Translational Aspects of the Immunology of *Clostridioides difficile* Infection: Implications for Pediatric Populations

Larry K. Kociolek, Joseph P. Zackular, and Tor Savidge

*Department of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA, Division of Infectious Diseases, Ann & Robert H. Lurie Children’s Hospital of Chicago, Chicago, Illinois, USA, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA, Division of Protective Immunity, Department of Pathology and Laboratory Medicine, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania, USA, Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA, and Department of Pathology & Immunology, Baylor College of Medicine & Texas Children’s Hospital, Houston, Texas, USA

*Clostridioides difficile* has become the most common healthcare-associated pathogen in the United States, leading the US Centers for Disease Control and Prevention (CDC) to classify *C. difficile* as an “urgent” public health threat that requires “urgent and aggressive action.” This call to action has led to new discoveries that have advanced our understanding of *Clostridioides difficile* infection (CDI) immunology and clinical development of immunologic-based therapies for CDI prevention. However, CDI immunology research has been limited in pediatric populations, and several unanswered questions remain regarding the function of host immune response in pediatric CDI pathogenesis and the potential role of immunologic-based therapies in children. This review summarizes the innate and adaptive immune responses previously characterized in animals and humans and provides a current update on clinical development of immunologic-based therapies for CDI prevention in adults and children. These data inform the future research needs for children.

**Key words:** antibody; *C. difficile*; immunity; pediatric; vaccine

The pathogenesis of CDI is complex and multifactorial, but a clear understanding of disease mechanisms informs potential treatment and prevention strategies (Figure 1) [6]. Acquisition of *C. difficile* (ie, the microorganism), either in the healthcare setting or in the community, is essential but not sufficient to cause CDI (ie, the infection or disease state). This is particularly evident in infants and young children in whom frequent *C. difficile* carriage is evident without clinical symptoms. Intestinal dysbiosis, commonly related to antibiotic exposure before or after *C. difficile* acquisition, permits *C. difficile* overgrowth and toxin production. Failure of dysbiosis to resolve, commonly resulting from ongoing antibiotic exposure for CDI or other concomitant infections, increases likelihood of recurrences. For these reasons, avoiding antibiotics and/or restoring the healthy microbiome are important strategies for preventing recurrences. However, because antibiotics are necessary for many patients, and because many patients may not be candidates for microbiome restoration through fecal microbiota transplantation (FMT) or other biologic therapies, there is increasing need for prevention strategies that target other aspects of CDI pathogenesis, such as the host immune response.

The understanding of the host immune response to CDI has expanded substantially over the past two decades. Translational research observations in animals and humans have led to the clinical development of several immunologic therapies for CDI in adults. This review summarizes the immunological aspects of CDI, the clinical development of immunologic therapies, and ongoing research and clinical needs for immunological therapies for CDI in children.
HOST IMMUNE RESPONSES TO *C. DIFFICILE*

Innate Immune Responses

The host immune response to *C. difficile* is widely implicated in mediating many of the clinical symptoms of CDI. A large body of evidence demonstrates that the immunostimulatory activity of toxins (TcdA and TcdB) released by *C. difficile* activate innate and adaptive immune responses targeting the colon and disrupting intestinal barrier function [7]. This toxin-induced proinflammatory response is mediated by the inactivation of small intracellular Rho GTPases and promotes innate immune cell infiltration. Neutrophils constitute a major histopathological feature of pseudomembranous colitis evident in many patients with CDI, including children. When appropriately regulated, the innate inflammatory response protects the host from CDI by clearing the pathogen from the intestine and mediating mucosal repair to restore colonic integrity. Effective innate immunity is therefore crucial in protecting the host from infectious colitis, but if left unchecked, results in disease exacerbation and/or susceptibility to reinfection.

Even though neutrophils represent a classical host innate immune response against CDI, their role in CDI pathogenesis remains controversial. Following infection, inactivated neutrophils are recruited from the circulation and are primed into a state of enhanced responsiveness promoting pathogen clearance via complement-mediated pathways [8]. Innate immune sensors, including TLR4, Nod1, and the MyD88 adapter protein, are required for attenuation of clinical disease in experimental CDI models by controlling *C. difficile* spore production and preventing luminal translocation of pathobionts, which are commensal organisms that can induce disease only under certain conditions. Intestinal epithelial NLRP3 inflammasome activation is also reported as a protective proinflammatory signal to neutrophils, and as such serves as an innate immune cell type that directly interfaces with the gut lumen and rapidly responds to *C. difficile* toxin exposure by activating IL-1 and IL-6 signaling circuits [9]. Other toxin-induced innate immune responses include activation of transcription factors such as HIF1-α that regulate anti-inflammatory responses [10, 11]. This includes initiating respiratory bursts and the production of nitric oxide reactive species that can protect the host against CDI by covalently modifying and inactivating the *C. difficile* toxins [12]. Increased pathobiont translocation and mortality...
are also evident in experimental CDI models where neutrophils are depleted. Further, the metal-chelating S100 protein, calprotectin, makes up over 50% of the cytoplasmic protein fraction of neutrophils and is highly abundant in the gut during CDI. Calprotectin has antimicrobial activity against numerous pathogens, including *C. difficile*, and in experimental CDI models is essential for controlling severe CDI [13, 14].

The important and multifaceted role of neutrophils in risk of and host response to CDI is highlighted in patients with severe neutropenia and/or granulocyte deficiency who lack disease protection from toxigenic *C. difficile*, as well as in some patients with CDI in whom excessive neutrophil infiltration and activation can increase the risk for disease complications and mortality. This critical balance appears in part to be regulated by the cytokine IL-22, which also promotes the expression of antimicrobial peptides and other factors that promote intestinal barrier integrity and stabilizes the gut microbiota community composition [15]. Neutrophil activation is also responsive to IL-33 production, which represents another critical cytokine regulator in experimental models of CDI. Group 3 innate lymphoid cells (ILC-3) and eosinophils represent another source of protective IL-33 in experimental CDI [16, 17], but their role in patients is not yet established. In mouse models, ILC-3s rapidly regulate innate immune responses and facilitate tissue repair in response to inflammation [18, 19]. Similar to neutrophils, over-activation of ILC-3 cytokine production in mice can exacerbate colitis. Further studies are required to better understand the protective crosstalk between a myriad of innate immune cell types that confer immune protection against CDI and to assess the translational potential of these mechanisms in patients. Drug repurposing studies offer a promising strategy to rapidly identify new leads that can activate protective pathways against CDI, for example, intestinal IL-33 neutrophil responses that are localized to the site of infection [20]. Further, targeted and transient activation of these innate immune responses may alleviate potential side effects of systemic IL-33 therapy, thereby avoiding excessive tissue regeneration that can lead to fibrosis.

**Adaptive Immune Responses**

Recent work also demonstrates that adaptive immune responses to *C. difficile* antigens play important protective roles against CDI. Adaptive immune responses are very well characterized against toxins A and B [21, 22], but increasingly so for other bacterial components as well, including surface layer and flagellar proteins [23]. The humoral immune responses to *C. difficile* toxins have been well characterized, with predominant work focusing on protective immunity against CDI provided through antibody neutralization of toxin A and B. Toxin-specific antibodies are found in serum >60% of adults and children older than 2 years [24], and colonization by toxigenic *C. difficile* in infants is associated with development of antibodies against toxins A and B [25]. Neutralizing antibodies have been identified in umbilical cord blood sera from 9/50 (18%) consecutive deliveries at a large birthing center, but IgG titers against *C. difficile* toxins were significantly less in cord blood sera than that seen in infants following *C. difficile* colonization [25]. Given the ubiquitous absence of CDI in the infant population, transplacental transfer of antibodies is not considered to occur frequently enough to be a plausible explanation for this infant clinical phenomenon.

Passive transfer of toxin-specific IgG decreases recurrent CDI. Whereas serum toxin-IgG correlates with protection from recurrent CDI in most studies (eg, asymptomatic colonization [21] and recovery from CDI with the absence of relapse [26, 27], other immunoglobulin isotypes and especially IgA were less frequently assessed in these studies. While the role of IgA in protecting against CDI has not been clearly established, data suggest that it may be important. In humans with CDI, toxin-IgA is the primary serum neutralizing antibody [28]. Stool toxin-IgA correlates with protection; patients who did not relapse had more colonic IgA+ cells than in those who did [29]. Further, in a pilot study of 16 adult subjects, toxin-IgA given passively as a whey protein concentrate prevented CDI relapse in 15/16 (94%) participants; relapse was defined as symptomatic CDI and detection of stool *C. difficile* toxins during the duration of follow-up for each patient (median [range] 333 [33-365] days) [30]. In vitro, toxin-IgA inhibits toxin A binding to epithelial cells and improves toxin A neutralization, which confers greater protection of intestinal epithelial cells against toxin than by toxin-IgG alone [31]. For example, following a *C. difficile* challenge, IgG-deficient mice were protected from CDI after developing only IgA against *C. difficile* toxins [32]. Polymeric immunoglobulin receptor knockout mice that develop exceptionally high serum titers of toxin-IgA and neutralizing antibody IgA secreting cells in the gut are protected from CDI, likely following paracellular leakage of toxin-IgA across damaged epithelium, as reported for IgG. None of these studies addressed the mechanistic necessity of IgA in CDI immunity and further studies are required to establish its role in protective immunity. Recent studies now also implicate dysbiosis as a potential regulator of protective IgA responses, with microbiota-derived branched-chain amino acids promoting IgA production in the intestine [33].

The gut microbiota also plays an important protective role against CDI through adaptive immune responses [34]. Although FMT is heralded as a hugely successful treatment against CDI, it is less effective in complicated cases where treatment failure is associated with unsuccessful engraftment of donor microbiota and many of these cases are associated with immunodeficiency in the patient [35]. Indeed, emerging data demonstrate that host immune status is an important consideration in successful FMT, but precise mechanisms have not been delineated. A recent study exploring the role of regulatory T cells (Tregs) in the CDI symptom resolution and clearance of *C. difficile* in a murine model of infectious colitis demonstrated that these cells play a critical role in determining the success of
FMT [36]. Notably, T- and B-cell (Rag1)-deficient mice, in contrast to co-housed immunocompetent littermate control mice, failed to resolve CDI after FMT. Further studies revealed a necessary role for CD4+ T cells, specifically Tregs, but not B cells or CD8+ T cells, in the clearance of C. difficile following FMT. Mouse genotypes that were nonresponsive to FMT treatment exhibited exacerbated intestinal inflammation, an impaired capacity to engraft the FMT microbial communities, and a failure to restore intestinal metabolites that are associated with susceptibility to C. difficile-induced disease, for example, secondary bile acids. These new observations support the host adaptive immune system being a key factor in determining the success of microbiota-based therapy against CDI, by promoting complex interactions between the intestinal immune system, microbiome, and the transplanted bacterial inoculum. It is now important to establish whether Treg insufficiency could represent a host immune deficiency in children who are susceptible to CDI and in restoring responsiveness of microbial therapy in recurrent and refractory cases. There are increasing data of safety of FMT in adults and children and continually evolving knowledge of the role of intestinal microbiota in immunological response to pathogens. Thus, microbiome manipulation may emerge as a future strategy to improve immune response against C. difficile or to C. difficile vaccines, which are described in more detail below.

**CLINICAL DEVELOPMENT OF IMMUNOLOGIC THERAPIES**

**Clinical Aspects of CDI and Host Immune Response in Adults**

Knowledge of the host immune response to CDI and colonization in adult patients has established immunologic therapies as a potential prevention strategy and prompted clinical development of multiple immunologic therapeutics. In hospitalized adults who become colonized with C. difficile, those who develop CDI have significantly lower levels of serum IgG against toxin A compared to those who remain asymptomatic after C. difficile colonization [21]. Further, in adults who are treated for CDI, those who later develop recurrent CDI have significantly lower levels of serum IgM against toxins A and B 3 days after infection and significantly lower levels of serum IgG against toxin A 12 days after infection compared to those with sustained CDI resolution [27]. Given the morbidity and mortality associated with severe CDI and CDI recurrences in adults, the association of clinical outcomes with the natural humoral immunological response to C. difficile toxins subsequently led to clinical development of passive immunization and vaccination strategies for CDI prevention, which are described in more detail below.

**Clinical Aspects of CDI and Host Immune Response in Children**

The host immune response to C. difficile in children has been described in some pediatric populations. In a cohort of 85 children with inflammatory bowel disease (IBD) [37], asymptomatic carriage of C. difficile was noted in 17% of patients with IBD compared to 3% of control children without IBD. Accordingly, the population of children with IBD had higher levels of IgA against toxin B than controls, suggesting the humoral immune response was protective against symptomatic CDI. Infants remain a unique population regarding CDI risk because of comparatively high rates of toxigenic C. difficile colonization and toxin A and B exposure despite the near absence of symptoms. In a cohort of 32 healthy infants followed throughout the first year of life, colonization with toxigenic C. difficile occurred in 50% of infants [25]. Compared to those without toxigenic C. difficile colonization, infants colonized with toxigenic C. difficile prior to 9-12 months of age subsequently had significantly greater serum IgA and IgG against toxins A and B. Further, 42% of colonized infants, but none of those without colonization, had detectable neutralizing antibody titers against toxin B. The clinical implications of this natural immunization event following C. difficile exposure is unknown and is an important area of future investigation. Considering the high frequency of asymptomatic C. difficile colonization in groups of older children, including children with cancer [38], IBD [37], and hospitalization [39], as well as substantially lower risk of severe and fulminant CDI in children compared to adults, it is possible that a humoral immune response during infancy provides protection against symptoms resulting from C. difficile toxin exposure later in childhood.

**Passive Immunization Strategies**

Passive immunization against C. difficile by administration of polyclonal or monoclonal antibodies that target toxins A and/or B is a strategy that has been employed for CDI prevention with varying success. The rationale is that, based on observational studies described above, temporary protection against illness induced by C. difficile toxins can be achieved through administration of antibody-based therapies.

Intravenous immunoglobulin (IVIG) contains varying levels of polyclonal neutralizing IgG against toxins A and B [40]. Among 11 small case reports and case series of patients with CDI, 40 of 46 (87%) adult patients had clinical resolution of diarrhea, and 14% had a CDI recurrence following receipt of IVIG at variable dosing regimens [41]. IVIG has not yet been assessed in a randomized controlled clinical trial (RCT); convincing evidence of IVIG effectiveness for CDI is limited. Further, it is not known whether sufficient antitoxin neutralizing activity is maintained with IVIG. Thus, IVIG is not a recommended treatment for CDI at this time [42].

Nonetheless, several studies in animals have supported the rationale for development of immunologic therapies in humans. Previously, hamsters were protected against CDI following passive immunization with various products, including chicken IgY against toxin A [43], hyperimmune bovine colostrum (ie,
from cows immunized against *C. difficile* toxins A and B) [44], and through breastmilk from *C. difficile* immunized mother hamsters to their infants [45]. Protection against CDI following passive immunization of gnotobiotic mice [46] with a toxin A monoclonal antibody and gnotobiotic piglets with hyperimmune bovine colostrum [47] has also been described, although more recent studies in piglets demonstrated that protection against toxin B is required to ameliorate clinical symptoms [48]. Subsequently, protection against CDI was investigated using a panel of human monoclonal antibodies against toxins A and B that were produced by transgenic mice carrying human immunoglobulin genes. These mice were vaccinated against toxoid A and recombinant toxin B fragments. In this study, protection against CDI in a hamster model was most pronounced with an antitoxin A antibody (eventually named actoxumab), but the addition of an antitoxin B (eventually named bezlotoxumab) enhanced protection [49].

These humanized antibodies from transgenic mice were subsequently investigated in clinical trials, but a similar pattern of protection was not noted. Two phase 3 RCTs (MODIFY I and MODIFY II) of actoxumab and bezlotoxumab were completed. In MODIFY I [50], 1452 adults receiving antibiotic therapy for primary or recurrent CDI were randomized to receive either actoxumab alone, bezlotoxumab alone, both monoclonal antibodies together, or placebo. CDI recurrence was less frequent in patients receiving both monoclonal antibodies (15.9%, \( P < .0001 \)) or bezlotoxumab alone (17.4%, \( P = .0003 \)) compared to placebo (27.6%). Despite its promise in hamsters, actoxumab was associated with a similar CDI recurrence rate as placebo (25.9%) so it was discontinued for further development as monotherapy. In MODIFY II [51], 1203 adults receiving antibiotic therapy for primary or recurrent CDI were randomized to receive bezlotoxumab alone, both monoclonal antibodies together, or a placebo. Symptomatic CDI recurrence was less frequent in patients receiving both monoclonal antibodies (14.9%, \( P < .0001 \)) or bezlotoxumab alone (15.7%, \( P = .0003 \)) compared with those receiving a placebo (25.7%). The CDI recurrence rate among those receiving both monoclonal antibodies and those receiving bezlotoxumab alone were similar. Attributable safety events were not identified.

A follow-up study of the MODIFY I/II studies assessed genetic polymorphisms associated with failure to respond to bezlotoxumab. In patients who received bezlotoxumab, several specific allelic changes in the extended major histocompatibility complex on chromosome 6 predicted recurrent CDI in the bezlotoxumab group, but not in the placebo group, suggesting these polymorphisms are associated with bezlotoxumab response but not risk of recurrent CDI generally [52]. Bezlotoxumab ultimately was approved by the FDA and is recommended by the Infectious Diseases Society of America and Society for Healthcare Epidemiology of America as an adjunctive treatment to fidaxomicin in patients with first or subsequent episodes of CDI recurrence [42]. A phase 3 RCT comparing bezlotoxumab to a placebo in children receiving antibiotics for CDI (MODIFY III) is currently enrolling (ClinicalTrials.gov Identifier: NCT03182907).

**Vaccination**

Passive immunization with bezlotoxumab, which has a half-life of approximately 19 days, provides temporary protection against *C. difficile* toxins. The potential to induce longer-lasting active immunity through vaccination is an attractive approach. Several vaccine candidates are in clinical development, although results to date suggest limited success.

Sanofi Pasteur recently published results of a phase 3 placebo-controlled RCT of an aluminum-adjuvanted toxoid vaccine derived from toxins A and B (in a 3:2 ratio of A:B) in adults age 50 years and older with various CDI risk factors [53]. Three doses were administered on days 0, 7, and 30, and participants were followed for 3 years to primarily identify incidence of PCR-confirmed symptomatic CDI in each group. Over a 4-year period, 9302 subjects were enrolled (6201 received vaccine, 3101 received placebo). Among 6201 subjects receiving vaccine, 34 developed CDI (0.29 infections per 100 person-years [95% CI 0.20-0.41]). Among 3101 subjects receiving placebo, 16 developed CDI (0.28 infections per 100 person-years [0.16-0.45]). This, vaccine efficacy was approximately −5.2% (95% CI −104.1 to 43.5). After these disappointing results, the study was terminated after meeting criteria for futility, and clinical development stopped. Given success in early phase trials and the general success of toxoid vaccines in humans, these results were surprising and disappointing. Although the reasons for failure are not yet known, some have suggested that the following could be contributing factors: inappropriate adjuvant; inappropriately low ratio and immunogenicity of toxin B:A in the toxoid vaccine; insufficient patient neutralizing responses against toxin B; differences in toxinotypes between vaccine and strains circulating during the study; too long of a follow-up period (3 years) for vaccine durability; and misdiagnosis of CDI using overly sensitive PCR testing. Further, because injectable vaccines do not elicit a strong IgA response, immunity from injectable delivery may not provide equivalent protection to immunity generated from a mucosal response to a vaccine delivered enterally.

Another toxoid vaccine candidate from Pfizer is currently being investigated in a phase 3 placebo-controlled RCT. This vaccine is an aluminum-adjuvanted formulation of toxoids derived from toxins A and B and is produced by expressing genetically deactivated toxins in *C. difficile* and chemically treating the purified antigens to remove residual cytotoxicity. In their phase 2 study [54], 855 adults age 65-85 years at high risk for CDI were randomized to either receive vaccine at different dosing regimens or to receive a placebo. The 200 µg regimen given on days 0, 30, and 180 was associated with the most robust immune response as measured by neutralizing antibody titers.
against toxins A and B; this regimen was advanced to phase 3 investigation (ClinicalTrials.gov Identifier: NCT03090191). As of April 2021, more than 17,000 adults age 50 years and older at high risk for CDI have been randomized to receive vaccine or placebo and will be followed for 3 years to primarily identify incidence of CDI in each group. Results are not yet available.

While we await results of the Pfizer vaccine phase 3 RCT, ongoing efforts are underway to identify other promising vaccine candidates. Novel approaches to improving antigenicity of *C. difficile* toxin-based vaccine targets, such as using recombinant chimeric inactive mutants of toxin A and B [55], may show promise in the future. Further, a mucosal vaccine could more closely mimic the immune response elicited during natural disease [56].

A disadvantage of toxin-based vaccines is that they only protect against symptomatic infection and not against colonization. Thus, toxin-based vaccines would not be expected to prevent transmission, potentially limiting the public health value of those vaccine candidates. Many non-toxin antigens, including those responsible for *C. difficile* colonization in the gut, induce an immune response in animals and humans. Thus, immunization to some non-toxin antigens could potentially interrupt colonization and human-to-human transmission [23, 57]. However, a non-toxin antigen vaccine that can successfully prevent colonization has not yet advanced to human clinical trials.

**Innate Immunomodulatory Therapies**

Manifestations of CDI and several correlates of clinical outcomes of infection are associated with the innate immune response during CDI. Thus, modulation of innate immunity has promise for treatment and prevention of both acute and recurrent CDI. However, immunomodulatory therapies targeting this axis of immunity must overcome the paradox that innate immunity can be both beneficial and detrimental, depending on context. Striking the balance between favorable and harmful effects of innate immune pathways and identifying the patient cohort for these treatments remain challenges for therapeutics. Proposed strategies for innate immunomodulation have included both blockade of proinflammatory signals and delivery of anti-inflammatory signals. Currently there are no data to support administration of medications to block specific proinflammatory cytokine pathways, such as tumor necrosis factor (TNF)-alpha, for treatment of CDI. This is a central theme in the treatment of IBD, where patients are at increased risk for CDI following treatment with biologic therapies [58] and these treatments can worsen underlying disease course. This leaves clinicians with a dilemma for treatment of CDI in patients with IBD, which is particularly problematic in children, in whom *C. difficile* carriage is frequent [37]. Introduction of recombinant IL-22 has been suggested as a potential therapeutic for CDI, as this cytokine seems to play a central role in controlling infection. In mice, introduction of recombinant IL-22 improves survival following CDI and decreases translocation of bacteria into the bloodstream [59]. This therapy has not been studied in humans and further mechanistic studies are needed since discordant systemic vs intestinal cytokine levels are often evident.

Potential therapeutic targets for innate immunomodulatory therapies are prostaglandins (PGs) and their cognate receptor signaling pathways (EP receptors). PGs are important inflammatory lipid mediators in the gastrointestinal tract and have been shown to be important for homeostasis of the epithelium and repair of the mucosa following infection [60]. Recent epidemiological studies have shown a correlation between CDI and nonsteroidal anti-inflammatory drug (NSAID) use, which inhibits the activity of cyclooxygenase enzymes and production of PGs. Furthermore, experimental models of CDI demonstrate that indomethacin exacerbates disease severity, increases mortality associated with infection [61, 62], and leads to a loss of epithelial barrier integrity. These studies suggest PGs may play a central role in the innate immune response to CDI and may have promise for therapeutic intervention. Indeed, in experimental models, the FDA-approved PGE1 analog, misoprostol, protects mice from severe CDI and enhances barrier function [61]. Misoprostol is currently in phase 2 clinical trials for the prevention of recurrent CDI (ClinicalTrials.gov Identifier: NCT03617172). More work is needed to understand the molecular mechanisms associated with NSAID exacerbation of CDI and PG-mediated protection.

**CLINICAL AND RESEARCH PRIORITIES IN PEDIATRIC POPULATIONS**

Immunologic aspects of CDI in pediatric populations remain understudied and constitute a priority for future CDI research. Current CDI prevention strategies in children are focused on infection prevention and antimicrobial stewardship. However, avoiding antibiotics may not be feasible in many children, particularly those who are immunocompromised. Further, while restoration of the intestinal microbiota through FMT has been shown to be safe and effective in many children, concerns about long-term safety, safety in immunocompromised children, and operational challenges at many centers increase the need for other pediatric prevention strategies, such as immunologic therapies. To advance this work in children, the following research priorities should be addressed:

- Natural History of the Immune Response to *C. difficile* Colonization and Infection in Children
- The Role of Microbiota Replacement as an Immunomodulatory Intervention
- Advancing Pediatric Clinical Trials of Existing Immunomodulatory Therapies
- Identification of Priority Populations for Immunologic Therapies
Natural History of the Immune Response to *C. difficile* Colonization and Infection in Children

Because of the previously described work demonstrating the association between humoral response and asymptomatic colonization in adults [21], and the high frequency of colonization described in various groups of children at high risk of CDI, including children with cancer [38], IBD [37], and hospitalization [39], it is possible that humoral immunity is frequent and durable. This may be true even among children with these immunocompromising conditions. Immunity against *C. difficile* toxins may be derived from early life exposure [25]. Thus, an important area of future investigation will be to better understand the natural history of *C. difficile* immunity related both to early life exposure and from symptomatic CDI later in childhood and adolescence. This knowledge may guide the identification of priority populations for future clinical trials of various immunologic strategies for *C. difficile* prevention.

The Role of Microbiota Replacement as an Immunomodulatory Intervention

The gut microbiome plays an important protective role against CDI through adaptive immune responses, particularly through Treg activity. Further understanding of the complex interplay between the microbiota and host immune system may identify the role of FMT beyond correction of dysbiosis and include immunomodulation. Characterization of dysbiosis and Treg activity may also provide an opportunity for a precision medicine approach by identifying those who may be most likely to benefit from FMT.

Advancing Pediatric Clinical Trials of Existing Immunologic Therapies

Results of the pediatric phase 3 bezlotoxumab trial (ClinicalTrials.gov Identifier: NCT03182907), as well as adult vaccine trials that may then advance to trials in pediatric populations, are eagerly awaited.

Identification of Priority Populations for Immunologic Therapies

Once clinical trial data are available for immunologic therapies, those data, in conjunction with data regarding the natural history of the pediatric immune response to CDI in healthy children and those with co-morbidities, will permit investigation of comparative and cost-effectiveness of immunologic therapies in priority pediatric populations.

CONCLUSION

There has been tremendous advancement in the understanding of the host immune response to CDI in adults that has led to clinical development of immunologic therapies for CDI prevention. Although research and clinical development in this area have been limited in children, the knowledge gained has informed clinical and research priorities for children.

Notes

**Financial support.** This work was supported, in part, by grants from the National Institutes of Health/National Institute of Allergy and Infectious Diseases to L. K. K. (R03-AI149000), J. P. Z. (K22-AI22720), and T. S. (U01-A124290, P01-AI152999, and R01-AI10091401), from the National Institutes of Health/National Institute of General Medical Sciences to J. P. Z. (R35-GM138369), and from the Texas Medical Center Digestive Disease Center to T. S. (P30-DK6338).

**Supplement sponsorship.** This supplement was sponsored by Pfizer, Merck, and Azteryx.

**Potential conflicts of interest.** L. K. K. reports research grant support from Merck, unrelated to the content of this work. J. P. Z. reports research grant support from BioNTech. T. S. reports research grant support from Merck, Nivalis, Cubist, Mead Johnson, Rebiotix, BioFire, Assembly Biosciences, and he has served on the advisory board for Rebiotix and BioFire. All authors have submitted the ICMJE Form for Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**

1. Magill SS, O’Leary E, Janelle SJ, et al.; Emerging Infections Program Hospital Prevalence Survey Team. Changes in prevalence of health care-associated infections in U.S. Hospitals. N Engl J Med 2018; 379:1732–44.
2. Lessa FC, Mu Y, Ramberg WM, et al. Burden of *Clostridium difficile* infection in the United States. N Engl J Med 2015; 372:825–34.
3. Tschudin-Sutter S, Tamma PD, Naegeli AN, et al. Distinguishing community-associated from hospital-associated *Clostridium difficile* infections in children: implications for public health surveillance. Clin Infect Dis 2015; 57:1665–72.
4. Sandora TJ, Fung M, Flaherty K, et al. Epidemiology and risk factors for *Clostridium difficile* infection in children. Pediatr Infect Dis J 2011; 30:580–4.
5. Mehrotra P, Jang J, Ng D, et al. Attributable cost of *Clostridium difficile* infection in pediatric patients. Infect Control Hosp Epidemiol 2017; 38:1472–7.
6. Kociolek LK, Gerdling DN. Breakthroughs in the treatment and prevention of *Clostridium difficile* infection. Nat Rev Gastroenterol Hepatol 2016; 13:150–60.
7. Sun X, Hirota SA. The roles of host and pathogen factors and the innate immune response in the pathogenesis of *Clostridium difficile* infection. Mol Immunol 2015; 65:193–202.
8. Peniche AG, Spinler JR, Boonman P, et al. Aging impairs protective host defenses against *Clostridoides* (*Clostridium*) difficile infection in mice by suppressing neutrophil and IL-22 mediated immunity. Anaerobe 2018; 54:83–91.
9. Ng J, Hirota SA, Gross O, et al. *Clostridium difficile* toxin-induced inflammation and intestinal injury are mediated by the inflammasome. Gastroenterology 2010; 139:542–52, 52.e1-3.
10. Hirota SA, Fines K, Ng J, et al. Hypoxia-inducible factor signaling provides protection in *Clostridium difficile*-induced intestinal injury. Gastroenterology 2010; 139(1): 259–69.e3.
11. Lee JY, Hirota SA, Glover LE, et al. Effects of nitric oxide and reactive oxygen species on HIF-1α stabilization following *Clostridium difficile* toxin exposure of the Caco-2 epithelial cell line. Cell Physiol Biochem 2010; 26:317–30.
12. Savidge TC, Utz V, Oegema N, et al. Host 5-nitrosoylation inhibits clostridial small molecule-activated glucosylating toxins. Nat Med 2011; 17:1136–41.
13. Zackular JP, Skaar EP. The role of zinc and nutritional immunity in *Clostridium difficile* infection. Trends Biochem Sci 2011; 36:281–9.
14. Zackular JP, Moore KL, Jordan AT, et al. Dietary zinc alters the microbiota and decreases resistance to *Clostridium difficile* infection. Infect Immun 2014; 82:4095–106.
15. Sahel MM, Petri WA Jr. Type 3 immunity during *Clostridoids* difficile infection: too much of a good thing? Infect Immun 2019; 88.
16. Frisbee AL, Saleh MM, Young MK, et al. IL-33 drives group 2 innate lymphoid cell-mediated protection during *Clostridium difficile* infection. Nat Commun 2019; 10:2712.
17. Abemarley-Close L, Dieterle MG, Vendrow KC, et al. Aging dampens the intestinal innate immune response during severe *Clostridoids* difficile infection and is associated with altered cytokine levels and granulocyte mobilization. Infect Immun 2020; 88.
18. Abt MC, Lewis BB, Caballero S, et al. Innate immune defenses mediated by two ILC3 subsets are critical for protection against acute *Clostridium difficile* infection. Cell Host Microbe 2015; 18:27–37.
19. Fachi JL, Sécca C, Rodrigues PB, et al. Acetate coordinates neutrophil and ILC3 responses against *C. difficile* through FFA2. J Exp Med 2020; 217.
20. Andersson IA, Peniche AG, Galindo CL, et al. New host-directed therapeutics for the treatment of *Clostridoids* difficile infection. mBio 2020; 11.
21. Kyne I, Warney M, Qamar A, Kelly CP. Asymptomatic carriage of Clostridium difficile and serum levels of IgA antibody against toxin A. N Engl J Med 2000; 342:390–7.

22. Savidge TC, Pan WH, Newam P, et al. Clostridium difficile toxin B is an inflammatory enterotoxin in human intestine. Gastroenterology 2003; 125:413–20.

23. Péchini S, Bruxelle JP, Janoir C, Collignon A. Targeting Clostridium difficile surface components to develop immunotherapeutic strategies against Clostridium difficile infection. Front Microbiol 2018; 9:1009.

24. Viscidi R, Laughon BE, Yelenk D, et al. Serum antibody response to toxins A and B of Clostridium difficile. J Infect Dis 1983; 148:93–100.

25. Kocisolek LK, Espinosa RO, Gerding DN, et al. Natural Clostridoides difficile toxin immunization in colonized infants. Clin Infect Dis 2019.

26. Leav BA, Blair B, Leney M, et al. Serum anti-toxin B antibody correlates with protection from recurrent Clostridium difficile infection (CDI). Vaccine 2010; 28:965–9.

27. Kyne I, Warney M, Qamar A, Kelly CP. Association between antibody response to toxin A and protection against recurrent Clostridium difficile diarrhea. Lancet 2001; 357:189–93.

28. Johnson S, Sypura WD, Gerding DN, et al. Selective neutralization of a bacterial enterotoxin by serum immunoglobulin A in response to mucosal disease. Infect Immun 1995; 63:3166–73.

29. 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; 57:973–9.

30. van Dissel JT, de Groot N, Hensgens CM, et al. Bovine antibody-enriched whey to aid in the prevention of a relapse of Clostridium difficile-associated diarrhoea: preclinical and preliminary clinical data. J Med Microbiol 2005; 54:197–205.

31. Stubbe H, Bendor J, Kraehn ushulj JP, Corthésy B. Polymeric IgA is superior to monoclonic IgG and IgA carrying the same variable domain in preventing Clostridium difficile toxin A damaging of TH4 mononuclears. J Immunol 2000; 164:1952–60.

32. Johnston PF, Gerding DN, Knight KL. Protection from Clostridium difficile infection in CD4 T cell- and polymeric immunoglobulin receptor-deficient mice. Infect Immun 2014; 82:522–31.

33. Zhang S, Zeng X, Ren M, et al. Novel metabolic and physiological functions of branched chain amino acids: a review. J Anim Sci Biotechnol 2017; 8:10.

34. de Bruyn G, Gordon DL, Steiner T, et al. Safety, immunogenicity, and efficacy of a Clostridium difficile toxoid vaccine candidate: a phase 3 multicentre, observer-blind, randomised, controlled trial. Lancet Infect Dis 2021; 21:252–62.

35. 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.

36. Wang H, Sun X, Zhang Y, et al. A chimeric toxin vaccine protects against primary and recurrent Clostridium difficile infection. Infect Immun 2012; 80:2678–88.

37. Wang Y, Wang S, Bouillaut L, et al. Oral immunization with nontoxicogen Clostridium difficile strains expressing chimeric fragments of TcdA and TcdB elicits protective immunity against C. difficile infection in both mice and hamsters. Infect Immun 2018; 86.

38. Rebeaud F, Bachmann MF. Immunization strategies for Clostridium difficile infections. Expert Rev Vaccines 2012; 11:469–79.

39. Ghomil-Mostafaei FS, Yadegar A, Aghaieha HA, et al. Anti-TNF containing regiments may be associated with increased risk of Clostridium difficile infection in patients with underlying inflammatory bowel disease. Curr Res Transl Med 2020; 68:125–30.

40. Hasegawa M, Tada S, Liu MZ, et al. Interleukin-22 regulates the complement system to promote resistance against pathobionts after pathogen-induced intestinal damage. immunity 2014; 61:620–32.

41. Grainger JR, Wohlfert EA, Fuss IJ, et al. Inflammatory monocytes regulate patho-genic responses to commensals during acute gastrointestinal infection. Nat Med 2015; San Diego, CA, USA.

42. Wilcox M, Gerding D, Poxton I, et al. Phase 3 double-blind study of actoxumab (ACT) and bezlotoxumab (BEZ) for prevention of recurrent C. difficile infection (rCDI) in patients on standard of care (SoC) antibiotics (MODIFY I) [abstract]. Paper presented at: Interscience Conference on Antimicrobial Agents and Chemotherapy; September 17–21, 2015; San Diego, CA, USA.

43. Kink JA, Williams JA. Antibodies to recombinant Clostridium difficile toxins A and B are an effective treatment and prevent relapse of C. difficile-associated disease in a hamster model of infection. Infect Immun 1998; 66:2018–25.

44. Kelly CP, Pothenalakis C, Varva F, et al. Anti-Clostridium difficile bovine immunoglobulin concentrate inhibits cytotoxicity and enterotoxicity of C. difficile toxins. Antimicrob Agents Chemother 1996; 40:373–9.

45. Kim PH, Iaconis JP, Rollie RD. Immunization of adult hamsters against Clostridium difficile-associated ileocolitis and transfer of protection to infant hamsters. Infect Immun 1987; 55:2984–92.

46. Corrigan MC, Tullis TC, Roberts DM, et al. Protection against experimental pseudomembranous colitis in gnotobiotic mice by use of monoclonal antibodies against Clostridium difficile toxin A. Infect Immun 1994; 60:1192–5.

47. Spenscher JK, Steele JA, Schmidt DJ, et al. Hyperimmune bovine colostrum as a novel therapy to combat Clostridium difficile infection. J Infect Dis 2015; 211:1334–41.

48. Steele J, Mukherjee J, Parry N, Triapori S. Antibody against TcdB, but not TcdA, prevents development of gastrointestinal and systemic Clostridium difficile disease. J Infect Dis 2013; 207:323–30.

49. Babcock GI, 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; 74:6339–47.

50. Wilcox M, Gerding D, Poxton I, et al. Phase 3 double-blind study of actoxumab (ACT) and bezlotoxumab (BEZ) for prevention of recurrent C. difficile infection (rCDI) in patients on standard of care (SoC) antibiotics (MODIFY I) [abstract]. Paper presented at: Interscience Conference on Antimicrobial Agents and Chemotherapy; September 17–21, 2015; San Diego, CA, USA.

51. de Bruyn G, Gordon DL, Steiner T, et al. Safety, immunogenicity, and efficacy of a Clostridium difficile toxoid vaccine candidate: a phase 3 multicentre, observer-blind, randomised, controlled trial. Lancet Infect Dis 2021; 21:252–62.

52. 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.

53. Wang H, Sun X, Zhang Y, et al. A chimeric toxin vaccine protects against primary and recurrent Clostridium difficile infection. Infect Immun 2012; 80:2678–88.

54. Wang Y, Wang S, Bouillaut L, et al. Oral immunization with nontoxicogen Clostridium difficile strains expressing chimeric fragments of TcdA and TcdB elicits protective immunity against C. difficile infection in both mice and hamsters. Infect Immun 2018; 86.

55. Rebeaud F, Bachmann MF. Immunization strategies for Clostridium difficile infections. Expert Rev Vaccines 2012; 11:469–79.

56. Ghomil-Mostafaei FS, Yadegar A, Aghaieha HA, et al. Anti-TNF containing regiments may be associated with increased risk of Clostridium difficile infection in patients with underlying inflammatory bowel disease. Curr Res Transl Med 2020; 68:125–30.

57. Hasegawa M, Tada S, Liu MZ, et al. Interleukin-22 regulates the complement system to promote resistance against pathobionts after pathogen-induced intestinal damage. immunity 2014; 61:620–32.

58. Grainger JR, Wohlfert EA, Fuss IJ, et al. Inflammatory monocytes regulate pathogenic responses to commensals during acute gastrointestinal infection. Nat Med 2013; 19:713–21.

59. Zackular JP, Kirk L, Trindade BC, et al. Misoprostol protects mice against severe Clostridium difficile infection and promotes recovery of the gut microbiota after antibiotic perturbation. Anaerobe 2019; 58:89–94.

60. Muñoz-Miralles J, Trindade BC, Castro-Córdova P, et al. Indomethacin increases severity of Clostridium difficile infection in mouse model. Future Microbiol 2018; 13:721–71.