

CLINICAL UPDATE

Advances in *Clostridium difficile* Infection

Clostridium difficile Infection and the Role of Adaptive Immunity in the Microbiome



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G&H What is the pathogenesis of *Clostridium difficile* infection?

MF *Clostridium difficile* infection usually occurs in susceptible patients after antibiotic therapy kills a good portion of the native microbiota. In these vulnerable individuals, when *C difficile* spores are ingested, they germinate upon exposure to primary bile acids in the small bowel and travel down to the colon. There, the vegetative forms proliferate and produce enterotoxins that cause severe inflammation, resulting in debilitating diarrheal disease.

G&H What is the current understanding of the relationship between *C difficile* infection and the microbiome?

MF The microbiota's protective effect is the reason that most people who are regularly exposed to *C difficile*, such as health care workers, do not become infected very often. There are several potential mechanisms of action by which the native microbiota protect against *C difficile* infection. One mechanism involves the niche effect. In homeostasis, when the native microbiota are intact, there is intense competition for food and space, which makes it difficult for *C difficile* to develop. Another mechanism involves the production of antimicrobial peptides by the microbiota, the so-called bacteriocin. These peptides have a bactericidal property that also keeps *C difficile* in check.

Via another mechanism, the innate microbiota alter primary bile acids into secondary bile acids, creating a bile acid milieu in the colon that is mostly inhibitory to *C difficile* infection. This inhibits germination of the spores, as well as growth of the vegetative form. If, on the other hand, antibiotic treatment destroys the innate microbiota, then the primary bile acids will dominate the milieu in the colon, which conversely will stimulate germination of the spores and growth of the vegetative form.

In addition, the indigenous microbiota are known to play an important role in the maintenance of the gut barrier. This barrier consists of an organized mucous layer (which is quite thick in the colon), colonocytes, and tight junctions in between the colonocytes. The microbiome has a so-called tonic or stimulatory effect on the mucosal immune system, which then plays an important role in maintaining the gut barrier. Two cytokines, transforming growth factor β and interleukin-22, help fortify the gut barrier defenses.

If all of these mechanisms are in place at homeostasis, the indigenous gut microbiota are compartmentalized within the intestinal lumen and separated from the host's mucosal immune cells. Thus, the innate and adaptive immune systems do not readily have access to the gut microbiota and will likely not attack luminal microorganisms or start an inflammatory process in the mucosa.

G&H What is the next step in the mechanism of action in the development of *C difficile* infection and colitis?

MF The next step is that the gut barrier breaks down, mainly due to toxin A and B, which are produced by *C difficile* infection. These toxins lead to weakening of the tight junctions and apoptosis of the colonocytes. The mucin layer is already injured and broken down in part because of prior antibiotic treatment. The indigenous flora can then translocate into the mucosa and be exposed to the immune cells. This will recruit neutrophil granulocytes, which are an important part of the innate immune reaction, as they are responsible for starting a fairly significant primary inflammatory response. Interestingly, in order to patch up the holes in the gut barrier, neutrophil granulocytes create what are called neutrophil extracellular traps, which are the building blocks of the pseudomembranes that are a typical finding or hallmark of *C difficile* colitis.

Thus, the *C difficile* toxins or enterotoxins are not necessarily the cause of all the harm or injury to the colon; the majority of the damage is actually occurring due to the innate immune system's strong immune response to *C difficile* toxins A and B and the translocated microbiota. It is important to emphasize that as much as *C difficile* is responsible for the infection, the severity and clinical course of *C difficile* infection is just as much influenced, or even more, by the reaction of the immune system.

G&H Can the adaptive immune response help explain why some people exposed to *C difficile* are asymptomatic and others are symptomatic carriers?

MF The adaptive immune response to *C difficile* includes T cells and B cells, leading to the production of antitoxins A and B, which can be secreted into the gut through immunoglobulin (Ig) A or in the blood (via IgM or IgG). The antitoxin A and B Igs play an important role in diminishing symptoms or preventing people from developing severe disease or recurrent episodes of *C difficile* infection.

Essentially, the adaptive immune response is the production of antitoxin A and B Ig. If a patient has a good adaptive immune response (ie, produces high levels of antitoxin A and B Igs), then he or she might become colonized but is less likely to develop symptoms from *C difficile* infection. Some people are predisposed to developing recurrent *C difficile* infection. It is clear that the role of the antitoxins is to prevent development of disease, but they do not prevent colonization.

Specifically, Dr Ciaran Kelly's work showed that people who became colonized with *C difficile* in the hospital and had higher antitoxin A and B Ig levels were less likely to develop *C difficile* infection or symptoms compared to people who had low antitoxin A or B levels. He also showed that antitoxin A and B production decreased the risk of developing recurrent infection, although it did not prevent colonization.

G&H What prevents *C difficile* colonization?

MF It is the microbiota that keep *C difficile* away or in check via the mechanisms previously discussed and provides the so-called colonization resistance. Thus, if the microbiota are healthy, we think that *C difficile* does not have a chance to take hold and is less likely to colonize. Does the innate immune system or the adaptive immune system have a role in preventing colonization? The answer is not known. The future generation of vaccines against *C difficile* should target not only induction of antitoxin A and B production to decrease the risk of developing *C difficile* infection, but also prevention of colonization of *C difficile* in the first place.

G&H Could you discuss the traditional therapeutic approach to *C difficile* infection and the associated benefits and limitations?

MF Currently, *C difficile* infection is treated with the antibiotics metronidazole, vancomycin, and fidaxomicin (Difcid, Merck). First-line therapy for nonsevere *C difficile* in the United States is metronidazole. For severe disease, society guidelines currently recommend oral vancomycin. Fidaxomicin is also approved for the treatment of *C difficile* infection and would be the most advantageous antibiotic because the recurrence rate after its use is lower than with metronidazole or vancomycin. For example, the recurrence rate following a single vancomycin therapy for a first episode of *C difficile* infection is approximately 25% in comparison to approximately 15% with fidaxomicin.

Overall, the majority of patients respond to these agents; approximately 80% of patients are cured and do not have a recurrent episode of *C difficile* following a single treatment course with these antibiotics. The cost varies: metronidazole is particularly cheap, as it costs \$30 for a 10-day course, whereas oral vancomycin capsules cost approximately \$1300 and fidaxomicin is the most expensive, at approximately \$2600 for a 10-day therapy.

The limitations with these antibiotics are the primary nonresponse of *C difficile* (metronidazole), significant recurrence rate (highest following metronidazole and lowest after fidaxomicin), and promotion of antibiotic resistance. In addition, patients with severe or fulminant *C difficile* infection often do not respond to antibiotics. However, the most significant disadvantage of these antibiotics is that, while they kill *C difficile* infection, they also kill a good portion of the indigenous microbiota that keep *C difficile* in check. Essentially, an antibiotic-induced disease (ie, *C difficile* infection) is being treated with another antibiotic.

G&H How, specifically, do these antibiotics affect the gut microbiota?

MF Several studies have shown that the biodiversity and function of the indigenous microbiota are significantly diminished by metronidazole and vancomycin. This also applies to fidaxomicin, but to a lesser degree due to its narrower spectrum. With each episode of *C difficile* that a patient has and each antibiotic course he or she receives for the infection, the worse the dysbiosis becomes, and the vicious cycle keeps continuing.

G&H Are there any new therapeutic approaches currently being used or developed that target the adaptive immune response to *C difficile*?

MF New approaches involve active immunization (eg, a vaccine), which would induce an individual to produce

antitoxins when exposed to *C difficile* infection, and passive immunization, which would be given in a form of neutralizing antitoxin Igs. Bezlotoxumab (Zinplava, Merck) is an agent recently approved by the US Food and Drug Administration that transfers passive immunity (via antitoxin B) to patients who are already infected with *C difficile*. Over the course of several trials, researchers found that antitoxin B is protective against *C difficile* and decreases the risk of recurrence from approximately 26% to approximately 16%, whereas antitoxin A (actoxumab) is not beneficial. Thus, bezlotoxumab can be given to patients who have *C difficile* infection, in particular to those at high risk for recurrence, with the aim of decreasing the risk of recurrent disease.

As for agents using active immunization, vaccines are currently in development by Sanofi, Pfizer, and Valneva. The development of a *C difficile* vaccine has proven to be complex and has taken many years due to roadblocks and failed studies. The most recent vaccine news is that Valneva announced positive results this past January from its phase 2 study. A phase 3 study is currently being planned, but there will likely not be a vaccine available this or next year.

G&H What is the rationale for developing a vaccine for *C difficile* infection rather than using one of the other therapeutic approaches?

MF The other therapeutic approaches treat active infection or decrease the risk of recurrence but do not confer immunity against *C difficile*. Thus, the patient will be at risk for developing *C difficile* infection every time he or she is exposed to a systemic antibiotic. With a vaccine, the immune system is trained to react to *C difficile* infection every time it is exposed. Thus, vaccines may be able to produce the most long-lasting protective effect.

G&H Which patients could potentially receive a vaccine for *C difficile*?

MF There are approximately 500,000 cases of *C difficile* infection in the United States yearly. However, a recent meta-analysis has shown that even among hospitalized high-risk patients on antibiotics, only approximately 3% will develop *C difficile* infection. To maximize the efficiency of vaccination and to make economic sense, high-risk patients, such as patients undergoing elective surgery, long-term care facility residents, and discharged patients who acquired in-hospital *C difficile* infection, should be considered for vaccination in the first place.

G&H What is the rationale behind using monoclonal antibodies for treating *C difficile*?

MF Passive immunization with monoclonal antibodies that neutralize *C difficile* toxins has a prompt effect (as

opposed to vaccines). In addition, this therapeutic approach could potentially ameliorate disease severity and *C difficile*-related complications when given in combination with other therapies such as antibiotics or fecal microbiota transplantation, which would be particularly helpful in severe and complicated infections.

G&H Where will bezlotoxumab be positioned in the treatment armamentarium for *C difficile*?

MF I see bezlotoxumab playing an important role in the treatment of hospitalized patients who have severe infection or who are otherwise at high risk for *C difficile* recurrence, such as patients older than 65 years, immunocompromised patients, patients infected with the BI/NAP1/027 strain of *C difficile*, and patients with previous recurrences. In these patient groups, a recurrent episode would likely require hospital readmission, possibly including colectomy, and would carry a high risk of mortality. Thus, minimizing the risk of *C difficile* recurrence in these high-risk patients is very important. By identifying these patients and giving them passive immunity via bezlotoxumab, we could decrease their risk of having another episode of *C difficile* infection.

However, fecal microbiota transplantation also works in these patients and may be more cost-effective. Passive immunization is temporary; the transferred antitoxins will prevent a recurrence in the short term, but may not necessarily prevent recurrence in the long run. Fecal microbiota transplantation may provide longer-lasting protection because of the re-establishment of the premorbid microbiota. However, this protection will not be permanent either; once the individual is re-exposed to another antibiotic, he or she will be at risk of developing *C difficile* infection once again. In a recent study, we found that patients who underwent successful fecal microbiota transplantation with no recurrence at 8 weeks only had a 10% chance, an average, to have recurrent episodes of *C difficile* for the following year. Long-term data are needed for bezlotoxumab.

Dr Fischer has no relevant conflicts of interest to disclose.

Suggested Reading

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