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Immune response against *Clostridioides difficile* and translation to therapy

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***Abstract*:** The pathogenesis of *Clostridioides difficile* infection (CDI) has largely been attributed to the action of two major toxins – A and B. An enhanced systemic humoral immune response against these toxins has been shown to be protective against recurrent CDI. Over the years, fully human monoclonal antibodies against both of these toxins have been developed in an attempt to counter the increasing incidence of recurrent CDI. Clinical trials conducted to evaluate the efficacy of anti-toxin A monoclonal antibody, actoxumab, and anti-toxin B monoclonal antibody, bezlotoxumab, demonstrated that bezlotoxumab substantially lowered the rate of recurrent infection, while actoxumab did not. A significant therapeutic benefit was appreciated in patients with at least one high-risk factor for recurrence, including, age ⩾65 years, immunocompromised state, prior CDI and severe CDI. In light of toxins A and B being immunogenic, vaccine trials are underway with the aim to prevent primary infection.

Keywords: bezlotoxumab; C difficile; Clostridium difficile; diarrhea; immunity; pathogenesis

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Introduction

Clostridioides difficile was established as the causative agent of pseudomembranous colitis in 1978 and has since emerged as one of the most commonly encountered nosocomial infections in the United States.[1,2] A population- and laboratory-based surveillance study estimated that the national burden of *C. difficile* infection (CDI) in the United States was 462,100 cases in 2017.[3] Annual treatment costs related to CDI are estimated at US$4.8 billion in United States acute healthcare settings, with additional burden in the outpatient settings and long-term care facilities.[4] A recent systemic review and meta-analysis examined reports of CDI incidence rates to develop an estimate in the current global evidence of the infection. They estimated that the overall incidence rate of healthcare facility-associated CDI was 2.24 per 1000 admissions per year and 3.54 per 10,000 patient-days.[5] Another global systemic analysis, which included 195 countries, established that *C. difficile* was responsible for the most deaths among children younger than 5 years and among all age groups in countries with a higher socio-demographic index.[6]

The host response to the *C. difficile* bacterium ranges from asymptomatic carriage, mild diarrhea to life-threatening colitis and, in some cases, even death.[7] The recurrence of disease after an initial infection continues to pose one of the greatest challenges in its management. Recurrent CDI is seen in 15–35% of patients after a first infection and in 33–65% of patients who have had two or more infections.[8] The wide spectrum of outcomes is influenced by bacterial virulence factors including toxins that are encoded in the pathogenicity locus, and adherence and motility factors, as well as host comorbid conditions and immune responses.[9] The presence of a healthy gut microbiome also has a bearing on the development of CDI as it provides a resistance against *C. difficile* colonization.[10] The rates of asymptomatic colonization in healthy adults have been found to be up to 17.5%.[11–13] Asymptomatic colonizers may serve as potential disease carriers and have the risk of transmitting CDI to others or may progress to infection themselves if they carry the toxigenic strains.[14,15] Although disruption of the protective gut microbiome, mostly by the use of antibiotics, can predispose to CDI,[16] the host

immune system also determines the development of symptomatic disease and it is believed that repeated reinfection from the environment results in a protective antibody response in healthy adults.[17] The realization of the critical role that human immune response plays in the pathogenesis of the disease has also led to the development of drug therapies that target the immune system.

Pathogenesis of *C. difficile* infection

Transmission of the bacteria occurs by the fecal–oral route in the form of spores. Favorable conditions, such as gut microbiota perturbations, facilitate *C. difficile* to manifest as an infection. *C. difficile* spores survive the acidic environment of the stomach and reach the intestine, where they germinate and convert to vegetative, toxin-producing forms. Although there are a number of virulence factors possessed by *C. difficile*, including motility and adherence, the symptoms of infection correlate with the production of two large exotoxins, namely toxin A and toxin B.[18] These two major virulence factors are the toxin A and toxin B proteins, which are 308 and 270 kDa in size respectively, and they have 48% identical amino acid sequences. Once internalized into the human colonic epithelial cytosol, these toxins catalyze glycosylation and inactivation of Rho-GTPases, which are regulatory proteins of the actin cell cytoskeleton. This leads to the disruption of the cytoskeleton and dissociation of tight junctions between the colonic epithelial cells, causing a loss of epithelial integrity.[19]

On breaching the host intestinal epithelium, toxins A and B access the lamina propria and act on macrophages and peripheral blood mononuclear cells to trigger an immune response.[20] These cells further cause the release of proinflammatory cytokines including interleukin (IL)-1α, IL-1β, IL-6, IL-8 and tumor necrosis factor-α.[21,22] The proinflammatory cytokines enable the infiltration of neutrophil and monocytes from peripheral blood. This is followed by localized mast cell degranulation promoting histamine release, which increases vascular permeability, resulting in fluid loss and watery diarrhea. An initial exposure to *C. difficile* also results in the release of cytokines such as IL-10 and IL-4. These interleukins promote the maturation of naïve B cells into mature Ig-producing plasma cells and memory B cells.[22]

Host immune response to infection

The mammalian immune system manifests both innate and adaptive immune responses. The innate immune system constitutes non-specific defenses such as phagocytosis, oxidative killing and cytokine mediated responses, and the adaptive immune system is generally more specific with T- and B-lymphocyte mediated responses which entail antigen processing and presentation. The innate immune response to CDI has been exhaustively studied and characterized by using mouse models.[23–25] These studies have found that mice lacking components of the innate immune system, such as interleukin-1β or the nucleotide-binding oligomerization domain-containing protein 1 receptor, exhibit a high mortality after CDI.[23] A selective loss of innate lymphoid cells-3 or IL-22 reduces the resistance to acute CDI and the loss of interferon-γ markedly increases morbidity and mortality.[24] In addition, the adaptive immune response, especially antibody responses *via* IgA, IgM and IgG, have been extensively investigated. Efforts to understand the implications of these antibody responses in CDI and extend this knowledge into therapy have been underway.

The role of antibodies

Conceivably, immunoglobulins IgA, IgG and IgM may protect against CDI. Since IgM antibodies play a role in the initial immune response and have a lower antigen affinity, research evaluating IgM responses has been limited. A retrospective study that measured serial IgM responses to CDI concluded that IgM levels against both toxin A and toxin B were lower in patients with recurrent CDI compared with patients with an initial infection.[26]

Immunoglobulin A response against C. difficile

IgA is an antibody that is widely secreted across mucosal surfaces. Serum IgA exists as a monomer and lacks a secretory component, which enables it to bind to myeloid cells,[27] whereas secretory IgA exists as a dimer that is produced by plasma cells in the lamina propria and is taken up into the cell *via* endocytosis, where it passes through the cell before being secreted onto the mucosal surface.[28] Production of secretory IgA against specific mucosal antigens depends on sampling by M cells, dendritic cells, T-cell activation and mesenteric

lymph nodes.[29] Given the crucial role it plays in maintaining mucosal immunity, it would be expected to provide substantial protection against CDI. Two studies showed that low total fecal IgA levels and low total colonic IgA producing cells are associated not only with prolonged CDI symptoms, but also with higher rates of recurrence.[30,31]

Immunoglobulin G response against C. difficile

Higher IgG levels in response to colonization are thought to be protective against the development of CDI.[32,33] It has been demonstrated that serum IgG levels in response to toxin A, toxin B and non-toxin antigens are elevated on day 12 after the onset of diarrhea in patients who have a single episode of CDI as compared with recurrent CDI.[26] Further, another study found that subclasses of antitoxin A IgG antibodies, IgG2 and IgG3, were deficient in patients with recurrent CDI.[34]

Immunotherapy: a promising therapeutic venture

Through animal studies, it was demonstrated that passive immunization against both the toxins A and B confers better protection than against either toxin alone.[35,36] Initial studies focused on conferring protection against toxin A, as it was believed to be more pathogenic and animal models suggested that toxin B did not establish infection.[37] Toxin A was demonstrated to be lethal when administered intragastrically in hamster and mouse models.[38] Toxin B did not cause disease symptoms unless it was co-administered with toxin A or if intestinal damage was present.[38]

Another study constructed and characterized four independently derived toxin A and toxin B mutants of *C. difficile in vitro* and *in vivo*, in hamster models. They that highlighted toxin B is an essential virulence factor as disrupting the toxin B gene, *tcdB*, resulted in an attenuated virulence phenotype. Also, the presence of toxin A, even at higher levels, was not lethal. Isolates that lack toxin A can still cause disease, and toxin B does not require the presence of toxin A for its activity.[39]

Recent research using human colon also illustrated that toxin B is rather more toxigenic than toxin A.[40] Additionally, toxin A-negative, toxin B-positive strains of *C. difficile* have also recently emerged, suggesting that perhaps both toxins play an important role in the pathogenesis of CDI, rather than toxin A alone.[41]

Translation to therapy

Actoxumab and bezlotoxumab

Building on the role that toxins A and B potentially play in the causation of CDI, efforts to directly neutralize these toxins by passive immunization have been undertaken. Actoxumab and bezlotoxumab are fully humanized monoclonal IgG antibodies that bind to and neutralize toxin A and toxin B respectively. These monoclonal antibodies were developed in conjunction on the basis that neutralization of both toxins of *C. difficile* would provide the highest benefit. A number of animal and human studies have since been carried out to evaluate the efficacy of bezlotoxumab (Table 1). A murine model demonstrated that mice who were administered bezlotoxumab and actoxumab 1 h before the administration of toxins A and B were protected from toxin-induced death in a dose dependent manner.[42] Further, ileal samples taken from mice injected with the antibodies prior to toxin exhibited a reduction in fluid accumulation and histological evaluation of the ileal walls indicated protection from toxin-mediated damage and inflammation, as compared with mice pre-treated with placebo. Morbidity and mortality were lower in hamsters treated with bezlotoxumab plus actoxumab than in vehicle and vancomycin treated animals.[43] Similar results were also exhibited in a murine model.[43]

Although the mechanism of action of bezlotoxumab is not completely understood, it is speculated that the antibody is transported from the basolateral to the luminal compartment *via* paracellular transport after toxin mediated epithelial disruption.[47] As this epithelial damage induced by the toxin increases, it allows more antibody to enter the lumen to enable neutralization, epithelial recovery and consequently a decrease in antibody transportation.[47]

A randomized, double blinded, placebo-controlled phase II study was undertaken where patients on standard-of-care therapy received either monoclonal antibody to toxin A (CDA1) or placebo.[44] Twenty-nine patients were administered infusions of CDA1 while 17 patients received placebo. These patients were followed up for 56 days and serum antibodies against toxin

Table 1. Summary of animal studies and human clinical trials for actoxumab and bezlotoxumab.

Animal studies and clinical trials of actoxumab and bezlotoxumab				
Study no.	**Authors**	**Subjects**	**Design**	**Results**
1.	Yang *et al.*[42]	Mice	(a) Mice were treated i.p. with actoxumab and bezlotoxumab 1 h before being challenged with a mixture of toxin A and toxin B, saline was used as placebo.	(a) All saline treated mice challenged with the toxin mixture died, but mice pretreated with actoxumab and bezlotoxumab were protected from CDI in a dose-dependent manner.
			(b) Mice were injected with either actoxumab and bezlotoxumab mixture or saline. Ileal loops were inoculated with toxin A and toxin B or saline 24 h later. After 4 h the ileal loops were removed and also collected for histological examination.	(b) Fluid accumulation in the ileal loops in mice challenged with toxins was reduced in mice treated with actoxumab and bezlotxoumab. Mice treated with vehicle or actoxumab and bezlotoxumab had normal villous architecture, but untreated mice showed neutrophil infiltration, epithelial damage and erosion and loss of villi. Actoxumab and bezlotoxumab protect against inflammation and toxin mediated damage.
2.	Warn *et al.*[43]	Hamsters and mice	(a) Hamster model – pretreated with clindamycin followed by a challenge of *Clostridioides difficile* spores. Hamsters were treated with (i) s.c. administration of actoxumab and bezlotoxumab, (ii) orogastric vancomycin (iii) placebo.	(a) (i) Actoxumab and bezlotoxumab treated hamsters showed fewer signs of morbidity, were protected from intestinal damage on histology and spore counts in the feces and intestine initially rose until day 2, but then gradually declined below level of detection by the end of the study. (ii) Vancomycin treated animals had no morbidity signs until PID 10, with all animals succumbing by PID 14. Histology showed inflamed and enlarged intestines and spore counts remained low through the treatment period but rose by PID 10–14. (iii) Placebo treated animals had signs of morbidity and all died within 2 days, had severe intestinal pathology and had a rise in intestinal spore levels after PID 1.
			(b) Murine model – given cefoperazone followed by clindamycin and then challenged with *C. difficile* spores. Treated with (i) s.c. actoxumab/bezlotoxumab (ii) orogastric vancomycin (iii) vehicle.	(b) Similar results were observed in the mouse model with actoxumab/bezlotoxumab, vancomycin and placebo treatment.
3.	Leav *et al.*[44]	Humans	Phase II, randomized, double-blind, placebo-controlled trial in patients receiving standard of care for *C. difficile.* 29 patients received actoxumab and 17 received placebo and were evaluated for recurrence.	17% had recurrence in actoxumab group and 18% in the placebo group, with a delay in recurrence time in CDA1 group. All patients that recurred had lower anti-toxin B antibody concentrations as compared with those that did not. No significant difference in anti-toxin A antibody levels was seen in those that had recurrence and those that did not. Also, low concentrations of anti-toxin B and anti-toxin A antibody and infection with BI/NAP1/027 strain were associated with recurrence.
4.	Lowy *et al.*[45]	Humans	Randomized, double-blind, placebo-controlled study of actoxumab and bezlotoxumab in patients with symptomatic *C. difficile* infection who were receiving either metronidazole or vancomycin.	Rate of recurrence was lower among patients treated with monoclonal antibodies (7% *versus* 25%; 95% CI, 7–29; $p < 0.001$). Recurrence rates for BI/NAP1/027 strain were 8% for the antibody group and 32% for the placebo group ($p = 0.06$); in patients with ⩾1 previous episode of CDI, recurrence rates were 7% and 38%, respectively ($p = 0.006$). Mean duration of the initial hospitalization did not differ significantly between the antibody and placebo groups (9.5 and 9.4 days, respectively).
5.	Wilcox *et al.*[46]	Humans	Two double-blind, randomized, placebo-controlled, phase III trials, MODIFY I and MODIFY II, in patients receiving oral standard of care for primary or recurrent CDI. Participants received an infusion of bezlotoxumab, actoxumab plus bezlotoxumab or placebo.	Rate of recurrent CDI was significantly lower with bezlotoxumab alone than with placebo (MODIFY I: 17% *versus* 28%; 95% CI, −15.9 to −4.3; $p < 0.001$; MODIFY II: 16% *versus* 26%; 95% CI, −15.5 to −4.3; $p < 0.001$) and was significantly lower with actoxumab plus bezlotoxumab than with placebo (MODIFY I: 16% *versus* 28%; 95% CI, −17.4 to −5.9; $p < 0.001$; MODIFY II: 15% *versus* 26%; 95% CI, −16.4 to −5.1; $p < 0.001$). Rates of initial clinical cure: 80% with bezlotoxumab alone, 73% with actoxumab plus bezlotoxumab and 80% with placebo; the rates of sustained cure (initial clinical cure without recurrent infection in 12 weeks) were 64%, 58%, and 54%, respectively.

CDI, *Clostridioides difficile* infection; CI, confidence interval; i.p., intraperitoneal; PID, post-infection day; s.c., subcutaneous.

A and B were measured before and after the infusion. It was subsequently observed that the rates of recurrence were almost identical by the end of 8 weeks.[44] In the same trial, patients who did not develop recurrent CDI had higher levels of antitoxin B antibodies as compared with those who did. In another trial, patients were randomly assigned to receive standard-of-care therapy plus either actoxumab and bezlotoxumab or placebo.[45] By 12 weeks, the rate of recurrence was 7% in the treated group, as compared with 25% in the control group.

Two large, double-blind, randomized, placebo-controlled, phase III trials, a study of MK-3415 (human monoclonal antibody to *C. difficile* toxin A), MK-6072 (human monoclonal antibody to *C. difficile* toxin B) and MK-3415A (combination of human monoclonal antibodies to *C. difficile* toxin A and toxin B) in participants receiving antibiotic therapy for *Clostridium difficile* infection (MK-3415A-001) (MODIFY I) and a study of MK-6072 and MK-3415A in participants receiving antibiotic therapy for *C. difficile* infection (MK-3415A-002)(MODIFY II) were conducted, with adults receiving oral standard-of-care antibiotics for the treatment of primary or recurrent CDI.[46] Overall, 2655 adults were enrolled who were receiving oral standard-of-care antibiotics for primary or recurrent CDI. Out of these, 2580 were treated and 2559 were included in the modified intention-to-treat population. These patients were randomly assigned in a 1:1:1:1 ratio to receive a single dose of bezlotoxumab, actoxumab plus bezlotoxumab, placebo or actoxumab alone (in MODIFY I only). Of these, 773 patients received actoxumab plus bezlotoxumab infusion, 781 received bezlotoxumab, 232 received actoxumab alone and 773 received placebo and were followed up for a total of 12 weeks.

In both trials, the proportion of participants who had recurrent infection was lower in the bezlotoxumab group than in the placebo group (MODIFY I: 17% *versus* 28%; $p < 0.001$; MODIFY II: 16% *versus* 26%; $p < 0.001$) and was also lower in the actoxumab–bezlotoxumab group than in the placebo group (MODIFY I: 16% *versus* 28%; MODIFY II: 15% *versus* 26%; both $p < 0.001$).[46] There was no significant difference in the rate of recurrent infection between the actoxumab group and the placebo group in MODIFY I (26% and 28%, respectively; $p = 0.64$). In the pooled data set, the rate of sustained cure (initial clinical cure without recurrent infection in 12 weeks) was 64% with bezlotoxumab, 58% with actoxumab–bezlotoxumab and 54% with placebo.[46]

In summary, bezlotoxumab had a rate of recurrence that was 38% lower than that with standard-of-care therapy alone and actoxumab was not beneficial when administered alone, or even when used in conjunction with bezlotoxumab. These findings reiterated the fact that toxin B might be the main virulence factor for recurrent CDI. Since then, several studies have been carried out to assess the utility and benefit of bezlotoxumab.

Clinical utility of bezlotoxumab

Bezlotoxumab has been approved by the United States Food and Drug administration as a one-time intravenous therapy in patients receiving standard-of-care antibiotics for CDI to prevent recurrent CDI. Patient characteristics such as age $\geqslant$65 years, history or present use of immunosuppressive therapy, severe CDI and prior episode of CDI have been shown to increase the risk of recurrent CDI or CDI-related adverse events. A *post hoc* analysis of the data from the MODIFY I and MODIFY II trials analyzed the efficacy of bezlotoxumab in patients with these high-risk features.[48] The greatest risk reduction was seen in patients with at least three risk factors and patients with only one risk factor also benefit from the monoclonal antibody.[48] Patients without even one of the prespecified risk factors at the time of diagnosis of CDI are not likely to benefit from bezlotoxumab. Individuals with other risk factors that were not included in the study, such as renal impairment, inflammatory bowel disease or concomitant antibiotic use, may also benefit from bezlotoxumab.

A study estimated 30-day all-cause and CDI-associated hospital readmissions in participants that were enrolled in MODIFY I and MODIFY II.[49] Bezlotoxumab reduced the number of 30-day rehospitalizations associated with CDI by 6% overall and by 8% in subpopulations with a higher risk of recurrent CDI. A retrospective study of 46 patients in a university hospital setting in Finland aimed to assess the real-world efficacy of bezlotoxumab for preventing recurrent CDI.[50] Bezlotoxumab infusion was used as an adjunctive to standard-of-care therapy and was found to be effective in preventing recurrent CDI in 73% patients in the following 3 months, with a 71%

efficacy in immunocompromised patients. Also, 63% patients with severe CDI remained free of recurrence a follow-up period of 3 months. A similar study sought to evaluate the use of bezlotoxumab in the real world setting across 34 infusion centers in the United States.[51] This multicenter study produced results comparable to the MODIFY clinical trials and demonstrated that bezlotoxumab was successful in preventing recurrent CDI in 84.1% patients after a single dose in combination of standard-of-care therapy. The timing of infusion and type of standard of care therapy used did not affect the results in the study.

A study determined the cost-effectiveness of bezlotoxumab in conjunction with standard-of-care, compared with standard-of-care alone to prevent recurrence of CDI in high-risk patients.[52] Their cost-effectiveness model demonstrated that bezlotoxumab added 0.12 quality-adjusted life-years and was cost-effective in the prevention of recurrent CDI in the trial population. It was also cost-effective in subgroups of patients >65 years of age, immunocompromised and those with severe CDI.

To further evaluate the benefit of bezlotoxumab in relation with standard-of-care antibiotics for CDI, an analysis of the MODIFY I/II trial revealed that participants who received bezlotoxumab in addition to metronidazole or vancomycin had similar significant reductions in the rates of recurrent CDI as compared with those who received placebo.[53] A similar reduction rate was also observed in patients who received bezlotoxumab with fidaxomicin in the same analysis. However, to date, data for the use of bezlotoxumab with fidaxomicin are limited.

A *post hoc* analysis was conducted to investigate the impact of timing of bezlotoxumab infusion as compared with placebo, which found that there was a reduction in the incidence of recurrent CDI with bezlotoxumab regardless of the timing of infusion.[54] Bezlotoxumab did not improve the initial clinical cure rates or the time to resolution of diarrhea if administered early during the course of antibiotic treatment. This probably occurs because stool toxin B levels have been found to rise 9–25 days after the end of antimicrobial therapy, representing the start of the recurrent CDI risk period,[55] thus, making it essential to administer bezlotoxumab before the end of antimicrobial therapy for CDI to protect against the effect of the new toxin that is produced during the period of risk of recurrence.[54]

Current treatment guidelines for CDI do not yet include the use of bezlotoxumab for therapy. Since its use provides maximal benefit to patients who have high risk features such as age ⩾65 years, immunosuppression, prior CDI episode and severe CDI, its use could be reserved for this patient population. Individuals with one or more of these risk features could benefit from bezlotoxumab when used as an adjunct to existing standard-of-care therapy. Its administration should be withheld in patient groups without high risk features due to lack of proven clinical advantage.

Vaccine development

Several vaccine efforts have been undertaken that focus on immunogenicity. A few vaccine candidates with variable targets, patient populations, different dosages and formulations have undergone phase I and phase II studies. One vaccine candidate developed by the Centre for Applied Microbiological Research containing formalin inactivated *C. difficile* toxin A and B underwent a phase I study of 30 young healthy adults.[56] The vaccine resulted in detectable IgG antibodies against both toxins in 90% of patients and fecal IgA in half of the patients.[56] Another phase I dose-finding study of a toxoid vaccine with an adjuvant was carried out in two population groups – 18–55 years and ⩾65-year-old volunteers – who received three doses.[57] Seroconversion was observed for toxin A in the younger population by the second dose, but seroconversion rates seemed to be dose dependent in the elder population. This seroconversion was sustained in younger individuals. Seroconversion for toxin B was found to be lower than for toxin A in both groups and the rate increased with increasing dose levels. In a follow-up two staged phase II trial, adults were enrolled to receive a high dose with or without adjuvant or placebo.[58] In stage I, the immune response against both the toxins was highest in the high-dose plus adjuvant group, which was then chosen for stage II. In stage II, the immune response for this high-dose formulation was better than the previous two schedules to day 180. A phase III study evaluated the efficacy of this candidate to prevent primary CDI in individuals who were at risk [ClinicalTrials.gov identifier: NCT01887912]. Participants were randomized to receive either the vaccine (where

high dose with adjuvant was selected, based on the phase II study[58]) or placebo in three doses. The primary goal was to assess the efficacy of the vaccine in preventing the development of CDI. It was found that the percentage of participants with a ⩾2-fold increase in antibody levels against toxin A was 93.3% and for toxin B was 82.2%. The active group subsequently had 34 confirmed cases of CDI and the placebo group had 16, indicating no significant efficacy of the vaccine.[59] Due to futility this trial was terminated after an interim analysis.[59]

Another phase I dose-escalation study was conducted to evaluate the safety and immunogenicity of a another toxoid vaccine, administered alone or with adjuvant.[60] There was an immune response seen with a rise in antitoxin A- and antitoxin B-antibodies in both the groups. This rise was higher in the toxoid only groups as compared with the toxoid plus adjuvant groups. In a follow-up phase II trial, adults were randomized 3:3:1 to receive low-dose or high-dose vaccine or placebo in a 30-day or 6-month regimen.[61] The higher dose elicited a higher immune response and the 6-month regimen produced a stronger and more persistent response. A follow-up phase III trial of this vaccine is currently underway.

What is next?

Despite the development of various therapies to treat and prevent CDI, recurrence of infection continues to be one of the main problems. Bezlotoxumab seems to be a promising therapeutic option that can be used adjunctively in patients with CDI. Its use also seems to be beneficial in patients with high-risk features including age ⩾65 years, immunocompromised state, severe CDI and prior episode of CDI. However, whether formation of an immune response against CDI prevents primary infection still needs exploration.

With the discovery of *C. difficile* toxoids being immunogenic, several vaccine trials are being carried out. While the results of these trials are encouraging, it is still premature to conclude their efficacy. It is pertinent that vaccines confer long-term sustained immune responses and provide benefit to high-risk populations.

The knowledge of immune responses can also be used to formulate clinical prediction tools for recognizing patients who are at risk of developing primary CDI, at risk of recurrence and development of severe CDI and complications. These tools would then be able to categorize patients who would benefit maximally from the use of immunotherapeutic interventions.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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References

1. Bartlett JG, Chang TW, Gurwith M, *et al*. Antibiotic-associated pseudomembranous colitis due to toxin-producing clostridia. *N Engl J Med* 1978; 298: 531–534.
2. McDonald LC, Owings M and Jernigan DB. *Clostridium difficile* infection in patients discharged from US short-stay hospitals, 1996-2003. *Emerg Infect Dis* 2006; 12: 409–415.
3. Guh AY, Mu Y, Winston LG, *et al*. Trends in U.S. Burden of Clostridioides difficile infection and outcomes. *N Engl J Med* 2020; 382: 1320–1330.
4. Dubberke ER and Olsen MA. Burden of *Clostridium difficile* on the healthcare system. *Clin Infect Dis* 2012; 55(Suppl. 2): S88–S92.
5. Balsells E, Shi T, Leese C, *et al*. Global Burden of *Clostridium difficile* infections: a systematic review and meta-analysis. *J Glob Health* 2019; 9: 010407.
6. GBD 2016 Diarrhoeal Disease Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease study 2016. *Lancet Infect Dis* 2018; 18: 1211–1228.
7. Kelly CP and LaMont JT. *Clostridium difficile*--more difficult than ever. *N Engl J Med* 2008; 359: 1932–1940.
8. Gerding DN, Muto CA and Owens RC Jr. Treatment of *Clostridium difficile* infection. *Clin Infect Dis* 2008; 46(Suppl. 1): S32–S42.
9. Carter GP, Chakravorty A, Pham Nguyen TA, *et al*. Defining the roles of TcdA and TcdB in

localized gastrointestinal disease, systemic organ damage, and the host response during *Clostridium difficile* infections. *mBio* 2015; 6: e00551.

10. Britton RA and Young VB. Role of the intestinal microbiota in resistance to colonization by *Clostridium difficile*. *Gastroenterology* 2014; 146: 1547–1553.

11. Nakamura S, Mikawa M, Nakashio S, *et al*. Isolation of *Clostridium difficile* from the feces and the antibody in sera of young and elderly adults. *Microbiol Immunol* 1981; 25: 345–351.

12. Fekety R and Shah AB. Diagnosis and treatment of *Clostridium difficile* colitis. *JAMA* 1993; 269: 71–75.

13. Terveer EM, Crobach MJ, Sanders IM, *et al*. Detection of *Clostridium difficile* in feces of asymptomatic patients admitted to the hospital. *J Clin Microbiol* 2017; 55: 403–411.

14. McFarland LV, Mulligan ME, Kwok RY, *et al*. Nosocomial acquisition of *Clostridium difficile* infection. *N Engl J Med* 1989; 320: 204–210.

15. Curry SR, Muto CA, Schlackman JL, *et al*. Use of multilocus variable number of tandem repeats analysis genotyping to determine the role of asymptomatic carriers in *Clostridium difficile* transmission. *Clin Infect Dis* 2013; 57: 1094–1102.

16. Buffie CG, Jarchum I, Equinda M, *et al*. Profound alterations of intestinal microbiota following a single dose of clindamycin results in sustained susceptibility to *Clostridium difficile*-induced colitis. *Infect Immun* 2012; 80: 62–73.

17. Sánchez-Hurtado K, Corretge M, Mutlu E, *et al*. Systemic antibody response to *Clostridium difficile* in colonized patients with and without symptoms and matched controls. *J Med Microbiol* 2008; 57: 717–724.

18. Awad MM, Johanesen PA, Carter GP, *et al*. *Clostridium difficile* virulence factors: insights into an anaerobic spore-forming pathogen. *Gut Microbes* 2014; 5: 579–593.

19. Hunt JJ and Ballard JD. Variations in virulence and molecular biology among emerging strains of *Clostridium difficile*. *Microbiol Mol Biol Rev* 2013; 77: 567–581.

20. Pothoulakis C. Effects of *Clostridium difficile* toxins on epithelial cell barrier. *Ann N Y Acad Sci* 2000; 915: 347–356.

21. Flegel WA, Muller F, Daubener W, *et al*. Cytokine response by human monocytes to *Clostridium difficile* toxin A and toxin B. *Infect Immun* 1991; 59: 3659–3666.

22. Ryan A, Lynch M, Smith SM, *et al*. A role for TLR4 in *Clostridium difficile* infection and the recognition of surface layer proteins. *PLoS Pathog* 2011; 7: e1002076.

23. Hasegawa M, Yamazaki T, Kamada N, *et al*. Nucleotide-binding oligomerization domain 1 mediates recognition of *Clostridium difficile* and induces neutrophil recruitment and protection against the pathogen. *J Immunol* 2011; 186: 4872–4880.

24. Abt MC, Lewis BB, Caballero S, *et al*. Innate immune defenses mediated by two ILC subsets are critical for protection against acute *Clostridium difficile* infection. *Cell Host Microbe* 2015; 18: 27–37.

25. Hasegawa M, Yada S, Liu MZ, *et al*. Interleukin-22 regulates the complement system to promote resistance against pathobionts after pathogen-induced intestinal damage. *Immunity* 2014; 41: 620–632.

26. Kyne L, Warny M, Qamar A, *et al*. Association between antibody response to toxin A and protection against recurrent *Clostridium difficile* diarrhoea. *Lancet* 2001; 357: 189–193.

27. Steffen U, Koeleman CA, Sokolova MV, *et al*. IgA subclasses have different effector functions associated with distinct glycosylation profiles. *Nat Commun* 2020; 11: 120.

28. Kaetzel CS, Robinson JK, Chintalacharuvu KR, *et al*. The polymeric immunoglobulin receptor (secretory component) mediates transport of immune complexes across epithelial cells: a local defense function for IgA. *Proc Natl Acad Sci U S A* 1991; 88: 8796–8800.

29. Mantis NJ, Rol N and Corthesy B. Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunol* 2011; 4: 603–611.

30. Johal SS, Lambert CP, Hammond J, *et al*. Colonic IgA producing cells and macrophages are reduced in recurrent and non-recurrent *Clostridium difficile* associated diarrhoea. *J Clin Pathol* 2004; 57: 973–979.

31. Warny M, Vaerman JP, Avesani V, *et al*. Human antibody response to *Clostridium difficile* toxin A in relation to clinical course of infection. *Infect Immun* 1994; 62: 384–389.

32. Kyne L, Warny M, Qamar A, *et al*. Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. *N Engl J Med* 2000; 342: 390–397.

33. Pechine S, Gleizes A, Janoir C, *et al*. Immunological properties of surface proteins of

Clostridium difficile. *J Med Microbiol* 2005; 54: 193–196.

34. Katchar K, Taylor CP, Tummala S, *et al*. Association between IgG2 and IgG3 subclass responses to toxin A and recurrent *Clostridium difficile*-associated disease. *Clin Gastroenterol Hepatol* 2007; 5: 707–713.
35. Babcock GJ, Broering TJ, Hernandez HJ, *et al*. Human monoclonal antibodies directed against toxins A and B prevent *Clostridium difficile*-induced mortality in hamsters. *Infect Immun* 2006; 74: 6339–6347.
36. Fernie DS, Thomson RO, Batty I, *et al*. Active and passive immunization to protect against antibiotic associated caecitis in hamsters. *Dev Biol Stand* 1983; 53: 325–332.
37. Voth DE and Ballard JD. *Clostridium difficile* toxins: mechanism of action and role in disease. *Clin Microbiol Rev* 2005; 18: 247–263.
38. Lyerly DM, Saum KE, MacDonald DK, *et al*. Effects of *Clostridium difficile* toxins given intragastrically to animals. *Infect Immun* 1985; 47: 349–352.
39. Lyras D, O'Connor JR, Howarth PM, *et al*. Toxin B is essential for virulence of *Clostridium difficile*. *Nature* 2009; 458: 1176–1179.
40. Savidge TC, Pan WH, Newman P, *et al*. *Clostridium difficile* toxin B is an inflammatory enterotoxin in human intestine. *Gastroenterology* 2003; 125: 413–420.
41. Drudy D, Harnedy N, Fanning S, *et al*. Isolation and characterisation of toxin A-negative, toxin B-positive *Clostridium difficile* in Dublin, Ireland. *Clin Microbiol Infect* 2007; 13: 298–304.
42. Yang Z, Ramsey J, Hamza T, *et al*. Mechanisms of protection against *Clostridium difficile* infection by the monoclonal antitoxin antibodies actoxumab and bezlotoxumab. *Infect Immun* 2015; 83: 822–831.
43. Warn P, Thommes P, Sattar A, *et al*. Disease progression and resolution in rodent models of *Clostridium difficile* infection and impact of antitoxin antibodies and vancomycin. *Antimicrob Agents Chemother* 2016; 60: 6471–6482.
44. Leav BA, Blair B, Leney M, *et al*. Serum anti-toxin B antibody correlates with protection from recurrent *Clostridium difficile* infection (CDI). *Vaccine* 2010; 28: 965–969.
45. Lowy I, Molrine DC, Leav BA, *et al*. Treatment with monoclonal antibodies against *Clostridium difficile* toxins. *N Engl J Med* 2010; 362: 197–205.
46. Wilcox MH, Gerding DN, Poxton IR, *et al*. Bezlotoxumab for prevention of recurrent *Clostridium difficile* infection. *N Engl J Med* 2017; 376: 305–317.
47. Zhang Z, Chen X, Hernandez LD, *et al*. Toxin-mediated paracellular transport of antitoxin antibodies facilitates protection against *Clostridium difficile* infection. *Infect Immun* 2015; 83: 405–416.
48. Gerding DN, Kelly CP, Rahav G, *et al*. Bezlotoxumab for prevention of recurrent *Clostridium difficile* infection in patients at increased risk for recurrence. *Clin Infect Dis* 2018; 67: 649–656.
49. Prabhu VS, Cornely OA, Golan Y, *et al*. Thirty-day readmissions in hospitalized patients who received bezlotoxumab with antibacterial drug treatment for *Clostridium difficile* infection. *Clin Infect Dis* 2017; 65: 1218–1221.
50. Oksi J, Aalto A, Saila P, *et al*. Real-world efficacy of bezlotoxumab for prevention of recurrent *Clostridium difficile* infection: a retrospective study of 46 patients in five university hospitals in Finland. *Eur J Clin Microbiol Infect Dis* 2019; 38: 1947–1952.
51. Hengel RL, Ritter TE, Nathan RV, *et al*. Real-world experience of bezlotoxumab for prevention of Clostridioides difficile infection: a retrospective multicenter cohort study. *Open Forum Infect Dis* 2020; 7: ofaa097.
52. Prabhu VS, Dubberke ER, Dorr MB, *et al*. Cost-effectiveness of bezlotoxumab compared with placebo for the prevention of recurrent *Clostridium difficile* infection. *Clin Infect Dis* 2018; 66: 355–362.
53. Dubberke ER, Gerding DN, Kelly CP, *et al*. Efficacy of bezlotoxumab in participants receiving metronidazole, vancomycin, or fidaxomicin for treatment of clostridioides (Clostridium) difficile infection. *Open Forum Infect Dis* 2020; 7: ofaa157.
54. Birch T, Golan Y, Rizzardini G, *et al*. Efficacy of bezlotoxumab based on timing of administration relative to start of antibacterial therapy for *Clostridium difficile* infection. *J Antimicrob Chemother* 2018; 73: 2524–2528.
55. Thabit AK, Alam MJ, Khaleduzzaman M, *et al*. A pilot study to assess bacterial and toxin reduction in patients with *Clostridium difficile* infection given fidaxomicin or vancomycin. *Ann Clin Microbiol Antimicrob* 2016; 15: 22.
56. Kotloff KL, Wasserman SS, Losonsky GA, *et al*. Safety and immunogenicity of increasing doses of

a *Clostridium difficile* toxoid vaccine administered to healthy adults. *Infect Immun* 2001; 69: 988–995.

57. Greenberg RN, Marbury TC, Foglia G, *et al.* Phase I dose finding studies of an adjuvanted *Clostridium difficile* toxoid vaccine. *Vaccine* 2012; 30: 2245–2249.

58. de Bruyn G, Saleh J, Workman D, *et al.* Defining the optimal formulation and schedule of a candidate toxoid vaccine against *Clostridium difficile* infection: a randomized phase 2 clinical trial. *Vaccine* 2016; 34: 2170–2178.

59. de Bruyn G, Gordon DL, Steiner T, *et al.* Safety, immunogenicity, and efficacy of a Clostridioides difficile toxoid vaccine candidate: a phase 3 multicentre, observer-blind, randomised, controlled trial. *Lancet Infect Dis* 2021; 21: 252–262.

60. Sheldon E, Kitchin N, Peng Y, *et al.* A phase 1, placebo-controlled, randomized study of the safety, tolerability, and immunogenicity of a *Clostridium difficile* vaccine administered with or without aluminum hydroxide in healthy adults. *Vaccine* 2016; 34: 2082–2091.

61. Kitchin N, Remich SA, Peterson J, *et al.* A phase 2 study evaluating the safety, tolerability, and immunogenicity of two 3-dose regimens of a *Clostridium difficile* vaccine in healthy US adults aged 65 to 85 years. *Clin Infect Dis* 2020; 70: 1–10.