INTRODUCTION

Fecal microbiota transplantation–early steps on a long journey ahead

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“In the early days of oral antibiotics we were plagued by frequent diarrhea in our patients due presumably to killing off intestinal bacteria. I was Chief of Surgery at the VA and simplistically considered merely reintroducing normal organisms to counter such absence. Those were days when if one had an idea, we simply tried. It seemed to work and I wrote it up. It made a small splash…Best Wishes.”

Ben Eiseman. Emeritus Professor of Surgery. (E-mail, September 20, 2011)

In 1958 Eiseman and colleagues reported a case series of 4 patients with pseudomembranous enterocolitis promptly cured with a “simple therapeutic measure,” fecal enemas.1 The condition was almost certainly caused by Clostridium difficile, a bacterium linked to pseudomembranous colitis only in the 1970s. However, the association with the usage of antibiotics was well recognized earlier and the fecal enema treatment was postulated to “re-establish the balance of nature” by “reintroduction of the bacteria, viruses, and bacteriophage normally found in the colon.” In the 1950s pseudomembranous colitis was a dreadful disease with nearly 75% mortality. The 30-day mortality rate associated with surgical excision of the colon, which remains the current standard of care for antibiotic-refractory severe-complicated C. difficile infection (CDI), still stands at approximately 50%.2,3 In this issue of Gut Microbes, in their updated single center experience, Fischer and colleagues report 91% clinical cure of antibiotic-refractory severe-complicated CDI using FMT in 33 consecutive patients.4 For practitioners who have personally witnessed the remarkably rapid reversal of the deteriorating clinical course of C. difficile-triggered toxic megacolon following FMT, standard surgical care may no longer seem an ethically justifiable first treatment choice. In fact, fecal enemas were initially rapidly adopted in the care of pseudomembranous colitis in some centers following the Eiseman publication, only to retreat into near obscurity after introduction of vancomycin.5

During the past decade FMT has become widely used once again in response to the enormous clinical challenges posed by CDI, which all too often can no longer be cured with antibiotics alone. However, this latest re-emergence of FMT has coincided with concurrent paradigm shifts in our scientific understanding of the human microbiota and the growing recognition of its interactions with the human host. Therefore, this entire issue of Gut Microbes is focused on FMT, a treatment approach that has literally opened up a new frontier of scientific investigation and therapeutics development.

The scientific revolution that led to the modern studies of the indigenous microbial communities was enabled by the development of high-throughput DNA sequence analysis, various other -omics technologies, and computational advancements. It is now clear that the pathogen-centric germ theory of disease reigning over medicine since the 19th century has not adequately acknowledged the essential roles played by the resident microbiota in the body physiology. Thus, over many decades physicians have been prescribing antibiotics with little consideration for their potential long-term effects that could result from altering the body microbiota. Even greater tonnage of antibiotics has been used in agriculture, where ironically their benefits had little to do with pathogens and everything to do with the role of indigenous microbiota in growth
and energy metabolism of the host. As a consequence, we are now facing an emerging tsunami of multidrug resistant pathogens. Moreover, it is widely speculated that the the ongoing epidemics of obesity, autoimmunity, and allergic diseases may be in part be related to population-wide shifts in the composition of gut microbiota.

Most antibiotic-based therapies challenge the long-established, mutually beneficial relationship between microbiota and its host. In the gut the host provides a hospitable home and a steady nutrient flow for the microbiota, while the microbiota promotes colonization resistance against pathogens and participates in the development and maintenance of the immune system and energy metabolism of the host. This mutualist relationship between host and its microbiota has been nurtured and perfected over millions of years. However, antibiotic drugs violate the terms of this ancient contract, and accelerate pathogen evolution by creating a hostile host environment for the microbiota. As a result, some of the most difficult emerging pathogens originate from the commensals. These microorganisms maintain their prowess to operate within their native host environment, but are also more likely to survive outside (e.g., C. difficile spores) and more likely to be relatively promiscuous, able to infect different host species (e.g., various strains of pathogenic Escherichia coli). Being no longer invested in the well-being of the original host, they develop strategies to take advantage of host resources and often optimize their ability to spread by causing disease. Development of new antibiotics against these commensal-derived pathogens is increasingly difficult because even relatively narrowly targeted drugs also affect many related, non-pathogenic species within the indigenous microbiota. Emergent pathogenic, multidrug resistant organisms now present a public health threat that needs urgent solutions to avert a growing crisis.

The distal gut is an especially fertile location for encouraging emergence of antibiotic-resistant pathogens because of its dense microbial population, which increases the opportunities for transfer of antibiotic resistance and virulence genes by phage-mediated transduction, conjugation-mediated plasmid exchange, and direct uptake of free DNA (transformation). Patients with recurrent CDI (rCDI) provide a compelling illustration. They receive multiple sequential courses of antibiotics, which ultimately decimate the indigenous microbiota, especially the obligate anaerobes, while allowing for blooms of normally minor constituents of the distal gut microbiota, such as Gammaproteobacteria. This class of bacteria is endowed with a strikingly rich genomic assortment of elements that enable horizontal gene transfer, which makes it especially adept at gene acquisition and diversification. Since horizontal gene transfer is even more efficient among related microorganisms, the commonly observed dramatic expansion of Gammaproteobacteria in rCDI patients is particularly troubling. Madsen and colleagues have previously shown that the abundance and diversity of antibiotic resistance genes (the ‘resistome’) is markedly increased in rCDI patients, and that FMT results in resistome contraction. In this issue the team explores FMT as a potential strategy to combat antibiotic resistant pathogens through normalization of microbial gut ecology by restoring mucosal host defenses and competitive interactions among microbes. Their preliminary results should encourage larger trials to test this idea in other common clinical situations associated with heavy antibiotic burden, e.g., patients undergoing intensive myeloablative chemotherapy where blooms of specific enteric bacteria appear to result in bloodstream infections.

The spectacular success of FMT in treating patients with rCDI has triggered a great deal of interest in trying out a similar approach in other conditions associated with altered composition and functionality of the gut microbiota. These include inflammatory bowel disease and metabolic syndrome, which are discussed in this issue. The potential causal links between altered microbiota and disease pathogenesis are compelling in both diseases. However, it is already clear that the path to success in these areas is going to be long and difficult. Unlike problems that are directly related to destruction of microbiota by antibiotics, patients affected by these diseases possess stable indigenous microbial communities that may be highly resistant to invasion or replacement. In fact, a single infusion of donor microbiota, which can be sufficient to achieve mostly donor-like microbiota composition in rCDI patients, allows only limited engraftment in absence of rigorous antibiotic conditioning. Yet, in addition to challenges in solving the technical issues of microbiota engraftment, there are many other unknowns, which include criteria for optimal selection of both donors and recipients. A skeptic may even speculate that most donors raised in the industrialized
countries may already have suboptimal microbiota for these indications because of altered microbiota functionality associated with population-wide loss of helminths and microbial diversity.\textsuperscript{20-22} Furthermore, timing of treatment may be critical because microbiota participates in host development and some immunologic and metabolic circuits may be irreversibly hard-wired even before onset of phenotypic disease.\textsuperscript{23} Clearly, systematic progress can only be made if mechanistic work is performed alongside the clinical FMT trials. Such investigations require careful characterizations of shifts in microbiota composition and functionality, combined with hypothesis-driven analyses to achieve greater understanding. Notably, while diversity among donors may be seen as a potential weakness in development of FMT products, it is an important opportunity for mechanistic research that can lead to elucidation of clinically relevant specific attributes of different microbial communities.

Given the many important roles microbiota play in host physiology, donor selection is a fundamentally important issue in FMT regardless of indication. Historically, patients were asked to identify their own donors, which commonly were their intimate partners or family members. Although this remains an approach still applied in clinical practice, most FMT treatments today use material prepared from standardized, healthy donors. Thus far, the basic methodology for qualifying donors based on donor health has changed little since its original descriptions,\textsuperscript{24,25} which was based on a model borrowed from the solid organ transplant field. The assumption is that healthy donors also have healthy microbiota, while the analysis of microbiota itself is limited to infectious disease testing. In this issue Woodworth and colleagues examine many challenges in the FMT donor selection process.\textsuperscript{26} These include not just mitigating risks of infectious disease, but also minimizing the risks of triggering autoimmunity, metabolic disorders, and transferring antibiotic resistance. The shortcomings of relying purely on the clinical phenotype of the donor are also highlighted by a brief report by Fischer and colleagues.\textsuperscript{27} They followed 31 recipients of fecal microbiota derived from a 28-year old asymptomatic donor who subsequently developed Crohn’s disease. Fortunately, none of the patients developed autoimmunity or inflammatory bowel disease in follow-up. However, this clinical anecdote clearly illustrates that youth is not equivalent to health. As the field moves forward, we should expect that the modern investigative tools, such as next-generation sequencing and metabolomics, will become incorporated into clinical laboratory testing. There is even potential that metrics based on these technologies will be developed to pair donors and recipients for optimal health outcomes and personalized medicine may become the model of FMT in the future.

It is important to appreciate that we are still in relative infancy in characterizing the gut microbiome. Sadowsky and colleagues compare the current technological advances in sequencing technologies to the revolution in biology ushered in by Antonie van Leeuwenhoek’s development of the microscope.\textsuperscript{28} Undoubtedly, van Leeuwenhoek would be impressed with the range and power of various microscopy techniques developed since his day. Similarly, although the current metagenomic technologies have enabled initial studies of complex microbial communities, the commonly used 16S rRNA amplicon-based analyses have serious limitations. Specifically, they cannot allow taxonomic assignment beyond a genus level and may contain biases associated with primer sequences and target amplification by PCR. Here, Sadowsky and colleagues discuss a new sequencing platform, which can produce much longer read lengths, allowing species-level resolution of bacteria and unbiased sequencing results.\textsuperscript{28} This advance may be compared with introduction of next-generation compound microscopes, which ultimately overtook the single lens instruments made by van Leeuwenhoek. However, as history of microscopy illustrates, we can expect many steps on this journey. Technological innovation and development will undoubtedly continue and enable increasingly better-resolved images of the microbiota.

Of course, exclusive attention to bacteria yields a very incomplete view of the gut microbiota, which contains members from all 3 domains of life and a rich viral population. The human enteric virome consists of bacteriophages and eukaryotic viruses. Both can play direct roles in intestinal physiology and state of mucosal inflammation. Bacteriophages also shape the composition of bacterial communities through predator-prey relationships. Interestingly, disease-specific enteric viromes have been described for ulcerative colitis, Crohn’s disease, and obesity.\textsuperscript{29,30} The literature on viral transfer following FMT remains sparse, but based on initial case reports from rCDI patients it appears to be
substantial.\textsuperscript{31,32} Furthermore, a recent provocative case series of 5 patients suggested that patients with rCDI may be successfully treated with sterile filtrates of donor stool, which do not contain intact bacteria, but do contain viruses.\textsuperscript{33} In fact, transfer of donor-associated bacteriophages was documented in the same study.\textsuperscript{33} In this issue Broecker and colleagues extend their previous analysis of post-FMT virome from an rCDI patient treated some 4.5 y ago.\textsuperscript{34} Interestingly, they found that the patient’s bacteriophage population became not only donor-like, but remained more stable than the shifting bacterial communities. At the moment we’re still reading the first pages of the chapter on the enteric virome in FMT, but it is shaping up to be a fascinating read.

Back in the clinic, the daunting complexity of the microbiota does not make patients having dysbiosis-associated disorders any less real. The CDI alone affects nearly half a million patients every year just in the US, and a substantial fraction goes on to develop the rCDI syndrome.\textsuperscript{35} FMT has entered mainstream medicine and became included in standard treatment guidelines.\textsuperscript{36} This was enabled by development of methodologies to bank cryopreserved microbiota material from standardized donors.\textsuperscript{32} Recently, oral encapsulated preparations, anticipated by Eiseman back in 1958,\textsuperscript{1} have also been used by several groups.\textsuperscript{37,38} In this issue Staley et al. describe the dynamics of microbiota shifts following treatment of rCDI patients with the encapsulated freeze-dried preparation.\textsuperscript{39} Although full donor-like normalization of microbiota in patients was somewhat delayed relative to previous studies with colonoscopic administration, the clinical efficacy of this preparation was comparable to the colonoscopic route. Importantly, this preliminary study showed that a relatively small dose, merely several capsules taken once, was equally potent in achieving clinical cure to a dose an order of magnitude greater. This finding suggests that manufacture of FMT products can be easily scaled up to meet a very large patient demand.

Emergence of FMT as a widely available treatment challenges the physicians and regulators to take a crash course in the new microbiome science. Most are still under the spell of the pathogen-centric germ theory of disease, which largely ignores the indigenous microbiota. At the same time many physicians and patients uncritically accept the mirror construct of the germ theory of disease, the notion of “good microbes,” which are marketed as ‘probiotics’. It is a compelling human idea – if there are bad microbes, there must be good microbes, too. In some ways, the probiotics science resembles alchemy, which was driven by a great promise despite a weak theoretical foundation, but enabled the ultimate emergence of chemistry (arguably, commercial makers of probiotics have been more successful than alchemists in making gold). In contrast, FMT as a therapeutic approach embraces the science of microbial ecology and acknowledges that in nature microbes exist as members of complex microbial communities, where each member has distinct relationships with many other members as well as the host. It treats microbiota as a whole, not a consortium of independent microorganisms. In fact, it recognizes gut microbiota as an organ integral to host physiology.\textsuperscript{40} One can make a strong scientific argument that it can be thought of as a human organ since it is uniquely adapted to human hosts.\textsuperscript{41} Notably, the Food and Drug Administration has classified FMT as a drug because it is used to treat, mitigate, or prevent disease. It ruled out the alternative classification of FMT as a human tissue transplant because it felt that microbiota is not human. In this issue Hoffman and coworkers provide an interim report on their important efforts to clarify the definition of what constitutes FMT.\textsuperscript{42} Clearly, terminology can be critically important, and even a single word can determine the trajectory for development of an entire field.

The regulators have a formidable task to develop policies that optimize access to important therapies, ensure their safety and efficacy, and allow innovation so that next-generation products can benefit future patients even more. It is clear that FMT opened a new frontier of medicine and demonstrated the healing powers within our own bodies. It is important that we are guided by up-to-date science on this journey. At the same time we need to be careful not to allow limited scientific knowledge turn into arrogance. We are still taking only the initial steps, even though they have already saved thousands of lives. The current issue of Gut Microbes provides a partial snapshot of the many topical problems and fascinating questions that are being pursued in this very young and active field to allow the next steps to follow.

\textbf{Disclosure of potential conflicts of interest}

No potential conflicts of interest were disclosed.
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