Overall, the results suggest improved remission rates for patients treated with FMT, possibly dependent on donor fecal

composition, the use of multiple FMTs, and early treatment of UC.

A second recent randomized study also investigated the use of FMT in UC. Investigators from the Academic Medical Center in Amsterdam performed FMT with nasoduodenal tube placement following full bowel lavage twice over a 3 week period.[112] At the second interim, analysis by the DSMB recommended cessation of the trial due to futility. In the intention to treat analysis, at week 12, 30% of those who received FMT from a donor were in remission vs. 20% of those who received placebo FMT (autologous stool transplant). Regardless of treatment group, responders demonstrated an increase in diversity of fecal microbiota at week 12, whereas non-responders in either group did not have any change in diversity.

These 2 trials differed in dose, frequency, and administration of FMT, as well as type of placebo used, making direct comparisons difficult. However, in light of the results available from case series, these findings suggest that donor selection, disease duration, and successful engraftment of microbiota may all be significant factors affecting clinical outcome following FMT. A recently published abstract describing a third blinded study of 81 patients treated with an intense, multiple FMT regimen (fecal enemas 5 d per week for 8 weeks) also noted significantly improved remission in patients treated with donor fecal material (27% vs. 8% of placebo-treated controls, $p = 0.02$), suggesting that the number of transplants may also play a role in the success of this therapy.[113] Clearly, more work is needed to understand how the microbiota influences mucosal inflammation in ulcerative colitis.

Crohn's disease

Data on FMT for Crohn's disease is somewhat more limited than UC. Case reports have demonstrated mixed results with some suggesting clinical and endoscopic remission while others demonstrated no effect.[107,114-116] The earliest report of FMT for Crohn's disease noted symptomatic improvement in one patient over 4 months.[117] A more recent case report of a patient with severe, complicated CD also noted successful treatment with FMT.[118] A cohort of 30 patients with refractory mid-gut CD (defined as Harvey Bradshaw Index [HBI] $\geq$ 7) demonstrated 77% clinical remission at one month following a single FMT via nasoduodenal route.[119] A small but significant benefit was noted in hemoglobin and albumin at 3 months post-FMT in this study. FMT may also be a potential treatment of pediatric CD, as one recent case series noted remission in 5 of 9 patients (56%) after FMT, with 7 of 9 patients (78%) demonstrating engraftment of donor microbiota.[120] Interestingly, although one study did not find a significant decrease in Crohn's disease severity following FMT, patients reported significantly increased quality of life scores after the procedure.[108]

Prior work from one of us for FMT in active Crohn's disease (defined as HBI $\geq$ 5) had mixed results.[121] Of the 20 subjects enrolled for FMT, 19 had complete follow-up data. While most subjects improved post-FMT, the clinical course was variable with one subject with severe disease before FMT proceeding to colectomy following FMT. Similar to the randomized trials in UC, microbiota diversity increased for clinical responders. Additionally, clinical responders assumed more of their donor profile as measured by week 4 Bray Curtis similarity index. However, the small number and lack of a control group limit the ability to draw conclusions about efficacy in this study. Overall, these findings suggest that FMT may also be a potential therapy for CD. However, much more work is needed to assess both clinical efficacy as well as changes to the gut microbiota of these patients.

Pouchitis

Patients with UC who undergo colectomy may develop inflammation of the surgically-created ileal pouch, a condition known as pouchitis. Although created from small intestinal tissue, the microbiota of the pouch frequently resembles that of the colon.[122] Like UC itself, however, patients with pouchitis may have a pouch microbiota that is distinct from that of patients without active inflammation.[122,123] Although this suggests that FMT may also be a treatment of pouchitis, small studies have failed to demonstrate remission following FMT.[124] One key limitation in FMT for pouchitis is the size of the pouch, which may limit engraftment of a donor microbiota. Although current data are not promising, more data are necessary to understand the effects of FMT on pouchitis.

Systematic reviews of FMT in IBD

Given the overall limited data, some investigators have pooled available data to attempt to discern a signal from the noise. One review found promising results for FMT in IBD, with the majority of patients achieving reduction of symptoms, cessation of IBD medications, and/or clinical remission.[125] Another review found similar results, with 78% of patients, both pediatric and adult, achieving remission.[126] However, these studies only included data from case reports or series, which are not controlled and subject to publication bias. A more recent systematic review which included a randomized trial for FMT in IBD noted less promising results, with 45% of patients achieving remission (22% of patients with UC, 60.5% of patients with CD).[127] The variability in these findings likely reflects the underlying heterogeneity in the primary studies of FMT for IBD, which in turn may be related to several factors such as donor selection, FMT preparation and delivery, and length of diagnosis or severity of disease.

Safety of FMT

In addition to efficacy, safety of the procedure is an important factor in assessing the usefulness of FMT for IBD. Although minimal side effects are typically reported for FMT for *C. difficile* infection, one case report did note a UC flare following FMT in a patient who was previously in remission for over 20 y before the procedure.[128] Similarly, a recent case series noted that 2.9% of patients treated for *C. difficile* who had underlying IBD required hospitalization for IBD flare after FMT.[129] This suggests that the potential for adverse effects in FMT may be greater in IBD than for recurrent *C. difficile* infection. In studies examining FMT for IBD without *C. difficile* infection, side effects are typically mild and self-limiting. In one systematic review, no serious adverse events were reported in any included study; all mild to moderate adverse events (such as fever, abdominal tenderness, and CRP elevation) were self-limiting outside a single patient with fever, who was successfully treated with acetaminophen.[127] Another study in pediatric patients reported only mild adverse events with one case of moderate abdominal pain, all of which were self-limiting.[120] These findings suggest that FMT for IBD is a safe procedure although the numbers in each trial are small. Given the decreased efficacy of FMT for *C. difficile* infection in patients with underlying IBD,[130] it is possible that the increased side-effects noted in this population are confounded by the presence of both diseases.

Future directions

With increasing numbers of studies, including randomized trials, of FMT for IBD (39 registered studies on clinicaltrials.gov as of this writing), we will be faced with more clinical data in an area where a mechanistic understanding is lacking. At the moment, FMT cannot be considered a consistent therapy across trials. In fact, trials to date vary on the method of FMT delivery, bacterial dose, method of stool filtration, frequency of administration, and do not control for other donor factors such as diet. This diversity may ultimately be beneficial for deriving a mechanistic underpinning for FMT in IBD, though currently it limits our interpretation of FMT as a clinical success or failure.

One essential issue to clarify and define is that of engraftment of a microbiota. A common theme among published studies is that those who achieve normalization of the gut microbiota, or are more similar to their donor, typically experience improved clinical outcomes.[107,109,112,120,121] A key issue moving forward, therefore, will be to understand how donor microbiota engraftment can be improved, as this alone may lead to increased success with FMT. Perhaps one reason why some studies have had more encouraging results is due to multiple FMTs, which suggests those studies may have achieved a higher rate of engraftment.[111]

Related to the issue of engraftment is the question of whether or not a so-called "Western" microbiota may even be useful in the treatment of IBD. The prevalence of IBD in developed countries is typically much higher, and increasing at a much steeper rate, compared to developing nations,[131] although IBD appears to be increasing in these countries as well.[132] Additionally the gut microbiota of individuals in developed countries is substantially different compared to individuals in primitive hunter-gatherer societies.[133-135] These changes are usually characterized as a loss of bacterial diversity, suggesting a possible link between the gut microbiota and the rising prevalence of IBD and other autoimmune and inflammatory conditions in developed countries. Recent data from a mouse model suggests that a shift toward a more "Western" diet results in extinction of certain bacteria within the

gut,[136] further suggesting that a "Western" microbiota may lack key organisms which might be critical to preventing or treating IBD. All of these findings imply that "Western" fecal donors may overall be sub-optimal for treating IBD patients.

Beyond the issues of engraftment and donor choice, a critical issue will be to understand the functions of the microbiota following FMT in those patients who achieve remission. Several studies have suggested that patients with IBD have decreased populations of bacteria capable of producing butyrate, a known anti-inflammatory molecule also used as an energy source by the colonic epithelium.[70,137-139] One study has already found that IBD patients who responded to FMT replenished these butyrate-producing bacteria, suggesting that this may a key mechanism to target in the future.[109] Ideally, future work will lead to targeted microbial therapies aimed at restoring intestinal microbiota functions currently diminished in patients with IBD.

Lastly, the issue of cause and effect is still an unanswered question. Despite the fact that treatment-naïve patients with IBD have dysbiosis,[72] it is still not fully understood if the effects of the early inflammation cause dysbiosis or if the dysbiosis precedes inflammation. Perhaps as IBD appears to be an abnormal immune response to the microbiota, the microbial composition is irrelevant as any generic microbial stimulus will perpetuate inflammation. The truth is likely in between a cure and no effect at all; and while overall the use of FMT for IBD is promising, clearly the course will not be the same as FMT for recurrent *C. difficile* infection. Determining microbial biomarkers (specific bacterial taxa, pathways, or metabolites) associated with IBD will be essential to establish key outcomes for clinical trials. Continued clinical trials of FMT in IBD without teasing out these underlying mechanisms will continue to result in variable and difficult-to-interpret results. Beyond the potential therapeutic application, microbial patterns may allow us to identify those at risk for IBD, predict phenotypes or disease courses, and perhaps even predict complications of IBD, such as PSC or dysplasia. In conclusion, IBD is clearly associated with dysbiosis, and the available data suggest there may be a role for manipulating the intestinal microbiota in the treatment of these devastating diseases.

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