

Therapeutic transplantation of the distal gut microbiota

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Although it is generally accepted that the distal gut microbiota are relatively stable in healthy adult individuals, a collapse of the microbial community structure resulting from antibiotic therapy or pathogen presence can lead to gut dysfunction. However, recent findings demonstrate that it is possible to engraft new microbiota from a donor source, resulting in the restoration of gut functionality and improvement in health. This builds upon decades of case reports and series in which fecal transfers were used to successfully treat refractory and recurrent *Clostridium difficile* infection. As fecal transplantation becomes part of mainstream medicine, it will likely provide a unique opportunity to study the interactions of humans with their attendant microbiota and allow greater insights into their synergistic functionality.

Although antibiotic therapy is routine in modern medicine and surgery, it may lead to unanticipated negative consequences. Opportunistic infections can complicate antibiotic treatments. An important example of this problem is pseudomembranous colitis, which is caused by a pathogen that is allowed to grow unopposed by the antibiotic-suppressed normal flora of the distal gut. Despite the best supportive measures, the mean mortality of fulminant pseudomembranous colitis is ~75%.¹ The increasing incidence of this condition calls for some novel treatment options. One possible therapeutic solution seems rather simple—to re-establish the normal intestinal flora by administration of fecal material obtained from a healthy donor into the colon of patients with the disease.

The preceding paragraph summarizes how Eiseman *et al.*,¹ a team of surgeons at the University of Colorado and the Veterans Administration Hospital, introduced their paper in 1958, in which they described a case series of four patients treated with fecal enemas. Although some of the once powerful antibiotics listed in their paper have since been discontinued and are no longer useful, others, such as streptomycin and oxytetracycline, have changed their status from life-saving medicines to biocides for use on fruit trees.²

The patients described by Eiseman *et al.* were 45–68 years of age. Their symptoms developed shortly after exposure to multiple antibiotics. Three of four patients had fulminant pseudomembranous colitis and their critical condition continued to worsen despite “heroic” use of hydration,

vasopressors, hydrocortisone, more antibiotics, and even probiotics such as “acidophilus milk” containing *Lactobacillus acidophilus*. Clearly in desperation, the physicians used fecal retention enemas, consisting of feces collected from healthy donors without recent antibiotic exposure. Remarkably, all patients experienced prompt recovery and were discharged from the hospital within days of enema administration. In their report, the team expressed their “hope that more complete evaluation of this simple therapeutic measure can be given further clinical trial by others.” They further noted that once “more precise” microorganisms are identified, their administration in “enteric-coated capsules might be both more aesthetic and more effective.”

At present, we know that the main causative agent of pseudomembranous colitis is *Clostridium difficile*, a Gram-positive bacterium, which results in disease by the production of toxins that directly disrupt the epithelial layer and promote inflammatory responses in the colon.³ Advances in medicine have not kept up with the evolution of this pathogen. Both the incidence and the severity of *C. difficile* infection (CDI) have been steadily increasing due, at least in part, to the emergence of increasingly toxigenic strains of the bacterium that are resistant to most commonly used antibiotics. It is currently estimated that there are ~500,000 cases of CDI every year in the United States alone, and that 15,000–20,000 of these patients die as a result of this condition.⁴ This is comparable with the number of deaths in the United States attributable to HIV (human immunodeficiency virus).

Two of the most challenging clinical problems associated with CDI include fulminant disease and recurrent infection.⁵

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Unfortunately, the hope of Eiseman *et al.* that others would further test the use of bacteriotherapy for treatment of fulminant pseudomembranous colitis has not materialized over the intervening decades. The standard of practice for this form of CDI is emergent resection of the colon. However, despite all clinical advances, the current overall mortality rate for fulminant CDI is ~50%, which is only a modest improvement over that seen in 1958.⁶ Fortunately, most cases of CDI present with mild or moderate diarrhea rather than with severe illness, and the majority of patients can be treated with metronidazole or vancomycin. However, ~20% of these cases suffer recurrence of the infection, and the risk of recurrence increases twofold with each successive episode.⁵ Prolonged tapering and pulse dosing with vancomycin have been found to be effective in some of the most difficult cases. This strategy aims to keep the re-growth of *C. difficile* suppressed, while allowing some recovery of the normal intestinal microflora. However, the strategy can fail, and persistent infection can cause chronic, debilitating, symptoms, and ultimately even death.

Over the last several decades, fecal bacteriotherapy has been offered to some patients with recurrent, refractory CDI and there are now ~200 cases presented in the literature.^{7–9} The cumulative success rate of this treatment is ~90%, and no ill effects have yet been reported. Despite its remarkable success, why is it that a relatively simple and highly curative method for such a common and menacing problem never made it among the standard options of treatment? Perhaps it is simply because of aesthetic reasons and a lack of understanding of the composition and critical functions of the distal gut microbiota. Indeed, substantive investigations of function and composition of the intestinal microbiota have been mostly precluded by the technical limitations of standard microbiological techniques. In the absence of much science, the idea probably could not overcome the instinctive avoidance of something seemingly repulsive. However, recent developments in new genome sequencing technologies and the necessary bioinformatic tools to analyze large sequence sets have radically transformed this area of science.

Our microbiota have become the subject of intense investigations as evidenced by the emergence of a number of dedicated scientific journals, such as *Cell Host and Microbe*, *Mucosal Immunology*, *Gut Microbes*, and major initiatives, such the Human Microbiome Project in the United States and the Metagenomics of the Human Intestinal Tract project financed by the Seventh Framework Programme of the European Commission.^{10,11}

At present, we appreciate that the intestinal microbiota constitute a true organ, one that has pivotal roles in the body's metabolism and immune function.¹² This organ performs unique digestive functions that simply cannot be accomplished by a germ-free gastrointestinal (GI) tract. The cells that constitute this microbial organ are specialized, highly evolved to their environment, and form metabolic and signaling networks with each other and with their host. We have learned from studies conducted using germ-free and various mutant mice that the intestinal microbiota also interact closely with the rest of the GI tract and regulate key functions of nutrient absorption, mucosal barrier maintenance, and immune defense.^{13–15} Chronic inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are associated with an altered intestinal microflora, and are hypothesized to result from their imbalanced relationship with the host's immune system.¹⁶ However, the importance of the intestinal microbiota extends beyond the intestine. A number of studies conducted during the past decade suggest that the intestinal microbiota may have a role in the pathogenesis of metabolic syndrome and obesity.¹⁷ It has also been postulated that the altered gut microbiota represent a partially inherited factor that is contributing to the current obesity epidemic and contribute to the pathogenesis of systemic autoimmunity.¹⁸ Indeed, it is reasonable to speculate that a shift in the composition of the intestinal microbiota by factors identified in the hygiene hypothesis is contributing to the increasing prevalence of autoimmunity and allergic diseases.¹⁹

Naturally, there is intense interest in ways to modify the microbiota to treat disease and promote health. This idea

is generally associated with probiotics and in modern times dates back to the work of Metchnikoff.²⁰ Probiotics are live microorganisms that are added to foods claimed to improve the balance of the intestinal microbiota. In general, there is little evidence for clinical benefit of commercial probiotics, despite it being a multibillion-dollar industry.²¹ However, the challenges for the probiotic approach to alter the distal gut microbiota are truly formidable. The large intestine is inhabited by ~100 trillion bacterial cells, as well as by a number of members of the archaea and eukarya domains of life. In contrast, a typical dose of a commercial probiotic may contain only ~10 billion live organisms (10,000-fold less), and the majority of those eaten probably do not even reach the colon during their transit through the digestive tract. Moreover, microorganisms indigenous to the GI tract are adapted to their intestinal environment and have co-evolved with each other and their host species. Consequently, they live as complex microbial communities in all available intestinal niches. Although some microorganisms used in probiotics, such as bifidobacteria and Nissle *Escherichia coli*, were isolated from human intestines, it is unlikely that single microbial cultures have the ability to compete with the complex and established microbial community in the intestine. Even in pseudomembranous colitis, a condition triggered by suppression of native bacteria by antibiotics, the evidence for the ability of various probiotics to provide therapeutic benefit is lacking.²² Thus, until our understanding of the microbial organ within our intestine increases by many orders of magnitude, the simplest and most obvious method to alter its composition may be by transplantation of the entire microbiota by bacteriotherapy.

About 2 years ago, we started a clinical program to treat patients with recurrent, refractory CDI with fecal transplantation.²³ As part of our protocol, we sampled the feces of the donor and patients before and at various time points after the procedure. The samples then underwent metagenomic analysis to determine the taxonomic distribution of bacterial DNAs in feces. Our initial results demonstrated that patients with recurrent CDI have

marked disruption of their intestinal microbiota. This is perhaps not surprising, given multiple rounds of antibiotics and possible further inhibition of the native microbiota by recurrent growth of *C. difficile* bacteria. Remarkably, however, the donor bacteria engrafted very quickly into their new host and persisted for at least a month (our last examined time point so far) as the new dominant microflora. More importantly, and from the patients' perspective, engrafting results in prompt resolution of symptoms associated with CDI. These results suggest that patients with recurrent CDI are suffering the consequences of failure of the distal gut microbial organ. When new microbial communities taken from a healthy donor are introduced into the patient's colon, they quickly occupy their proper ecological niches and reconstitute the gut organ ecosystem. This is similar to the reconstitution of the hematopoietic system after bone marrow transplantation.

There are, of course, some differences in the types of problems associated with fecal transplantation than those associated with most other organ transplantation. For example, as the gut lacks a formidable immune barrier to microbial engraftment, immunological matching of the donor and the recipient is theoretically not required. This makes the number of possible donors almost limitless. However, the criteria for donor selection should nevertheless be stringent. At this time, donor health is the best guide we have for assuming a healthy gut microbiota. The obvious exclusion criteria for donors include the presence of known intestinal pathogens and chronic systemic viral infections, such as hepatitis and HIV. In addition, donors are typically excluded if they have a history of any GI problems, including inflammatory bowel disease, irritable bowel syndrome, abdominal pains or altered bowel movements, or extensive travel history.⁷ We also exclude donors if they have a history of colon polyps, GI malignancy, bowel surgery, systemic autoimmunity, any liver test abnormalities, or any features of metabolic syndrome. As mentioned above, the composition of the gut microbiota may have a causal link with most of these problems.

It is currently unknown how the composition of the transplanted microbiota changes over time in the new host. However, it is possible that it may largely be shaped by the mucosal immune system through various innate mechanisms and through the secreted antibody.¹⁶ In fact, shepherding the composition and activity of our mutualistic microflora may well be one of the core functions of the intestinal mucosal immune system, in addition to its well-established role in protection against pathogen invasion. Additional physiological, anatomical, and genetic factors intrinsic to the new host may also impact the final composition of the transplanted intestinal microbiota.²⁴ Most obviously, however, individual experiences and behaviors of the host, such as diet and exercise, are also expected to have a considerable influence on the composition of the intestinal microbiota.

Further development of clinical microbial transplantation programs can create new opportunities to address important questions central to mucosal immunology and clinical medicine. We can see some of these opportunities potentially arising from our current work with recurrent CDI. Patients with inflammatory bowel disease are at increased risk for contracting CDI.²⁵ So far, among our refractory CDI patients, there seems to be an increased prevalence of patients with Crohn's disease and microscopic colitis. This opens the door to numerous questions, including: Could fecal transplantation have an impact on its underlying inflammatory bowel disease? What will be the fate of the new microbiota over the long term? How do the new microbiota affect overall health and weight gain? Will the composition evolve differently from that seen in patients without discernible underlying intestinal inflammation? Investigating complex patients such as these could provide some interesting pilot data.

A more direct approach to test the therapeutic potential of fecal transplantation for some diseases may also be justifiable, and accompanied by basic investigations. In 1989, Bennet and Brinkman²⁶ described a case report of the successful treatment of refractory ulcerative colitis by fecal bacteriotherapy following antibiotic "sterilization." Borody *et al.* published

a case series of six patients with refractory ulcerative colitis who have apparently achieved complete (clinical and histological), medication-free, remission using a similar procedure. No disease recurrence was noted in 1–13 years of follow-up.²⁷ However, no follow-up controlled trials have yet emerged, and similar to fulminant CDI, the standard care for refractory ulcerative colitis remains surgical removal of the colon. Nevertheless, replacement of the host's intestinal microbiota by fecal transplantation following deliberate antibiotic treatment and durable persistence of donor bacteria for up to 24 weeks has recently been documented.²⁸ This provides evidence for the plausibility of using this therapeutic approach for conditions that may be caused by the pathogenic microbiota.

Although long recognized for their importance, the human microbiota have been largely ignored by science because of their complexity and technical limitations of classic microbiological techniques. However, genomic sequencing technologies, computational methods, as well as large-scale proteomic and metabolomic analyses, are finally opening up this world to scientific investigation. Microbial ecologists, clinicians, immunologists, physiologists, nutritionists, and computer scientists are now beginning to work together, learn from each other, and are building this new science. Clearly, however, the mere cataloguing of all the different microbial species at various locations of the human body and the association of specific microbial signatures with diseases will not by themselves establish mechanisms of disease causation or provide novel therapies. Rather, combining metagenomic and metabolomic studies with intentional modification of the microbiota are necessary to achieve these goals. Transplantation of the microbiota in animal models, particularly using gnotobiotic mice, have already led to major new insights into host interactions with its microflora and is contributing to the development of new hypotheses and disease paradigms.^{14,29} However, transplantation of the microbiota in humans has also been occasionally performed for selected indications in the clinic with very promising results for many decades.

It is time to realize the hopes expressed by Eiseman *et al.* over half a century ago. This requires careful design and the use of larger and well-controlled clinical trials involving transplantation of the distal gut microbiota. We can ultimately get a clearer understanding of this important microbial organ by combining clinical data obtained in these studies with modern metagenomic analyses of the gut microbiome.

DISCLOSURE

The authors declared no conflict of interest.

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