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Fecal microbiota transplantation: effectiveness, complexities, and lingering concerns

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The mammalian colon is home to a microbial ecosystem that enhances resistance to infection, stimulates mucosal immune defenses, synthesizes essential vitamins, and promotes caloric uptake by hydrolyzing complex carbohydrates. The bacterial populations inhabiting the gut are complex and vary between different individuals. Clinical and experimental studies reveal that the colonic microbiota can enhance or ameliorate intestinal and systemic inflammatory diseases. Because of its potential to enhance resistance to infection and to reduce inflammatory diseases, targeted manipulation of microbial populations is a growing focus of investigation. The most dramatic manipulation of the intestinal microbiota involves fecal microbiota transplantation (FMT) from healthy donors to individuals with specific diseases. Remarkable clinical effectiveness of FMT has been demonstrated for recurrent *Clostridium difficile* infection and ongoing studies are investigating FMT for other diseases. Transplantation of complex microbial populations to recipients likely triggers mucosal immune responses that, depending on the microbiota composition and the recipient's genotype, could range from pro- to anti-inflammatory. The impact of FMT on the recipient immune system is complex and unpredictable. Ongoing discovery of commensal microbes and investigations of their impact on the host will lead to the development of new probiotic agents and microbial consortia that will eventually replace FMT.

Our understanding of immune cells inhabiting the gut mucosa, particularly in the lower gastrointestinal tract, has increased markedly in recent years.[1–3] In parallel with the identification and characterization of intestinal T cell, innate lymphocyte and dendritic cell subsets, next-generation DNA sequencing platforms have enabled analyses of the complex microbial populations inhabiting the intestine and associating with the gut epithelium.[4,5] The discovery of clinically important and fascinating interactions between host immune cells and commensal microbes has drawn the fields of mucosal immunology and microbial ecology together.[1,3,6,7] Bacterial families, genera, and species that previously interested only microbiologists focusing on dietary fiber breakdown and short-chain fatty acid production are now also a focus of mucosal immunologists struggling to gain a command of ever-evolving microbial taxonomic classifications.[8,9]

Associations between intestinal microbes and inflammatory diseases of the gut have long been suspected and are increasingly being defined by metagenomic analyses of the gut microbiota. Mouse models have been particularly enlightening, in part because of the wide array of genetically defined mutant mouse strains and because the intestinal microbiota of mice can be aggressively manipulated by antibiotic treatment or administration of specific bacterial strains or complex microbial populations. Additionally, studies with germ-free and gnotobiotic mice enable experiments where the impact of specific bacteria on the immune system can be determined.[8,10] Studies with several mutant mouse strains demonstrated

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the impact of the innate immune system on the intestinal microbiota and the development of gut inflammation. For example, T- and B-cell-deficient mice lacking the T-bet transcription factor develop colitis with an altered microbiota that, upon transfer to wild-type-recipient mice, induces bowel inflammation.[11] Recent studies determined that *Helicobacter typhlonius* stimulates tumor necrosis factor production by intestinal dendritic cells in these mice.[12] Along similar lines, mice lacking the NLRP6 inflammasome subunit also develop bowel inflammation that is driven by an abnormal microbiota that induces colitis and enhances liver inflammation.[13,14] In some but not all studies, likely reflecting institutional differences in the microbial populations inhabiting mouse colonies, deficiency of Toll-like receptor 5 (TLR5) induces obesity and metabolic syndrome attributable to the intestinal microbiota composition.[15–17]

The intestinal microbiota has a major role in defense against infection. Work in the 1940s and 1950s demonstrated that antibiotic-mediated disruption of the intestinal microbiota markedly increases susceptibility of mice and other rodents to intestinal bacterial infection.[18] These studies also correlated the presence of obligate anaerobic bacteria in the colon with resistance to infection.[19–21] Over 50 years ago, Hentges and Freter[22] suggested that manipulation of the intestinal bacterial flora might represent an important therapeutic option for intestinal infections. Advances in this area, however, were limited because distinguishing different intestinal bacterial species and strains depended on *in vitro* culturing and an array of biochemical and metabolic tests that provided relatively low taxonomic resolution. The rate of progress in the field of intestinal microbial ecology accelerated, however, with the introduction of high-throughput nucleic acid sequencing platforms, which enabled rapid identification and quantitation of different intestinal bacterial species, even if they could not be cultured. Conventional sequencing of bacterial 16S rRNA genes cloned from the normal colon of three healthy individuals revealed complexity and diversity of bacterial populations inhabiting the gut,[23] and more recent applications of next-generation sequencing platforms have provided unprecedented views of the complexity and composition of intestinal microbial populations.[4,5] Using these and other high-throughput platforms, subsets of intestinal microbes that are associated with resistance to intestinal pathogens have been identified.[24–26] Furthermore, antiviral defenses are also reduced in germ-free or antibiotic-treated mice.[27]

Although it is known that antibiotic treatment of animals can increase their susceptibility to infection, our understanding of the impact of antibiotic administration on human susceptibility to infection is incomplete. Studies of humans treated with different antibiotics revealed that antibiotic administration results in expansion of aero-tolerant bacteria such as Enterococci and Proteobacteria in the intestine and correlates with the loss of obligate anaerobes.[28] Bacteria belonging to these groups are some of the most common pathogens of hospitalized patients, a likely result of frequent and often prolonged prophylactic or therapeutic antibiotic administration.

Antibiotic-associated diarrhea, which spans a clinical spectrum from mild to severe, is a widely recognized and common complication of antibiotic treatment. *Clostridium difficile*, an endospore-forming and toxin-producing bacterium that can thrive in the intestine of antibiotic-treated patients, is the most common cause of antibiotic-associated diarrhea.[29,30] In the past decade, *C. difficile* infections have become more frequent because of the rapid spread of a specific and presumably more transmissible strain throughout North American and European health-care institutions.[31] Treatment of *C. difficile* infection involves administration of antibiotics, most commonly Metronidazole or oral Vancomycin. The broad antibacterial spectrum of these antibiotics, however, further damages the microbiota, leading to high rates of *C. difficile* recurrence.[29] Indeed, some patients, once infected with *C. difficile*, become incurable with antibiotic therapy and suffer from frequent recurrences and prolonged, severe diarrhea.

The association of *C. difficile* infection with preceding antibiotic treatment suggests that damage to the intestinal microbial flora leads to infection and recurrences. Although it makes sense that administration of a "healthy" fecal microbiota to patients might ameliorate recurrent *C. difficile* infections, the medical community has a deeply ingrained and well-justified aversion to exposing patients to feces. Nevertheless, decades ago, a few pioneering physicians performed fecal transplants from healthy donors, often close relatives, to patients with recurrent *C. difficile* infections.[32] The results were dramatic and highly effective in roughly 90% of cases.[33,34] Numerous uncontrolled studies demonstrated the effectiveness of this procedure, and recently led to a controlled study that demonstrated the effectiveness of fecal transplantation compared with conventional antibiotic administration.[35]

Recurrent *C. difficile* infection is the only infection with randomized clinical trial evidence documenting fecal transplantation's effectiveness. Relapsing *C. difficile* infections can be highly debilitating and fecal microbiota transplantation (FMT) is often rapidly effective, thus favoring this intervention when risks and benefits are considered. The risks include potential exposure to unknown pathogens and/or potential transfer of a microbiota that predisposes the recipient to obesity, inflammatory bowel disease, or metabolic syndrome. One approach to limit the risks of fecal transplantation is to carefully screen fecal donors for transmissible diseases and inflammatory disorders that might be driven by the intestinal microbiota. Donor feces can be frozen and thawed without loss of effectiveness, enabling long-term banking.[36] To circumvent clinical concerns with FMT, a number of laboratories and groups are isolating specific bacterial species from the gut microbiota and testing their ability to provide resistance to *C. difficile* infection. Bacteria belonging to the

Lachnospiraceae family have been implicated in resistance to *C. difficile* infection and, upon transfer into germ-free mice, can reduce the intensity of infection.[37] Tvede and Rask-Madsen[38] demonstrated that a consortium of 10 different bacterial species, upon administration to patients with recurrent *C. difficile*, can be curative. Other investigators have assembled small consortia of intestinal bacteria to effectively treat recurrent infection in mice and humans.[39,40]

How does FMT cure chronic, recurrent *C. difficile* infection? Establishment of a complex, donor-derived microbiota is one likely result of FMT and is supported by analyses of the post-transplant recipient microbiota. Some studies, however, suggest that administration of donor bacteria also promotes re-expansion of recipient bacterial species, thus normalizing microbiota diversity. One mechanism by which the re-established intestinal microbiota might suppress *C. difficile* infection is by occupying space, thereby physically eliminating its niche. Experimental studies, however, do not support this conceptually simple model. A more likely scenario is that the newly established microbiota directly inhibits *C. difficile* or depletes substrates that promote *C. difficile* germination, growth, or toxin production.[1] Competition for nutrients can inhibit the growth of competing bacterial species. Suppression of pathogenic bacteria by commensal Bifidobacterium has been demonstrated to result from acetate production.[41] An additional potential mechanism involves the role of commensal bacteria in bile salt metabolism. Primary bile salts can induce *C. difficile* endospore germination, an important step in the pathogenesis of *C. difficile* infection.[42] Secondary bile salts are generated by bacterial modification of primary bile salts and can inhibit vegetative growth of *C. difficile*. Antibiotic treatment can eliminate bacterial populations that generate secondary bile salts, leading to enhanced vegetative growth.[43] Bacterial species or strains that promote secondary bile salt generation remain incompletely defined.

In addition to these mechanisms, induction of intestinal innate immune defenses by the transplanted microbiota may indirectly defend against *C. difficile* infection. Antibiotic administration reduces the density and alters the composition of the microbiota inhabiting the small and large intestine, resulting in reduced intestinal expression of RegIIIγ, a C-type lectin that is secreted by epithelial cells and that kills Gram-positive bacteria.[44,45] Recent studies demonstrated that reduced RegIIIγ expression following antibiotic treatment enhances intestinal colonization by vancomycin-resistant enterococcus (VRE).[46] Administration of oral lipopolysaccharide or systemic flagellin to antibiotic-treated mice re-induces RegIIIγ expression and reduces intestinal colonization with VRE.[46,47] Recent studies using the mouse model demonstrated that stimulation of TLR5 with flagellin increases resistance to *C. difficile* infection.[48] Although it is unclear whether TLR5-mediated protection against *C. difficile* infection is mediated by RegIIIγ, recent studies have demonstrated that MyD88-deficient mice are highly susceptible to *C. difficile* infection and that MyD88-signaling is required for neutrophil recruitment to the gut, an essential step for survival of infection.[49,50] In addition to TLR-driven innate immune activation, signaling via cytosolic NOD1 and expression of IL1β also contribute to defense against *C. difficile*.[51,52]

The effectiveness of fecal transplantation, therefore, may be direct, by inhibiting *C. difficile*, or indirect, by enhancing host factors such as bile salts or innate immune defenses. Further studies will be required to determine the relative contributions of these mechanisms to defense against *C. difficile* infection. Given the spectrum of *C. difficile* infection severity, it is likely that host defense is multifactorial, with the most severe and prolonged infections resulting from compromise of multiple parallel defensive pathways.

Will fecal transplantation evolve into a therapy for other diseases? There is great interest in the potential role of fecal transplantation for a wide range of diseases, particularly inflammatory bowel diseases.[53] The rationale for this interest is reasonable. Studies with mice have demonstrated that some intestinal bacterial species, such as segmented filamentous bacterium, induce the differentiation of the Th17 subset and promote intestinal and systemic inflammations.[10] On the other hand, other bacterial strains have been implicated in the induction of Tregs, which have been implicated in the reduction of inflammatory processes in the gut.[54] More recent studies have identified bacterial strains derived from human feces that induce Treg development.[8]

Fecal transplantation for inflammatory diseases might be effective if the recipient flora is pro-inflammatory and the donor flora is anti-inflammatory. Although some bacterial species have been identified that are pro- or anti-inflammatory, fecal samples from healthy donors are complex and composed of species that are incompletely defined or even completely undefined. Thus, it is not possible at this time to determine by compositional analysis whether a donor fecal sample will be net pro- or anti-inflammatory. Although there are reports of clinical success with fecal transplantation for inflammatory bowel disease, the consistency of effectiveness falls short of that demonstrated for treatment of recurrent *C. difficile* infection. Controlled clinical studies of fecal transplantation are being performed for inflammatory bowel disease, obesity, and other inflammatory diseases. Some studies have suggested that genomic complexity of the microbiome is an important factor determining host's state of metabolic activation.[55]

What is the future of fecal transplantation? Although our understanding of the microbiome and mucosal immune system is moving forward rapidly, the diversity of the fecal microbiota and the marked genomic variation even within well-defined bacterial species is making the design of optimal probiotic combinations challenging. Thus, at least for recurrent *C. difficile* infection, fecal transplantation is likely to be a last-ditch therapy for years to come. Eventually, with the development of probiotic combinations, fecal transplantation will be replaced by administration of probiotic consortia. Will it be possible to optimize microbiota to prevent

infections or to reconstitute the microbiota following antibiotic treatment? This seems likely, but the time frame for this is unclear. Should administered microbiota components be matched to the host's genotype, diet, or environment? This is a complex topic but it seems likely that a specific commensal bacterial species might cause disparate immune responses in different individuals. To what extent can dietary changes optimize the intestinal microbiota, and how will this influence the immune system? Diet has been demonstrated to alter the microbiota and enhance the ability of the microbiota to absorb calories,[56] and this is likely to also extend to relative immune activation. These are all questions that are of great interest to patients, clinicians, and microbiologists, and, given the known impact of the microbiota on gut-associated immune tissues and systemic immune development, they will remain the focus of subset of mucosal immunologists for many years to come.

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DISCLOSURE

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REFERENCES

1. Buffie, C.G. & Pamer, E.G. Microbiota-mediated colonization resistance against intestinal pathogens. *Nat. Rev. Immunol.* **13**, 790 (2013).
2. Spits, H. *et al.* Innate lymphoid cells–a proposal for uniform nomenclature. *Nat. Rev. Immunol.* **13**, 145 (2013).
3. Kamada, N. *et al.* Role of the gut microbiota in immunity and inflammatory disease. *Nat. Rev. Immunol.* **13**, 321 (2013).
4. Gevers, D. *et al.* The Human Microbiome Project: a community resource for the healthy human microbiome. *PLoS Biol.* **10**, e1001377 (2012).
5. Arumugam, M. *et al.* Enterotypes of the human gut microbiome. *Nature* **473**, 174 (2011).
6. Hooper, L.V., Littman, D.R. & Macpherson, A.J. Macpherson. Interactions between the microbiota and the immune system. *Science* **336**, 1268 (2012).
7. Honda, K. & Littman, D.R. The microbiome in infectious disease and inflammation. *Annu. Rev. Immunol.* **30**, 759 (2012).
8. Atarashi, K. *et al.* Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* **500**, 232 (2013).
9. Flint, H.J. *et al.* The role of the gut microbiota in nutrition and health. *Nat. Rev. Gastroenterol. Hepatol.* **9**, 577 (2012).
10. Ivanov, K. Il *et al.* Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* **139**, 485 (2009).
11. Garrett, W.S. *et al.* Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell* **131**, 33 (2007).
12. Powell, N. *et al.* The transcription factor T-bet regulates intestinal inflammation mediated by interleukin-7 receptor + innate lymphoid cells. *Immunity* **37**, 674 (2012).
13. Elinav, E. *et al.* NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* **145**, 745 (2011).
14. Henao-Mejia, J. *et al.* Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* **482**, 179 (2012).
15. Vijay-Kumar, M. *et al.* Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science* **328**, 228 (2010).
16. Ubeda, C. *et al.* Familial transmission rather than defective innate immunity shapes the distinct intestinal microbiota of TLR-deficient mice. *J Exp. Med.* **209**, 1445 (2012).
17. Letran, S.E. *et al.* TLR5-deficient mice lack basal inflammatory and metabolic defects but exhibit impaired CD4 T cell responses to a flagellated pathogen. *J. Immunol.* **186**, 5406 (2011).
18. Freter, R. The fatal enteric cholera infection in the guinea pig, achieved by inhibition of normal enteric flora. *J. Infect. Dis.* **97**, 57 (1955).
19. Bohnhoff, M. & Miller, C.P. Enhanced susceptibility to Salmonella infection in streptomycin-treated mice. *J. Infect. Dis.* **111**, 117 (1962).
20. Bohnhoff, M., Miller, C.P. & Martin, W.R. Resistance of the Mouse's Intestinal Tract to Experimental Salmonella Infection. Ii. Factors Responsible for Its Loss Following Streptomycin Treatment. *J. Exp. Med.* **120**, 817 (1964).
21. Bohnhoff, M., Miller, C.P. & Martin, W.R. Resistance of the Mouse's Intestinal Tract to Experimental Salmonella Infection. I. Factors Which Interfere with the Initiation of Infection by Oral Inoculation. *J. Exp. Med.* **120**, 805 (1964).
22. Hentges, D.J. & Freter, R. In vivo and in vitro antagonism of intestinal bacteria against Shigella flexneri. I. Correlation between various tests. *J. Infect. Dis.* **110**, 30 (1962).
23. Eckburg, P.B. *et al.* Diversity of the human intestinal microbial flora. *Science* **308**, 1635 (2005).
24. Sekirov, I. & Finlay, B.B. The role of the intestinal microbiota in enteric infection. *J. Physiol.* **587** (Pt 17), 4159 (2009).
25. Endt, K. *et al.* The microbiota mediates pathogen clearance from the gut lumen after non-typhoidal Salmonella diarrhea. *PLoS Pathog.* **6**, e1001097 (2010).
26. Ubeda, C. *et al.* Intestinal microbiota containing Barnesiella species cures vancomycin-resistant Enterococcus faecium colonization. *Infect. Immun.* **81**, 965 (2013).
27. Abt, M.C. *et al.* Commensal bacteria calibrate the activation threshold of innate antiviral immunity. *Immunity* **37**, 158 (2012).
28. Vollaard, E.J. & Clasener, H.A. Colonization resistance. *Antimicrob. Agents Chemother.* **38**, 409 (1994).
29. Kelly, C.P. & LaMont, J.T. Clostridium difficile–more difficult than ever. *N. Engl. J. Med.* **359**, 1932 (2008).
30. Rupnik, M., Wilcox, M.H. & Gerding, D.N. Clostridium difficile infection: new developments in epidemiology and pathogenesis. *Nat. Rev. Microbiol.* **7**, 526 (2009).
31. O'Connor, J.R., Johnson, S. & Gerding, D.N. Clostridium difficile infection caused by the epidemic Bl/NAP1/027 strain. *Gastroenterology* **136**, 1913 (2009).
32. Eiseman, B. *et al.* Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery* **44**, 854 (1958).
33. Gough, E., Shaikh, H. & Manges, A.R. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection. *Clin. Infect. Dis.* **53**, 994 (2011).
34. Kassam, Z. *et al.* Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. *Am. J. Gastroenterol.* **108**, 500 (2013).
35. van Nood, E. *et al.* Duodenal infusion of donor feces for recurrent Clostridium difficile. *N. Engl. J. Med.* **368**, 407 (2013).
36. Hamilton, M.J. *et al.* Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. *Am. J. Gastroenterol.* **107**, 761 (2012).
37. Reeves, A.E. *et al.* Suppression of Clostridium difficile in the gastrointestinal tracts of germfree mice inoculated with a murine isolate from the family Lachnospiraceae. *Infect. Immun.* **80**, 3786 (2012).
38. Tvede, M. & Rask-Madsen, J. Bacteriotherapy for chronic relapsing Clostridium difficile diarrhoea in six patients. *Lancet* **1**, 1156 (1989).
39. McDonald, J.A. *et al.* Evaluation of microbial community reproducibility, stability and composition in a human distal gut chemostat model. *J. Microbiol. Methods* **95**, 167 (2013).
40. Lawley, T.D. *et al.* Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing Clostridium difficile disease in mice. *PLoS Pathog.* **8**, e1002995 (2012).
41. Fukuda, S. *et al.* Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* **469**, 543 (2011).
42. Sorg, J.A. & Sonenshein, A.L. Inhibiting the initiation of Clostridium difficile spore germination using analogs of chenodeoxycholic acid, a bile acid. *J. Bacteriol.* **192**, 4983 (2010).
43. Giel, J.L. *et al.* Metabolism of bile salts in mice influences spore germination in Clostridium difficile. *PLoS One* **5**, e8740 (2010).
44. Cash, H.L. *et al.* Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science* **313**, 1126 (2006).
45. Brandl, K. *et al.* yD88-mediated signals induce the bactericidal lectin RegIII gamma and protect mice against intestinal Listeria monocytogenes infection. *J. Exp. Med.* **204**, 1891 (2007).
46. Brandl, K. *et al.* Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. *Nature* **455**, 804 (2008).

47. Kinnebrew, M.A. *et al.* Bacterial flagellin stimulates Toll-like receptor 5-dependent defense against vancomycin-resistant Enterococcus infection. *J. Infect. Dis.* **201**, 534 (2010).
48. Jarchum, I. *et al.* Toll-like receptor 5 stimulation protects mice from acute Clostridium difficile colitis. *Infect. Immun.* **79**, 1498 (2011).
49. Jarchum, I. *et al.* Critical role for MyD88-mediated neutrophil recruitment during Clostridium difficile colitis. *Infect. Immun.* **80**, 2989 (2012).
50. Lawley, T.D. *et al.* Antibiotic treatment of clostridium difficile carrier mice triggers a supershedder state, spore-mediated transmission, and severe disease in immunocompromised hosts. *Infect. Immun.* **77**, 3661 (2009).
51. Hasegawa, M. *et al.* Nucleotide-binding oligomerization domain 1 mediates recognition of Clostridium difficile and induces neutrophil recruitment and protection against the pathogen. *J. Immunol.* **186**, 4872 (2011).
52. Hasegawa, M. *et al.* Protective role of commensals against Clostridium difficile infection via an IL-1beta-mediated positive-feedback loop. *J. Immunol.* **189**, 3085(2012).
53. Smits, L.P. *et al.* Therapeutic Potential of Fecal Microbiota Transplantation. *Gastroenterology* **145**, 946 (2013).
54. Atarashi, K. *et al.* Induction of colonic regulatory T cells by indigenous Clostridium species. *Science* **331**, 337 (2011).
55. Le Chatelier, E. *et al.* Richness of human gut microbiome correlates with metabolic markers. *Nature* **500**, 541 (2013).
56. Gordon, J.I. *et al.* The human gut microbiota and undernutrition. *Sci. Transl. Med.* **4**, 137ps12 (2012).