CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor*

Toward a True Bacteriotherapy for *Clostridium difficile* Infection

Maja Rupnik, Ph.D.

Clostridium difficile is one of the most commonly reported pathogens in nosocomial infections in the United States and the European Union. It is associated with a disturbance in gut microbiota; symptoms range from mild diarrhea to colitis and pseudomembranous colitis or gut perforation. Although most cases of infection are treated with antibiotics (e.g., metronidazole, vancomycin, and fidaxomicin), a subgroup of patients who have had multiple relapses are treated through the restoration of gut microbiota.[1,2] In this approach, fecal matter from a healthy donor is suspended in solution and introduced into the gut of the patient. This procedure has multiple descriptions, including fecal transplantation, fecal replacement, fecal microbiota transplantation, and the more aesthetically pleasing bacteriotherapy.

Understanding the nature of the changes in microbiota during infection with *C. difficile* has become a major focus of research. Such investigations have included attempts to define changes in species diversity and to understand the mechanisms underlying microbiota-associated protection against *C. difficile* infection. A recent study by Buffie et al.[3] represents an advance on both fronts.

The authors first analyzed gut microbiota in both mice and humans. The healthy gut microbiota has three features: a large number of microorganisms (constituents), a large number of different species, and an increased representation of certain bacterial phyla, such as Firmicutes and Bacteroidetes, and a decreased representation of other phyla, such as Proteobacteria. The disruption of any of these features can result in dysfunctional gut microbiota, which can result in increased susceptibility to the growth of *C. difficile*. When *C. difficile* vegetative cells reach a sufficiently large number during infection, they produce two main toxins (A and B) that cause intestinal and inflammatory symptoms.

Buffie at al. found that different classes of antibiotics affect mouse gut bacteria in different ways. The actual numbers of bacterial cells were similar in antibiotic-treated mice and untreated mice, but the composition of the bacterial populations differed. The investigators also found that a high diversity in bacterial groups was not necessarily associated with protection against *C. difficile* and that a low diversity was not necessarily associated with susceptibility to the growth of *C. difficile*.

To test for associations between the presence of certain bacterial species and resistance to infection, Buffie at al. combined mathematical modeling approaches with data on the composition of gut microbiota in humans and mice with established *C. difficile* colonization, production of *C. difficile* toxins, and symptoms. For some bacterial species, the association with resistance to infection was observed only in humans; for other species, the association was observed only in mice; and for three bacterial species, the association was observed in both humans and mice.

An association between infection and a particular bacterium may result from a predilection of this organism for an environment similar to that favored by *C. difficile*. To tease causation from association, Buffie et al. selected the four bacterial species with the strongest associations with resistance to infection and tested each individually or in combination for their ability to prevent infection in a mouse model. The combination of the four bacterial species protected animals: growth of *C. difficile* was poor, levels of *C. difficile* toxins were low, and all animals survived. The protection provided by one of the four species, *C. scindens*, was only slightly inferior to that provided by the combination.

The authors therefore tried to elucidate the mechanism by which *C. scindens* protects against infection. *C. difficile* is a sporogenic microorganism: it is ingested as an aerotolerant spore and then germinates into a strictly anaerobic vegeta-

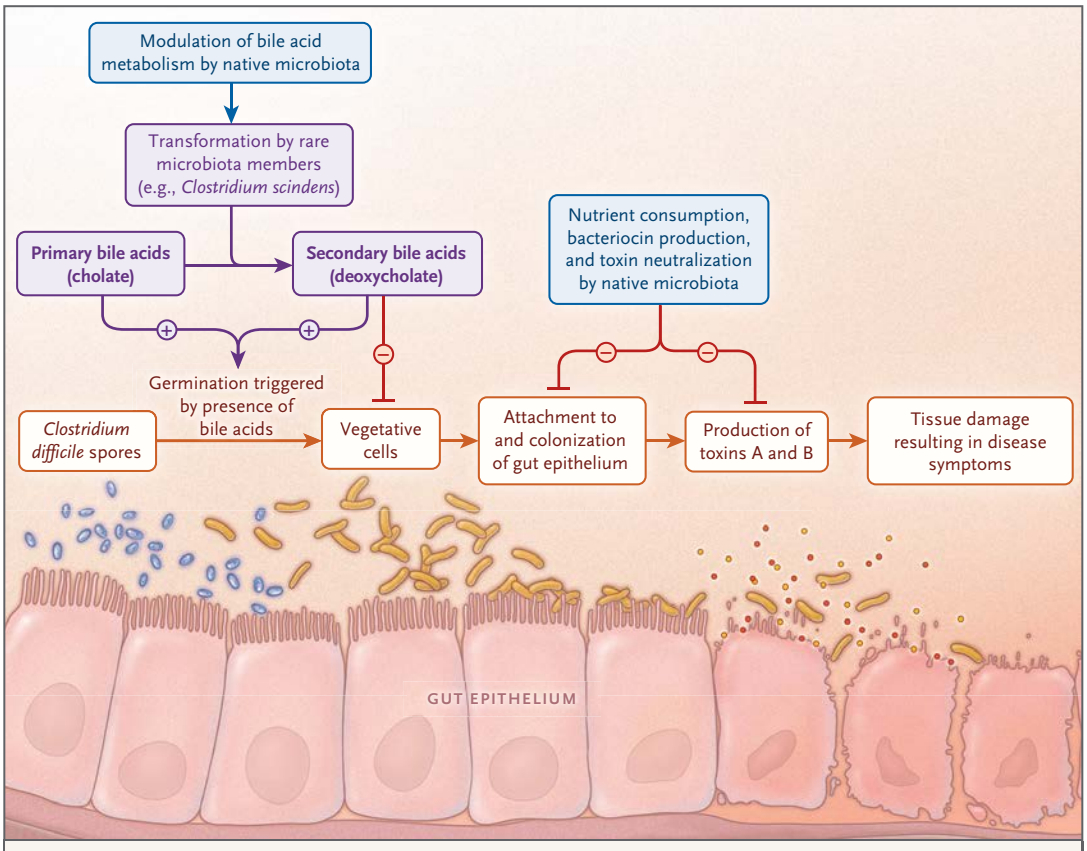

Figure 1. *Clostridium difficile* Colonization and Gut Microbiota.

Spore germination is the first step in the establishment of *C. difficile* infection in the mammalian gut and is dependent on the combination of certain bile acids (e.g., cholate and deoxycholate). Primary bile acids that are produced by human cells are transformed in the gut into secondary bile acids by a few bacterial species, including *C. scindens*. In vitro experiments have shown that some primary and secondary bile acids usually have positive effects on spore germination into a vegetative cell, but some secondary bile acids (e.g., deoxycholate) inhibit the growth of vegetative cells. The presence of gut bacteria that are responsible for the synthesis of these inhibitory bile acids (e.g., *C. scindens*) can therefore reduce the growth of *C. difficile*. The gut microbiota could also inhibit *C. difficile* by other putative mechanisms, such as competition for nutrients, production of bacteriocins, or neutralization of toxins.

tive cell (Fig. 1). Subsequently, it must colonize the gut and then produce toxins in order for symptoms to develop. Bile acids of the gut serve as a signal to the spore that it has reached the (anaerobic) intestinal tract, at which point the spore germinates. Different bile acids have different effects on spore germination, with some inhibiting and others stimulating the process.[4] In addition, secondary bile acids also inhibit the growth of vegetative cells. Only a small proportion of gut bacteria, including *C. scindens*, can convert stimulatory combinations of bile acids into inhibitory combinations of bile acids; this seems to be the mechanism through which *C. scindens* mediates resistance to *C. difficile* infection in mice.

Have we reached the acme of understanding of microbiota-mediated protection against *C. difficile* infection? Far from it. First, the relevance of the findings in mice must be tested for relevance in humans. Second, Buffie et al. observed that a combination of four different bacterial species provided better protection than did *C. scindens* alone. Combining different species of bacteria with different modes of action against *C. difficile* is probably a more effective strategy than one that focuses on a single species.[1] The work of Buffie et al. and others points to a future in

which a multispecies probiotic approach will be used to treat *C. difficile* infection, at which point fecal bacteriotherapy will have been replaced by a true bacteriotherapy.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

From the National Laboratory for Health, Environment and Food and the Faculty of Medicine, University of Maribor, Maribor, and the Center of Excellence for Integrated Approaches in Chemistry and Biology of Proteins, Ljubljana — all in Slovenia.

1. Adamu BO, Lawley TD. Bacteriotherapy for the treatment of intestinal dysbiosis caused by Clostridium difficile infection. Curr Opin Microbiol 2013;16:596-601.
2. Brandt LJ, Aroniadis OC. An overview of fecal microbiota transplantation: techniques, indications, and outcomes. Gastrointest Endosc 2013;78:240-9.
3. Buffie CG, Bucci V, Stein RR, et al. Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. Nature 2015;517:205-8.
4. Britton RA, Young VB. Role of the intestinal microbiota in resistance to colonization by Clostridium difficile. Gastroenterology 2014;146:1547-53.

DOI: 10.1056/NEJMcibr1500270