Fecal Microbiota Transplantation: A Review of Emerging Indications Beyond Relapsing *Clostridium difficile* Toxin Colitis

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Abstract: The symbiotic relationship between gut microbiota and humans has been forged over many millennia. This relationship has evolved to establish an intimate partnership that we are only beginning to understand. Gut microbiota were once considered pathogenic, but the concept of gut microbiota and their influence in human health is undergoing a major paradigm shift, as there is mounting evidence of their impact in the homeostasis of intestinal development, metabolic activities, and the immune system. The disruption of microbiota has been associated with many gastrointestinal and nongastrointestinal diseases, and the reconstitution of balanced microbiota has been postulated as a potential therapeutic strategy. Fecal microbiota transplantation (FMT), a unique method to reestablish a sustained balance in the disrupted microbiota of diseased intestine, has demonstrated great success in the treatment of recurrent *Clostridium difficile* infection and has gained increasing acceptance in clinical use. The possibility of dysfunctional microbiota playing a causative role in other gastrointestinal and nongastrointestinal diseases, therefore, has also been raised, and there are an increasing number of studies supporting this hypothesis. FMT is emerging as a feasible therapeutic option for several diseases; however, its efficacy remains in question, given the lack of clinical trial data. Altering microbiota with FMT holds great promise, but much research is needed to further define FMT's therapeutic role and optimize the microbiota delivery system.

Keywords
Fecal microbiota transplantation, *Clostridium difficile* infection, inflammatory bowel disease, irritable bowel disease, obesity

Human intestine provides a nutrient-rich environment to a vast number of microbes. The number and complexity of gut inhabitants are staggering. The human intestinal tract has been estimated to host, on average, 10^{14} microbes, the majority of which reside in the colon, where densities approach 10^{11} to 10^{12} cells per mL.[1] That number of microbes is approximately 10 times greater than the number of cells in the human body,[2] demonstrating that commensal bacteria in the human body vastly outnumber the total

Table. The Current Status of Research on Potential Clinical Indications for Fecal Microbiota Transplantation

Potential Clinical Indications	Current Research Status
Clostridium difficile infection	1 randomized controlled trial and multiple meta-analyses showing efficacy; currently in clinical use
Inflammatory bowel disease	Limited to case reports and case series; not in clinical use
Irritable bowel syndrome	Limited to case reports; not in clinical use
Obesity and diabetes mellitus	Limited to animal and human study; further research needed
Multiple sclerosis and Parkinson disease	Limited to animal and human study; further research needed
Atopy and rheumatoid arthritis	Limited to animal and human study; further research needed
Autism	1 open-label trial showing possible efficacy; further research needed
Depression	Limited to animal study; further research needed

number of cells they inhabit. In addition, microbes in the human intestine are impressively diverse. The majority of microbes in the human gut are strict anaerobes, and more than 80% of these microbes cannot be cultured under standard laboratory conditions.[3] Consequently, the utilization of new, culture-independent techniques such as bacterial 16S ribosomal RNA gene sequencing and DNA fingerprinting methods has shed light onto the remarkable diversity within the microbes.[3] The estimated number of bacterial species present in the human intestine has been thought to be between 500 and 1000[4]; however, a recent analysis suggested that the human gut microbiota may comprise more than 35,000 bacterial species.[5] Such an immense and diverse population of microbes is not without order and organization. There exists a marked and progressive distal increase in bacteria: 10^1 cells per mL in the stomach, 10^3 cells per mL in the duodenum, 10^4 cells per mL in the jejunum, 10^7 cells per mL in the ileum, and 10^{12} cells per mL in the colon.[6] Within the colon, the proximal, middle, and distal colonic segments are physiologically distinct with different bacterial interactions. There is also evidence to suggest spatial organization of microbiota.[7] Therefore, the human gut and its enormous number of microbes do not represent a simple tank filled with an array of floating bacteria; rather, they are a highly organized, diverse ecosystem with complex interactions and sophisticated control.

With the recent intensification of interest in human microbiota, their intrinsic role in human development and health is becoming increasingly recognized. The concept of human microbiota as inert flora in the human gut is obsolete, as there is growing evidence demonstrating their active role in postnatal structural and functional maturation of the gut, development of the immune system, and development of mesenteric vasculature, as well as their influence on the nervous system.[8-10] Data from studies using sterile animals deprived of natural microbiota (germ-free animals or gnotobiological models) have shown that these animals have a markedly enlarged cecum,[11] increased enterochromaffin cell area,[12] severely reduced villous capillary network,[8] and smaller villous thickness.[13] Microbiota also have a pivotal role in mucosal immunity, as illustrated by a series of studies that demonstrated a complete reversal of $CD4^+$ T-cell deficiency in germ-free mice after monocontamination with *Bacteroides fragilis*.[14] Gut microbiota are also known to produce antimicrobial proteins such as defensins, cathelicidins, and C-type lectins.[15,16]

Given the significant role of microbiota in the homeostasis of numerous physiologic processes, it is not surprising that an imbalance of microbiota has been implicated in many disease states, such as antibiotic-associated diarrhea and *Clostridium difficile* infection (CDI). Fecal microbiota transplantation (FMT), an innovative attempt to restore the disturbed microbiota by infusion of fecal suspension from a healthy individual, was first reported in the fourth century by Ge Hong, who described its use in treating food poisoning or severe diarrhea.[17] In modern medicine, the first use of FMT was described by Eiseman and colleagues for the treatment of pseudomembranous colitis in 1958,[18] and by Schwan and colleagues for the treatment of CDI in 1983.[19] Since then, numerous reports and clinical trials have demonstrated the impressive efficacy of FMT in the treatment of recurrent CDI. There are also emerging data on the potential clinical applicability of FMT beyond CDI in both gastrointestinal and nongastrointestinal conditions, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), diabetes mellitus, obesity, multiple sclerosis (MS), Parkinsonism, autism, and depression (Table). This article reviews the potential clinical indications of FMT.

Fecal Microbiota Transplantation Technique

Multiple techniques for FMT have been reported in the literature, but no single, standard protocol has been accepted. Regardless of the technique used, every FMT must first start with the identification of an appropriate donor.

FMT carries the risk of potential transmission of infectious agents. As a result, donor screening tests are

widely recommended.[20,21] Donor stool should be tested for the following: *C difficile* toxin or toxin genes by polymerase chain reaction assay (based on institution); stool culture for standard enteric pathogens as well as standard ova and parasites, with special evaluation for *Giardia* species antigen, cryptosporidium antigen, *Isospora* species (acid-fast stain), and *Helicobacter pylori* stool antigen; and consideration of rotavirus testing. Donor blood screening should include serologic testing for hepatitis A, B, and C, HIV-1 and -2, and syphilis. The cost of donor screening may be problematic based on the recipient's insurance.

In addition to laboratory testing, a medical history should be obtained to exclude donors if they have received antibiotics, a tattoo, or body piercing within the past 3 months, if they engage in high-risk sexual behavior, or if they have recently been incarcerated.[20,21] A history of IBD, IBS, constipation, chronic diarrhea, colonic polyps, colorectal cancer, immunocompromise, metabolic syndrome, morbid obesity, or chronic fatigue syndrome are additional donor exclusions, as these conditions may be associated with altered intestinal microbiota.[20] A history of food allergies for the intended recipient should be obtained, as the donor must avoid ingestion of the allergen(s) for several days prior to donation.[21]

Historically, the majority of FMT donors have been a spouse, relative, or close friend (patient-identified donors).[22-28] However, in some reports, unrelated, healthy individuals whom the recipient did not know have served as stool donors.[29-32] More recently, in order to minimize practical concerns associated with FMT, universal stool donors were identified and screened, fresh or frozen fecal material was used for FMT in patients with recurrent CDI, and the FMT results were compared with those using patient-identified donors.[33] No significant differences in outcomes were seen between patient-identified donor sources and universal donors.[34] Additionally, a meta-analysis specific to FMT in CDI found no difference in clinical outcomes using anonymous vs patient-selected donors.[35]

After an appropriate donor has been identified and screened, the preferred route of administration must be determined. The vast majority of FMT has been performed in patients with CDI, but the issues related to selecting a technique for FMT can apply to other indications. Several routes have been employed, including administration via enema or colonoscopy. Alternatively, the upper gastrointestinal tract has been accessed using an upper endoscope or nasogastric/nasoduodenal tube. Each FMT delivery approach has its advantages and disadvantages.

Fecal enemas are inexpensive and easy to administer, and they have reportedly been infused in patients' homes.[36] Fecal enemas have been shown to be successful in treating CDI.[29,36-38] However, a repeat enema may be required, and patients may have an aversion to handling stool.[39]

Although they may provide some logistic advantages, fecal enemas have a limited FMT distribution, as they can be used only in the splenic flexure, whereas FMT via colonoscopy can infuse stool throughout the colon and, in some instances, the terminal ileum. Multiple studies have reported successful FMT via colonoscopy, most with just a single administration.[27,32,34,40] Given the overall ease of administration in both outpatients and inpatients, the colonoscopic approach is currently the favored method of performing FMT. However, in patients with significant colonic distention or severe colitis, administration via colonoscopy may pose an increased risk of perforation. To date, there has not been a large prospective trial comparing fecal enema and FMT via colonoscopy.

The upper gastrointestinal tract can also be utilized in FMT via esophagogastroduodenoscopy.[41] Alternatively, FMT can also be delivered via nasogastric or nasoduodenal tube. Administration of donor feces via nasogastric or nasoduodenal tube is inexpensive, quick, and generally simple, eliminating the need for an endoscopic procedure. Smaller volumes of feces are typically infused to decrease the risk of vomiting or aspiration.[42-44] To date, there have not been any prospective trials comparing esophagogastroduodenoscopy vs nasogastric tube FMT delivery. The comparison of upper vs lower gastrointestinal FMT has been discussed in an article by Brandt and Aroniadis.[21]

When using the upper gastrointestinal tract for FMT, there is a theoretical risk for small intestinal bacterial overgrowth, but this has not been reported clinically. However, in the setting of intestinal motility disorders or anatomic variations that might promote stasis, such as jejunal diverticulosis, avoidance of FMT via the upper gastrointestinal tract should be considered.[21]

Potential Clinical Indications for Fecal Microbiota Transplantation

Clostridium difficile *Infection*

Given the high recurrence rate in CDI patients treated with antibiotics, FMT has been increasingly employed as an alternative treatment for CDI, with promising results. The most recent systematic review and meta-analysis, conducted by Kassam and colleagues, showed that 245 of 273 patients (90%) experienced clinical resolution.[35] Although it is the only existing study to date, a randomized, controlled trial by van Nood and colleagues also demonstrated the superiority of FMT, as the procedure cured 15 of 16 patients (94%, including 2 patients who required a second infusion to achieve cure), compared with curing 4 of 13 patients (31%) with standard vancomycin treatment and 3 of 13 patients (23%) using vancomycin plus bowel lavage.[42] Pre- and post-FMT fecal microbial analysis in 9 patients revealed

a consistently lower diversity of microbiota (based on Simpson's reciprocal index of diversity) pretransplant and a significant increase within 2 weeks of FMT, becoming indistinguishable from the fecal microbiota diversity level of the donor.[42] Impressive primary cure rates of CDI after FMT also appear to be durable.[40,41] A multicenter long-term follow-up study of 77 patients who had undergone FMT for recurrent CDI at least 3 months prior (with a mean follow-up period of 17 months) reported a primary cure rate of 91%, with all late recurrences of CDI (in 15 of 77 patients, 19%) occurring in the setting of antimicrobial therapy for an infection unrelated to *C difficile*.[40] Similar findings of relapse of primary responders only in the setting of receiving antibiotics for unrelated causes were also seen in a retrospective study by Mattila and colleagues.[32] In addition, favorable outcomes in CDI with the hypervirulent NAP1/ribotype 027 strain have been reported. In a retrospective review of 70 patients, 36 were identified with 027 CDI.[32] Thirty-two of the 36 patients (89%) had a favorable response to FMT.[32]

Inflammatory Bowel Disease

The consensus in the current literature is that IBD likely occurs in individuals with a genetic predisposition to developing an aberrant immune response to endoluminal bacteria. Interactions between the intestinal mucosa and the microbiota are now known to play a role in the development of the host immune system, with certain bacteria affecting the development of anti-inflammatory T-regulatory cells and others affecting the development of proinflammatory cells.[45] Dysbiosis, a shift in the composition of the microbiota, with reduced diversity of luminal microbiota, has been noted in patients with IBD, with decreases in Firmicutes and Bacteroidetes and a concomitant increase in mucosal-adherent bacteria such as Proteobacteria.[46-48] The diminished number of Firmicutes bacteria seen in IBD is notable, as Firmicutes are major producers of short-chain fatty acids such as butyrate, a substrate with immunoregulatory properties.[46,49] Furthermore, the dysbiosis in Crohn's disease has been associated with an increase in adherent/invasive *Escherichia coli* in the terminal ileum.[50] Crohn's disease has also been causally linked to *Mycobacterium avium* subsp *paratuberculosis*.[51]

Given the evidence that alterations in the gastrointestinal microbiota are correlated with inflammation in IBD, manipulation of the microbiota as a treatment for IBD has been investigated. The literature contains several case reports and case series, mainly about ulcerative colitis patients, but there have been no randomized, controlled trials of FMT as a therapy for IBD.[52] A recent systematic review found 9 articles, representing 26 patients (18 ulcerative colitis, 6 Crohn's disease, and 2 indeterminate) who had received FMT for management of IBD.[49] Results were reported for 17 of the 26 patients. Following FMT, 13 of 17 patients (76%) were able to discontinue all IBD medications within 6 weeks, and at 4 months, all had symptom reduction or resolution.[53] A systematic review also identified 8 articles, accounting for 15 patients with IBD who underwent FMT for CDI infection. Outcomes were reported for only 12 patients, and of these, all 12 experienced resolution of CDI, as measured by stool-specific testing.[49] No major adverse events were reported, but several patients experienced fever and abdominal pain.

A phase 1 clinical trial recently evaluated the feasibility, safety, and tolerability of FMT in children with ulcerative colitis while also measuring the effect of FMT on clinical disease activity.[54] FMT was administered via fecal enema daily for 5 days in 10 children ranging from 7 to 21 years of age. One subject could not retain the enemas. No serious adverse events were noted. After FMT, 7 of the 9 subjects showed a clinical response within 1 week, 6 of the 9 subjects maintained a response at 1 month, and 3 achieved clinical remission within 1 week.[54]

Not all studies of FMT for the treatment of IBD have had positive outcomes. Angelberger and colleagues analyzed the bacterial colonies present both pre- and post-FMT in 5 patients with severe to moderate ulcerative colitis.[55] In contrast to previous reports, none of the 5 patients achieved a remission by 12 weeks. Furthermore, only 1 of the 5 patients experienced a response, whereas 2 patients experienced further clinical deterioration of their condition at 4 weeks post-FMT.[55] Analysis of the microbiota composition noted that prior to FMT, the ulcerative colitis patients had an overrepresentation of Enterococcaceae and Enterobacteriaceae and an underrepresentation of Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae, compared with healthy donors.[55] Post-FMT, the microbiota of the patients became similar to those of the donors, but the duration of the change varied among the patients and appeared to be related to the clinical response. The 1 patient who experienced a response maintained similar microbiota compared with the donor at 12 weeks post-FMT. In contrast, the 2 patients who experienced clinical deterioration showed increased microbiota dissimilarity by 4 weeks post-FMT.

Irritable Bowel Syndrome

IBS is a chronic disease that may affect over 10% of the population and has a negative impact on patients both socially and economically.[56,57] Mechanisms by which dysbiosis could contribute to IBS include increasing visceral sensitivity, altering motility, developing small bowel bacterial overgrowth, or compromising intercellular junctions.[58] Several studies have noted differences in the fecal or mucosal microbiota in patients with IBS compared with healthy controls.[58-62] In one study, bacterial genomic

DNA was obtained from fecal samples of 24 patients with IBS as well as from 23 healthy control subjects.[59] The microbiota were altered in patients with IBS, and the composition varied depending on the predominant form of IBS.[59] In a separate study focused on patients with diarrhea-predominant IBS (IBS-D), bacterial DNA from fecal samples of 23 patients were compared with those of 23 healthy control subjects.[63] Overall, subjects with IBS-D had significantly higher levels of Enterobacteriaceae and significantly lower levels of *Faecalibacterium prausnitzii* compared with the healthy control subjects, suggesting an imbalance of protective and potentially harmful bacteria.[63] Similarly, Chassard and colleagues noted dysbiosis in patients with constipation-predominant IBS (IBS-C). In individuals with IBS-C, the number of Enterobacteriaceae was increased 10-fold compared with healthy control subjects.[64] Several other studies have reported decreased proportions of *Bifidobacterium* and *Lactobacillus* species in patients with IBS, with increased ratios of Firmicutes:Bacteroidetes in these patients.[58]

Not only does the luminal composition of microbiota appear altered in IBS patients, but biopsy studies have shown reduced mucosal microbiota diversity compared with control subjects.[65-67] In addition, one study reported that the number of mucosal bacteria in patients with IBS negatively correlated with the number of stools passed.[67] Examination of both mucosal and luminal diversity in patients with IBS-D and healthy control subjects found significantly lesser fecal microbial diversity in patients with IBS-D.[68]

Further supporting the hypothesis that dysbiosis is correlated with IBS is the observation that in some patients, the symptoms of IBS are preceded by an acute episode of gastroenteritis.[69] A meta-analysis of 8 studies reported an odds ratio of 7.3 (95% CI, 4.7-11.1) for developing postinfectious IBS after a gastrointestinal infection.[70] A subsequent systematic review of pooled data from 9 prospective studies reported that the odds ratio for developing postinfectious IBS was 5.9 (95% CI, 3.6-9.5).[71] The risk of postinfectious IBS remained elevated for up to 3 years.[71] The use of antibiotics for infectious diarrhea/gastroenteritis has also been noted to be a risk factor for developing subsequent IBS.[72,73]

Despite the recent findings of dysbiosis seen in IBS, the published evidence for FMT in IBS is limited. The majority of the literature consists of case reports of FMT to treat patients with IBS (either IBS-D or IBS-C).[74] One case series reported 3 patients with chronic constipation who underwent FMT. Post-FMT, all patients defecated at least every other day without the need for laxatives. In another study, 5 patients with IBS received colonic infusion of feces from a healthy donor.[75] Examination of stool posttransplant found that the microbiota resembled those of the donor and that the new microbiota composition remained stable over 24 weeks.[75] In a separate long-term follow-up study, 45 patients with chronic severe constipation were administered a liquid culture containing 20 species of nonpathogenic enteric aerobes and anaerobes via colonoscopy.[76] Thirty of the patients were followed during a period of 9 to 19 months. Improvements, including more frequent defecation and an absence of bloating and abdominal pain, were reported in 60% of patients (18/30).[76] More studies are needed to better understand the potential therapeutic benefit of FMT in IBS.

Obesity, Insulin Resistance, and Diabetes Mellitus

Obesity is an epidemic in the United States. According to the Centers for Disease Control and Prevention, as of 2012, 35% of the US population over the age of 20 years is obese.[77] Obesity-related diseases (such as atherosclerosis and nonalcoholic fatty liver disease) are leading causes of preventable death in the United States.[78] Abdominal obesity, in particular, is associated with insulin resistance (affecting glucose metabolism and fatty acid utilization), which may progress to diabetes mellitus type 2. The estimated annual cost from obesity and obesity-related diseases in the United States in 2008 was $147 billon.[77] The drivers of obesity are complex and are related to a confluence of behavioral, environmental, and genetic factors.

Studies in mice and humans have shown a relative abundance of Firmicutes with a corresponding decrease of Bacteroidetes in obese subjects.[79] In 2004, investigators found that the alteration of microbiota in mice leads to increased body fat in the recipient mice.[80] Microbiota were harvested from conventionally raised, genetically obese mice and were transferred to germ-free mice, which resulted in a 60% increase in body fat and the development of insulin resistance within 2 weeks in the germ-free mice with altered microbiota.[80] A separate study by Turnbaugh and colleagues demonstrated through metagenomic and biochemical analyses that these characteristics of the mouse gut microbiota (namely the relative abundance of Firmicutes bacteria) seen in obese animals affect the metabolic potential of the gut microbiota.[79] The obesity-associated microbiota appear to have an increased capacity to harvest energy from the diet.[78]

Vrieze and colleagues studied the effects of FMT on glucose metabolism in humans by infusing intestinal microbiota from lean donors to male recipients with metabolic syndrome.[81] The participants were randomly assigned to receive a small intestinal infusion of either allogeneic microbiota or autologous microbiota. Six weeks postinfusion, increased insulin sensitivity was noted in recipients of infusions from lean donors.[81] Although further studies in humans are needed, results suggest that alterations of the microbiota have potential for treating insulin resistance.

Central Nervous System Diseases: Multiple Sclerosis and Parkinson Disease

Alterations in the microbiota may lead to dysregulation of immune responses in the intestine. This, in turn, can result in a proinflammatory state, which may result in the development of autoimmune diseases. The link between microbiota and autoimmune disease has been shown by the effect of *B fragilis* on $CD4^+$ T cells. $CD4^+$ T cells are a major component of the immune system and are involved in all functions of the immune system, from reactions to infectious agents to the control of autoimmune reactions.[82] There are 2 subtypes of $CD4^+$ T cells, T-helper 1 (T_H1) and T-helper 2 (T_H2) cells, and the proper balance between these subtypes is crucial for a competent and controlled immune system. In a study using a germ-free murine model, Mazmanian and colleagues demonstrated that polysaccharide A (PSA), a surface polysaccharide unique to the major microbiota *B fragilis*, plays a crucial role in the development of $CD4^+$ T cells by showing the correction of impaired systemic $CD4^+$ T-cell maturation and aberrant T_H1/ T_H2 lineage differentiation in germ-free mice when colonized by *B fragilis.*[83] This association between microbiota and autoimmune disease has been further supported by an animal study of murine experimental autoimmune encephalomyelitis (EAE), the generally accepted experimental model for human MS. In this study, Ochoa-Repáraz and colleagues showed that wild-type *B fragilis* with intact PSA can protect against EAE in mice, whereas colonization of mice with a PSA-deficient *B fragilis* strain restored EAE susceptibility.[84] This interesting gut–central nervous system influence has been observed in humans, with near-complete and prolonged (>15 years) normalization of previously documented severe MS symptoms in 3 patients who underwent FMT for constipation.[85] However, there are no data beyond this anecdotal report on the efficacy of FMT in MS, and further studies are needed.

Data demonstrating an association between microbiota and movement disorders such as Parkinson disease are sparse. As with MS, there is an anecdotal report that showed remarkable resolution of Parkinson symptoms after alteration in the microbiota; tremors, glabellar tap reflex, and cogwheel rigidity resolved in a 73-year-old man after treatment with oral antibiotics (vancomycin and metronidazole) for his constipation.[86] A recent article hypothesized that the well-known epidemiologic pattern of smokers and coffee drinkers having a lower risk of Parkinson disease may be due to the differences in microbiota composition in these individuals.[87] The association between microbiota and Parkinson disease and the possible therapeutic role of FMT in Parkinson disease, as in MS, remain to be further elucidated.

Immune-Mediated Diseases: Atopy and Rheumatoid Arthritis

Previous studies have suggested that a low diversity in gut microbiota during infancy may be associated with the development of allergic disease. The proposed mechanism behind this association is the lack of microbial stimulation resulting in either a misbalance in T-helper cell–type responses or a misbalance in regulatory mechanisms.[88] The evaluation of fecal microbiota found that allergic infants were colonized less often with *Bacteroides* species and bifidobacteria and more often with *Staphylococcus aureus.*[89] A study by Abrahamsson and colleagues showed that infants with immunoglobulin E–associated eczema had a lower diversity of Bacteroidetes at 1 month and Proteobacteria at 12 months, demonstrating that the low intestinal microbial diversity during the first month of life was associated with subsequent atopic eczema.[90] An interesting study by Drago and colleagues showed that the treatment of moderate to severe atopic dermatitis patients with *Lactobacillus salivarius* LS01 probiotics reduced their Scoring Atopic Dermatitis severity scores and also resulted in significant decreases in staphylococci load in stool, further demonstrating the association between gut microbiota and atopic diseases.[91]

Similarly, disruption in gut microbiota has been implicated as a possible etiologic factor in the development of rheumatoid arthritis (RA). Infections and microbes have long been known as environmental factors affecting joints in the body, with microbes such as *Yersinia* species, *Salmonella* species, and *Shigella* species triggering reactive arthritis. The arthritogenic ability of bacterial cell walls had also been demonstrated when susceptible rat strains developed self-perpetuating arthritis, resembling RA, after a single intraperitoneal injection of *Streptococcus pyogenes* cell wall.[92] In addition, using computerized gas-liquid chromatography of bacterial cellular fatty acids to cluster bacterial flora, the investigators have found that patients with early RA had different intestinal microbiota compared with control subjects, further supporting the association between gut microbiota and RA.[93,94] A study by Vaahtovuo and colleagues showed that the intestinal microbe composition of patients with RA was significantly different from that of patients with fibromyalgia, with significantly less *B fragilis* and *Clostridium coccoides* found in the RA subgroup.[95]

There are multiple studies in the literature that link intestinal microbiota to atopic disease and RA. However, as in many other aforementioned disease processes, conclusive data demonstrating the efficacy of FMT in treating these diseases are lacking, and further research is required to define its role as a therapeutic option.

Autism

The association between autism and intestinal microorganisms was raised when the onset of the disease was often

observed following antimicrobial therapy, commonly in patients with gastrointestinal symptoms such as chronic diarrhea.[96] The link between autistic behavior and intestinal microbiota was further supported by a study that analyzed stool samples from autistic children and found higher counts of *Clostridium* species and *Ruminococcus* species as well as a unique species of these genera.[97] The same study also found that certain clusters of *Clostridium* species were present at concentrations 10-fold higher than in stool samples from healthy children.[97] Although the mechanism of gut microbiota affecting autism remains to be elucidated, one of the hypotheses is that disruption of normal microbiota results in overgrowth of neurotoxin-producing bacteria such as *Clostridium tetani*.[98] This hypothesis has been supported in a single case series in which short-term improvement was observed in autistic children after treatment with vancomycin.[99] In this case series, 11 children with regressive-onset autism were treated with an 8-week course of oral vancomycin, and their response was assessed with multiple blinded pre- and posttherapy evaluations by a clinical psychologist. Significant behavioral improvement was noted in 8 of 10 children (1 did not have video available for assessment); however, within 2 weeks of the discontinuation of vancomycin treatment, the behavioral improvement deteriorated. Unfortunately, the direct effect of FMT in autism has not yet been studied.

Depression

The close association between the digestive system and the brain has been recognized for many centuries. The brain-gut axis is a complex and dynamic neural network that communicates in bidirectional fashion rather than with a unidirectional somatosensory pathway from the gut to the brain.[100] Disruption in the brain-gut axis has been implicated in altered stress response and overall behavior, and the significant role of microbiota in homeostasis of this neural network has been an active area of research. An animal study showed that germ-free mice have an overactive hypothalamic-pituitary-adrenal axis in response to stress, which was reversed with colonization with *Bifidobacterium infantis*, a commonly used probiotic organism.[101] Another animal study demonstrated a reduction in the diversity of microbiota in maternally separated animals compared with nonseparated animals.[102] A similar pattern was observed in primate models, which showed a significant decrease in fecal lactobacilli on day 3 postseparation.[103] Despite the data from animal studies, there are no studies on the relative composition of the microbiota in depressed patients. However, major depression and anxiety states are common comorbidities associated with IBS, and studies have revealed abnormal intestinal microbiota profiles in IBS patients.[68,104]

The effect of FMT in altering behavior has been observed in an animal study, where increased exploratory behavior was seen in a strain of anxious mice after colonizing with microbiota from a normal strain.[105] The exploratory behavior was reduced when normal mice received microbiota from the anxious strain. However, there are no published studies to date examining the effect of FMT in depression.

Conclusion and Future Directions

In the past few years, there has been a paradigm shift in the way that the normal gut bacterial ecosystem is regarded. These bacteria are no longer considered passive flora, but rather a significant contributor to a variety of physiologic processes. Research continues to unveil links between dysregulation of microbiota and disease states, both gastrointestinal and non-gastrointestinal. Consequently, the use of FMT to reestablish a sustained balance in disrupted microbiota has proven to be impressively successful in treating recurrent CDI, and it is beginning to show great promise for treating many other diseases. However, much of the data on the efficacy of FMT in diseases other than recurrent CDI are limited to case reports or small studies, with very few randomized, controlled trials. Thus, further research is mandatory before the therapeutic role of FMT can be defined.

As FMT therapy moves forward, its delivery system is also evolving. In the past, most FMT recipients had to locate a willing and suitable donor. However, several institutions and companies have now developed stool banks with stool from prescreened donors, helping to eliminate the first barrier to FMT. Additionally, 2 recent studies have demonstrated that multispecies bacterial isolates from healthy donor stool were equally effective in curing recurrent CDI in animals and humans.[106,107] Researchers in Canada were able to formulate a stool substitute preparation, dubbed "RePOOPulate human probiotic," from purified intestinal bacterial cultures of a healthy human donor.[107] These proof-of-principle studies indicate that a selected mixture of bacterial isolates may replace stool infusions in FMT, pointing to a future where the delivery of FMT may be achieved via capsule or even food products such as yogurt. Indeed, future therapy may include artificial FMT capsules with defined bacterial payloads that target a specific disease state.

The authors have no relevant conflicts of interest to disclose.

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