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REVIEW

Host Immunity and Immunization Strategies for *Clostridioides difficile* Infection

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SUMMARY *Clostridioides difficile* infection (CDI) represents a significant challenge to public health. *C. difficile*-associated mortality and morbidity have led the U.S. CDC to designate it as an urgent threat. Moreover, recurrence or relapses can occur in up to a third of CDI patients, due in part to antibiotics being the primary treatment for CDI and the major cause of the disease. In this review, we summarize the current knowledge of innate immune responses, adaptive immune responses, and the link between innate and adaptive immune responses of the host against CDI. The other major determinants of CDI, such as *C. difficile* toxins, the host microbiota, and related treatments, are also described. Finally, we discuss the known therapeutic approaches and the current status of immunization strategies for CDI, which might help to bridge the knowledge gap in the generation of therapy against CDI.

KEYWORDS *Clostridium difficile*, fecal microbiota transplant, host immune response, innate lymphoid cells, microbiome, vaccine

INTRODUCTION

Clostridioides difficile is a Gram-positive, anaerobic, spore-forming, toxin-producing, rod-shaped bacterium that is the leading cause of nosocomial and gastroenteritis-associated death in developed countries (1, 2). *C. difficile* infection (CDI) accounts for around 460,000 illnesses and 20,000 deaths annually in the United States and 130,000 illnesses and 12,400 deaths in Europe (1, 3–9). It usually occurs in susceptible patients with dysbiosis due to prior antibiotic therapy. Other contributing risk factors include advanced age, recent hospitalization, gastrointestinal manipulations, drugs that lead

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to gastric acid suppression, the use of proton-pump inhibitors, and disruption of normal microbiota by chemotherapeutic agents (10–15). When *C. difficile* spores are ingested in these vulnerable individuals and reach the small bowel, their germination depends on various factors, including microbiota-host immune response, primary bile acids (mainly taurocholate), and different amino acids (16–18). From the small bowel, spores move down to the colon. Under favorable conditions, they germinate to vegetative cells producing enterotoxins to disrupt the intestinal epithelial barrier, cause tissue damage, gain nutrients, provoke or suppress inflammation, and contribute to colonization (14, 19–23). Disruption in the epithelial barrier by intoxication results in endogenous colonic flora translocating into the mucosa and becoming exposed to immune cells. This process activates immune responses, proinflammatory cytokines, and chemokine production that recruit neutrophils, mast cells, monocytes, and innate lymphoid cells (ILCs) (24). Neutrophils are responsible for the colonic pseudomembranes pathognomonic of the disease and in part via neutrophil extracellular traps (NET) attempt to protect the gut barrier (24). NET is known to limit the dissemination of pathogens by trapping them and creating an effective environment for an effective neutrophil microbicidal effect. Mast cell degranulation stimulates histamine release resulting in increased permeability of the intestinal barrier. Consequently, a substantial loss of fluid into the lumen causes severe diarrhea, cramps, dehydration, and in the most severe cases toxic megacolon (16, 25, 26). In addition, mucosal B cells, macrophages, and plasma cells are diminished in patients with CDI (27).

Repeat infections with *C. difficile* (rCDI) are also common, with over 15 to 35% of CDI patients suffering from one or more recurrent infections (28). It suggests that natural infection may not always stimulate a protective immune response (29). The lack of protection following primary CDI could be due to continued disruption of the normal microbiome from antibiotic therapy of CDI, insufficient antibody (Ab) production, and infection with an antigenically distinct strain (toxinotype) (30, 31). Although antibody responses directed against the toxins A and B (TcdA and TcdB) are associated with protection, primary CDI does not consistently induce T follicular helper cells (Tfh) cells or B memory (Bmem) cells that produce toxin-neutralizing antibodies (32–34). Many risk factors have been consistently associated with recurrent CDI, such as advanced age, (28, 35), chronic kidney disease, (28, 30), high leukocyte count, (28), baseline comorbidities (30, 31), continued use of non-*C. difficile* antibiotics after CDI diagnosis, and concomitant use of antacid medications (32–34).

CDI varies among individuals due to the complex relationships in the host immune response, host microbiota, and CDI. Some individuals develop asymptomatic colonization after acquiring *C. difficile* due to their strong immunity, whereas others individuals develop the symptomatic disease as they are vulnerable due to the above-discussed reasons. Some individuals suffer from recurrent disease as they are unable to mount enough of a protective adaptive response.

SUBTYPES OF THE TcdB TOXIN AND PHYLOGENETIC CLADES OF *C. difficile*

C. difficile secretes the closely related toxins TcdA and TcdB that bind to receptors on intestinal epithelial cells and act to disrupt the gut epithelial barrier (36, 37). In most strains of *C. difficile*, toxins are present on a pathogenicity locus (PaLoc) that encodes toxin genes (*tcdA* and *tcdB*) with regulatory (*tcdR* and *tcdC*) and secretory (*tcdE*) genes (38, 39). Epidemic strains may lack regulatory genes and additionally express a third toxin known as the binary toxin (CDT), in collaboration with septins that enhance the adhesion to the gut epithelia (20, 40–43). CDT enhances pathogenic host inflammation and has been demonstrated in the mouse model to be responsible for the increased virulence of epidemic strains by suppressing protective colonic eosinophils (20). CDT has two subunits, namely, CDTa and CDTb, where CDTb binds with the target cell and helps CDTa in cytoplasmic translocation. CDTa being an ADP ribosyltransferase disturbs the actin and microtubule balance to induce microtubule-based protrusions of

TABLE 1 Subtypes of the TcdB and disease associations

Clade	TcdB subtype	Associated host receptor	Diseases associated with hypervirulent sequence types
1	TcdB1 (classical)	Fzd, CSPG4	Hypervirulent ribotypes are notably absent.
2	TcdB2[a]	CSPG4, Fzd/TFP1	RT027 strains are well associated with worse outcomes in CDI (49, 50).
2	TcdB4	TFP1	Clinical data are very limited since TcdB4 was discovered only recently (44, 46).
3	TcdB1, TcdB1b	Fzd, CSPG4	Hypervirulent clade 3 strains showed rates of severe infection comparable to clades 2 and 5 and with significantly more bloody diarrhea (174) RT023 correlates with biomarkers of severity but not increased mortality (175).
4	TcdB1, TcdB3[b]	Fzd, CSPG4	Data from clade 4 outbreak settings with RT017 suggest potentially more severe disease compared with prior types (176, 177).
5	TcdB1c	Fzd, CSPG4	RT078 was associated with over twice the 14-day mortality compared with clade 1 (25% versus 12%, $P < 0.0001$) (175). Note that 078 also produces CDT.
5	TcdB5	Fzd, CSPG4	Just recently described, clinical data are not reported for this specific subtype (44).
Undefined	TcdB6, TcdB7, TcdB8	Fzd/ TFP1, CSPG4	Rarely found in outlier strains (44).

[a]One strain of clade 1 does express TcdB2.
[b]One strain of clade 1 does express TcdB3.

the cell membrane by catalyzing the modification of actin fibers. These protrusions enhance the adhesion of *C. difficile*.

TcdB as the key virulence factor of *C. difficile* undergoes accelerated evolution, likely in part to escape antibody-mediated neutralization (Table 1) (44). Clinically relevant strains of *C. difficile* are divided into 5 phylogenetic clades, and variants of TcdB were initially described in association with emerging hypervirulent clade 2 strains (i.e., TcdB2 [45] and TcdB4 [46]). Since then, at least 8 to12 TcdB subtypes have been identified with distinct biological activities (including a separate cell surface receptor in the case of tcdB2 and tcdB4 [47]). TcdB1 to 4 are the dominant subtypes and TcdB6, 7, and 8 are found in ribotypes not clearly associated with any of the 5 known clades. In addition, subtype variations of TcdB1 (TcdB1a/b/c) and TcdB2 (TcdB2a/b) exist (44). Among the dominant subtypes (1–4), TcdB1/3 use chondroitin sulfate proteoglycan 4 (CSPG4) and frizzled (FZD) receptors, TcdB2 uses CSPG4 and tissue factor pathway inhibitor (TFPI), and TcdB4 uses TFPI (47). Differences in cell entry of TcdB subtypes are thought to lead to divergent pathology (48). However, besides the clear association between TcdB2/4-producing clade 2 strains and more severe clinical outcomes, disease phenotypes associated with the less common toxin B subtypes are not well established (Table 1) (49, 50). Some have suggested that clade classifications are less important than specific virulence factors, such as binary toxin (CDT), and host factors (51). Importantly, the neutralizing capability of anti-toxin B monoclonal antibodies, including bezlotoxumab, may vary according to TcdB1 and TcdB2 as well as potentially other subtypes (52).

While CDT explains at least in part the heightened virulence of epidemic strains, TcdB plays a key role in causing gastrointestinal diseases in all strains since all pathogenic *C. difficile* strains contain functional *tcdB* genes, and lacking TcdB, but not TcdA, drastically attenuates the virulence of clinical strains in animal models (53, 54). Adaptive immune responses to one lineage may not result in protective immunity against other lineages (55). The induction of a strong toxin-neutralizing antibody response likely plays a key role in protection from disease, whereas immunity to cell surface antigens and other polysaccharides is likely important for promoting colonization resistance or pathogen clearance (35). Germination of *C. difficile* spores and colonization are critical steps in *C. difficile* pathogenesis, and adaptive immune responses that impact colonization have the potential to reduce transmission in health care facilities and communities (35).

C. difficile INFECTION AND THE MICROBIOME

The gut microbiota plays a central role in the prevention of CDI and colonization through several potential mechanisms. Some of the mechanisms involve primarily the impact of dysbiosis in abrogating otherwise protective mucosal type 2 immune responses.

The other mechanisms may also include competition for food and niche between *C. difficile* and commensal microbes (56, 57), the production of antimicrobial peptides (AMP), bacteriocin production by commensal bacteria (22, 58, 59), and deconjugation of primary bile acid into secondary bile acid to inhibit the germination of spores and growth of vegetative cells (60).

Susceptibility to CDI is due primarily to antibiotic-mediated dysbiosis disrupting the protective type 2 innate immune response in the gut. The intestinal microbiome has been shown to affect the production of cytokines, such as interleukin-25 (IL-25) (56), IL-33 (57), transforming growth factor β (TGF-β) (61), and IL-22 (62), which can ultimately alter CDI outcomes. Hence, fecal microbiota transplantation (FMT) to restore type 2 immunity is one of the current treatments to cure and manage refractory or recurrences of CDI (59). The safety and efficacies of the FMT procedure have been confirmed by randomized controlled clinical trials and meta-analyses (63–68). FMT also helps in the proliferation and differentiation of intestinal epithelial cells and fortification of the mucus layer to protect against disease recurrence (69). FMT can be delivered from different routes, including an upper gastrointestinal (GI) route (oral capsules, nasogastric tube, gastroscopy, or percutaneous endoscopic gastrostomy) and lower GI route (colonoscopy) with no significant difference in efficacy (70, 71). Along with the benefits of FMT, there are risks, including inflammatory and infectious complications in normal or immunocompromised and immunocompetent recipients (72–74). FMT has rarely been associated with ventilator-associated pneumonia, toxic megacolon, Crohn's disease, *Escherichia coli* bacteremia, drug-resistant *E. coli* bacteremia, transmitted septic shock, and death (75–79). Hence, the treatment of CDI patients with FMT must be examined critically to avoid such clinical repercussions. In this regard, live and purified *Firmicutes* bacterial spores (SER-109) have recently been clinically tested as a replacement for FMT (80, 81). Seres Therapeutics, Inc. (Nasdaq, MCRB) is a leading microbiome therapeutics company manufacturer of SER-109 that was granted Breakthrough Therapy designation and Orphan Drug designation for the prevention of rCDI by the U.S. Food and Drug Administration (FDA).

The severity of CDI and the efficacy of FMT also depend upon the host's immune response which varies from person to person. Hence, this variation may be the reason for not knowing the clear mechanism of FMT (82). Along with this information, intestinal IL-33, which is regulated by indigenous colonial microflora, prevents disease through the activation of ILC2s irrespective of the bacterial count. It is interesting to note that the production of IL-33 and IL-25 from the pericryptal myofibroblasts and intestinal endothelial cells is regulated by the commensal bacterium and FMT protects rCDI by restoring the signaling between the innate immune system and intestinal bacterium (56, 57). Downstream of IL-25 and IL-33, innate lymphoid cells were defined previously to protect from CDI. ILC1s are shown to protect CDI (83) and ILC2s too via tissue repair and eosinophil recruitment (56, 57). In addition, microbiota-derived acetate-induced ILC3s were also found to protect CDI through IL-22 production (84).

INNATE IMMUNE RESPONSES TO *Clostridioides difficile*

The first line of defense in the case of CDI is the mucosal layer and intestinal epithelium, including antimicrobial peptides secreted by the epithelial cells, Paneth cells, and commensal bacteria (58, 59, 85). The binding of *C. difficile* toxins to their receptors on colonocytes disrupts epithelial barrier integrity, including the cytoskeletal structure and tight junctions (86–89) that result in the translocation of commensals into the lamina propria. This process leads to inflammasome activation and the production of proinflammatory cytokines, such as IL-1β, IL-6, IL-12, IL-23, TNF-α, IFN-γ, and chemokines, including CXCL1, CXCL2, CXCL5, and IL-8, through the activation of nuclear factor-κB (NF-κB) and activator protein 1 (AP-1) pathways. They help in the production of antimicrobial peptides (AMP) and the recruitment of immune cells, including neutrophils, eosinophils, macrophages, ILCs, and dendritic cells (DCs), to the site of infection (90). TcdB-dependent inflammasome formation activates caspases that mediate proinflammatory

responses, such as IL-1β secretion. This inflammasome formation was found to be beneficial or harmful depending on context (23, 91, 92). It has also been noticed that plasminogen helps in the germination of vegetative cells by modulating the cell surface of *C. difficile* spores (93). In addition, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced by epithelial cells that disrupt the metabolism and attenuate the toxin potency of *C. difficile*, respectively (94, 95).

Neutrophils appear to play a dual role in reducing pathogen burden and mediating tissue damage (24, 96, 97). Macrophages secrete proinflammatory responses that help in bacterial clearance and the prevention of a recurrence of the disease. In contrast, *C. difficile* spores may survive within macrophages to avoid clearance and promote persistence in the gut (92, 98–100). Eosinophils remarkably showed a protective role in CDI, as in the mouse model, the adoptive transfer of eosinophils protected, and in humans, conversely, a peripheral eosinopenia at the time of CDI diagnosis was associated with higher mortality (56, 101). As per the available data, IL-4 plays a major role in tissue repair and the dampening of inflammatory responses (102, 103). Additionally, data from our lab also suggest the depletion of IL-4-producing eosinophils could be restored by IL-25, which enhances epithelial integrity and reduces disease severity in the recovery phase of CDI (56, 103). The role of IL-13 in the generation of protective anti-inflammatory alternatively activated macrophages in the recovery phase was also investigated in our lab (unpublished data). On the other side, we showed the pathogenic role of IL-23 signaling during CDI in both human and murine models. The IL-23 protein level increases in human colon biopsy specimens, and blocking IL-23 signaling increased the survivability of mice during CDI (104).

INTEGRATED DYNAMICS OF INNATE AND ADAPTIVE IMMUNITY OF *C. difficile*

ILCs Cross Talk with Type 2 and Type 3 Responses

Intestinal tissue-resident ILCs are known to integrate signals and communicate among microbiota, pathogens, and the host immune systems (84). ILC differentiation is dependent on IL-7, and their maintenance is done by IL-2 (105). ILCs can survive for 18 months *in vitro* in the presence of IL-2 and can survive lifelong in the host when transferred to $\gamma c^{-/-}$Rag2$^{-/-}$ mice (106). This feature is very specific for the ILCs which are not seen even in T and B lymphocytes which generally have a long life as memory cells. ILCs respond earlier than adaptive immune cells, developing against the invasion of bacteria. Commensals modulate intestinal homeostasis by interacting with ILCs (107, 108).

Nuclear factor, interleukin 3 regulated knockout (Nfil3$^{-/-}$) mice, which have a defect in ILC maturation, were found to be more susceptible to CDI (109). Ragyc$^{-/-}$ mice, which lack T and B cells along with ILCs, were also more susceptible and mortal to CDI than Rag2$^{-/-}$ mice, which lack only T and B cells (83), suggesting a major role of ILCs in protecting against CDI. In addition, ILC1-associated IFN-γ was demonstrated to be involved in CDI recovery (83). It is worth noting that IL-33 and IL-25 were shown to increase ILC2s (57). ILC2s could initiate an adaptive response and respond back to the signals produced by B cells and T cells (110). A study in a mouse model showed that both ILC2 and ILC3 express major histocompatibility complex (MHC) class II and promote T cell responses (111, 112). ILC2s also express costimulatory molecules CD80, CD86, PD-L1, OX40L, and inducible costimulator ligand (ICOS-L) that enable them to process and present antigens to CD4$^+$ T cells leading to their differentiation into Th2 phenotype in an IL-2-dependent manner (111). Along with this information, ILC2s can also perform endocytosis (111). Most of the studies available that describe cross talk between the ILC2s and the adaptive immune system were performed on mouse models (113–115). ILC2 can also activate B cells to undergo isotype switching, survival, and antibody production through cytokines and the interaction of ICOS with its ICOS-L ligand (116–118). *In vitro* data also showed that ILC2s dwelling in mesenteric fat could increase immunoglobulin A (IgA) production by peritoneal B cells (105). From the above knowledge, we may suggest that microbiota-induced IL-25/IL-33 regulates ILC2s that interact with T and B cells to protect against CDI/rCDI. Additionally, commensals produce acetate by fermenting dietary fibers. This

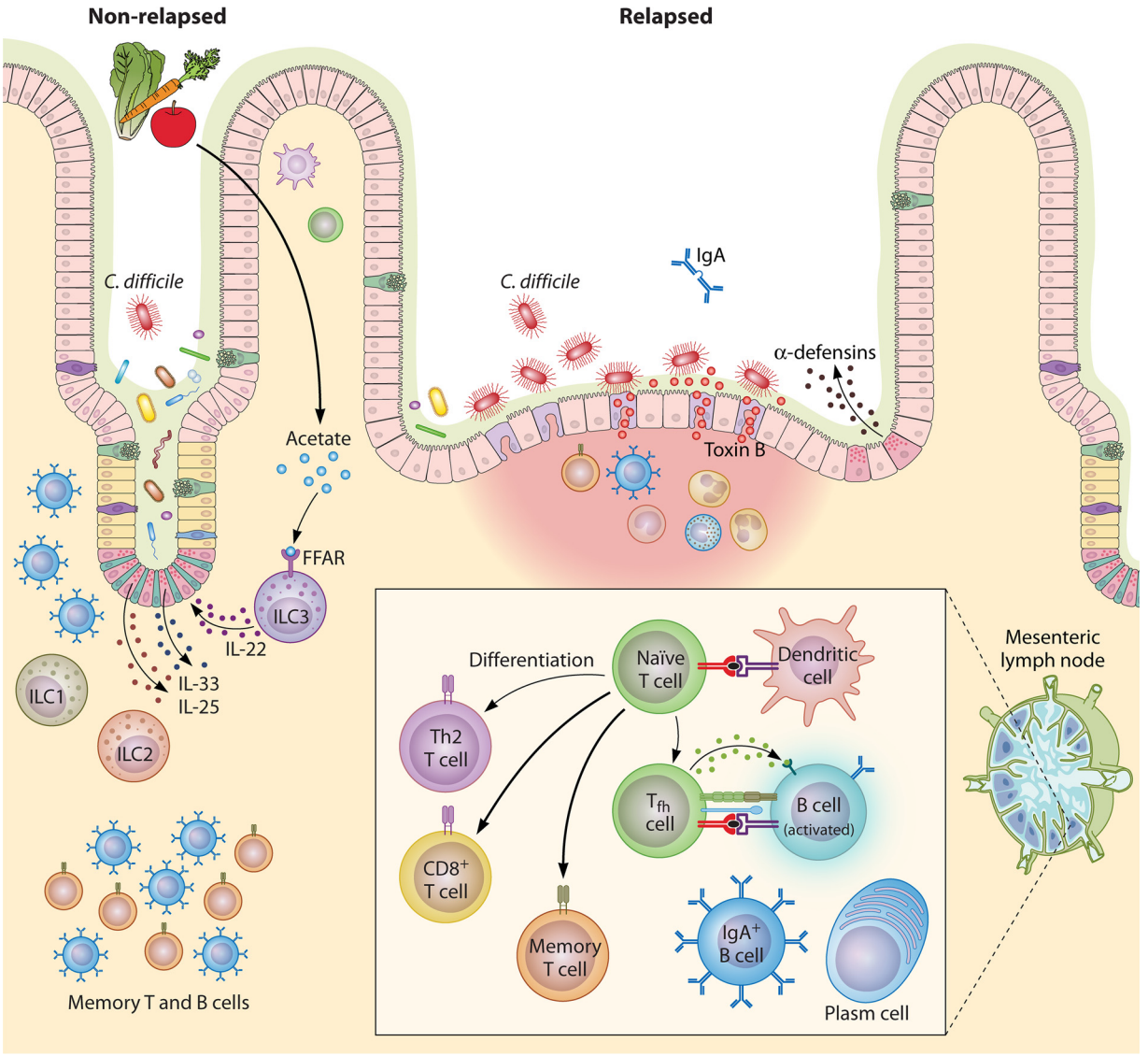

FIG 1 The gut microbiota orchestrates an immune circuit necessary for Tfh and Bmem cell function to generate protective adaptive immunity during CDI. (Left) With nonrecurrent cases, gut microbiota and epithelial cell-derived IL-33 and IL-25 induce Th2 responses and innate lymphoid cells type 2 (ILC2s). ILC2s interact with T cells via MHC-II, CCL5, PD-1/PD-L1, OX-40/OX-40L, CD80, and CD86. ILC2s interact with B cells via ICOS/ICOSL and IL-5, resulting in B cell activation, isotype switching, survival, self-renewal, and antibody secretion. Tfh and Bmem cells collaborate in the mesenteric lymph nodes, homing to the site of infection, and produce the anti-TcdB neutralizing antibody needed to contain CDI. In mesenteric lymph nodes or secondary lymphoid tissues, Tfh cells interact with B cells and favor the development of plasma cells. Afterward, these plasma cells reach the colonic lamina propria where they are able to produce and release large amounts of anti-TcdB neutralizing antibody leading to disease resolution. In the absence of appropriate gut microbiota, adaptive immune cells fail to respond to infection to generate sufficient immunity for a recall response during recurrent disease. (Right) (Relapsed) patients with recurrence episodes have a deficient memory B cell response. The reduction of these cells along with the failure in the production of IgA, IgG, and IgM Abs allows *C. difficile* to replicate and induce epithelial damage. Also, the microbiota produces acetate by fermenting dietary fibers that activate FFAR2 receptor signaling present on ILC3s. ILC3s promote T cell-dependent IgA production by regulating T cell homing to the gut and may help in epithelial healing and protection from *C. difficile* through the release of IL-22.

acetate activates Free Fatty Acid Receptor 2 (FFAR2) receptor signaling present on ILC3s, which helps in epithelial healing and CDI protection through the release of IL-22 (56, 57, 84) (Fig. 1).

Role of IL-33 and IL-25 in the Innate-Adaptive Immune Cross Talk

IL-33 signaling in CDI was found to be important in both mice and humans. The data from our lab showed in a *C. difficile* mice model the balance between type 17 and type 2-associated immunity governed by IL-33, which reduces neutrophils and increases eosinophils (57). To develop a mouse model of CDI that closely represents

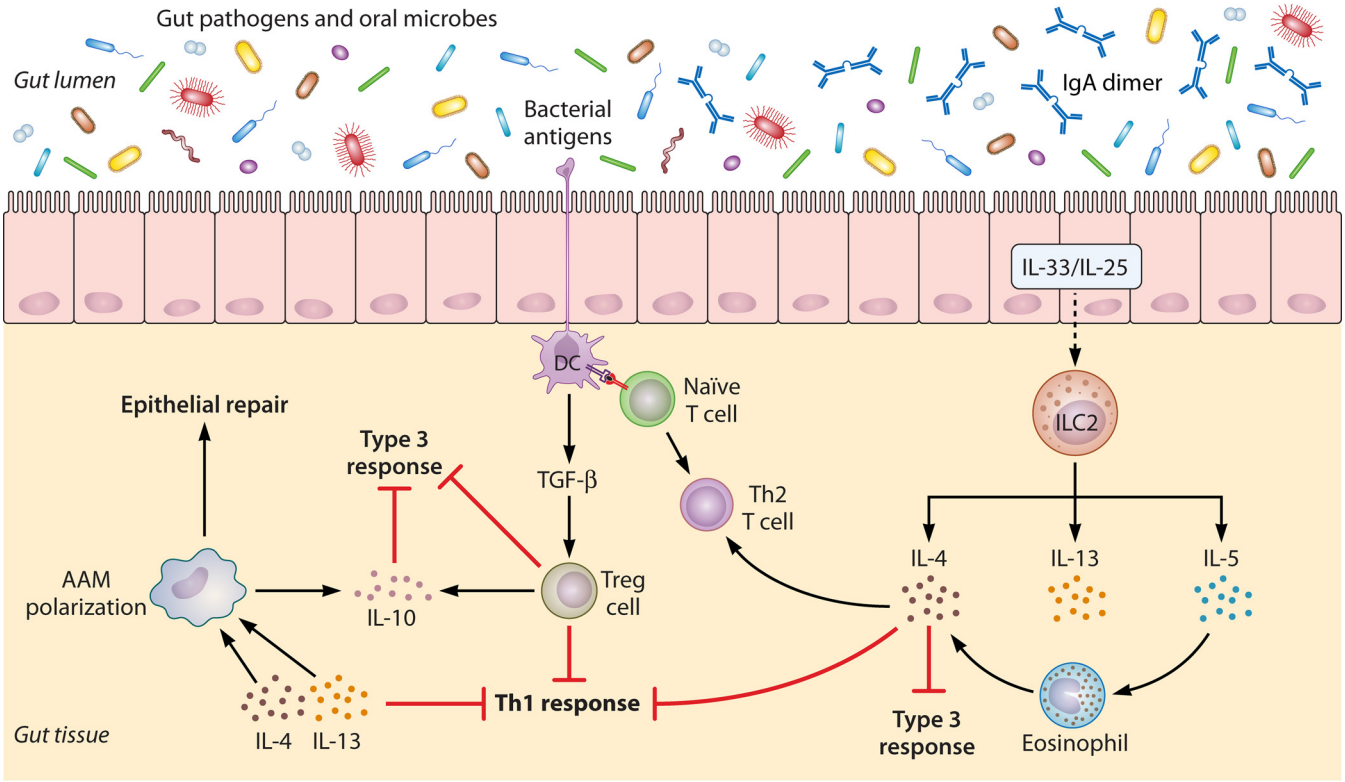

FIG 2 Microbiota-driven gut homeostasis. Commensal antigens induce tolerogenic DCs to produce TGF-β and retinoic acid, which contributes to Treg cell differentiation. Treg cells inhibit Th1 and Th17 responses to maintain gut homeostasis. On the other hand, activation of commensal-induced ILC2 produces IL-4, IL-5, and IL-13 and also activated the number of eosinophils that release IL-4, skewing the Th17 and Th1 to Th2 response that mediates protection. Furthermore, IL-13 and IL-4 induce alternatively activated macrophages that help in epithelial repair and produce IL-10. IL-10 further inhibits type 3 responses and maintains gut homeostasis.

the human disease, a mixture of antibiotics in water (gentamicin, colistin, metronidazole, and vancomycin) is given to C57BL6 mice for 3 days. Furthermore, clindamycin injections are given after 2 days of antibiotics treatment and 1 day before the *C. difficile* challenge. A higher IL-33 level has been detected in human colonic biopsy specimens and serum higher soluble IL-33 decoy receptor (sST2) was detected in human serum samples in severe CDI patients (57). The decoy receptor neutralizes IL-33. IL-33-mediated activation of ILC2s was found to improve $CD8^+$ T-cell-mediated tumor immunity (119). On the other hand, less mortality and less tissue pathology have been observed in the case of IL-25 irrespective of bacterial burden or toxin production (56). IL-25 was also found to maintain the balance between type 2 and type 17 through IL-4-producing eosinophils (56). Data from our lab on a IL-5-treated *C. difficile* mice model showed protection, whereas IL-13- and IL-4-treated mice showed faster recovery from CDI (120). Taking these data together, we can postulate that IL-33 and IL-25 induce ILC2s that interact with T cells and B cells to induce adaptive immune responses (Fig. 2). Additionally, microbiota utilizes dietary fibers to produce acetate, which activates ILC3s through FFAR receptor signaling. ILC3s can also induce T and B cells and help in mounting adaptive responses. In the recurrence model (Fig. 2, right), antibiotics deplete microbiota, and hence, no ILC activation leads to less effective innate and adaptive responses. Nevertheless, the question is whether ILCs interact with Tfh and Bmem cells to mount a stable immune response. Essentially, external injections of IL-25/IL-33 may be helpful to expand the Tfh cell compartment leading to the expansion in the Bmem cell compartment leading to the production of class-switched IgG responses that can neutralize toxins and cure CDI and rCDI. Leveraging CDI patient samples of recurrent and nonrecurrent to deeply study Tfh and Bmem cells is warranted. In addition, these induced ILC2s also produce IL-5, IL-4, and IL-13. The IL-5-derived eosinophils help with disease protection,

whereas IL-4 and IL-13 help in recovery from CDI. IL-4 also inhibits the type 3 immune response, and IL-13 induces alternatively activated macrophages that help in epithelial recovery and inhibit type 3 immune response (Fig. 2).

ADAPTIVE IMMUNE RESPONSE TO *Clostridioides difficile*

Rag1$^{-/-}$ mice that lack T and B cells did not show any difference in recovery from the acute phase of CDI but did show high CDI-associated mortality rates (83, 121). Resolution of the acute phase may depend on the innate immune response, but for rCDI, the inability to mount an effective adaptive immune response may also contribute (122). Interestingly, mice that lack CD4 showed protection from CDI and rCDI and were able to produce toxin-specific mucosal and serum IgA antibodies (123), suggesting CD4-independent antibody production in CDI. In contrast, mice, which lack MHC class II molecules, were more susceptible to CDI and rCDI and were not able to produce toxin-specific serum or mucosal antibodies (123).

Immunoglobulin A (IgA), IgG, and IgM are the main antibodies involved in protection, but in CDI, systemic IgG plays an important role in governing clinical outcomes (33). The higher the IgG against toxin B, the lower will be the disease severity and lower the chances of disease recurrence (2, 124). Similarly, monoclonal antibodies against TcdB used in the treatment of CDI are more effective than the monoclonal antibodies against TcdA, suggesting TcdB is more toxic than TcdA. CDI patients with no or low circulating TcdB-specific IgG were found to have multiple episodes of CDI (33, 125). However, it is worth noting that higher antibodies against TcdA or TcdB could protect against the disease and recurrence, but they did not prevent colonization by *C. difficile*.

Amani et al. (32) in rCDI murine models showed that the primary infection of VPI 10463 *C. difficile* had not induced an isotype-switched IgG response and during the second infection had not recalled IgA or IgG responses. Furthermore, it was demonstrated that toxinB-specific IgM and mucosal IgA did not protect against rCDI, but the serum IgG response was protective. In contrast, the generation of toxin-specific serum IgG, IgA, and mucosal IgA protected mice in the *C. difficile* model, while mucosal IgA was capable but not required for disease protection as the mice (pIgR$^{-/-}$), which lack the receptor to transcytose polymeric Ab across the epithelium, were protected from *C. difficile*-associated diseases (123). This finding suggests that serum IgG is the most important toxin-specific antibody that protects CDI and rCDI and that IgA may be capable of protecting rCDI but is not essential for disease protection. The plausible explanation for the contrasting results in the production of antibodies may be because of the difference in the mouse microbiota.

INVOLVEMENT OF CD4$^+$ T CELLS INCLUDING Tfh IN DISEASE RECURRENCE

Germinal center follicular helper (GC Tfh) cells govern the differentiation of activated B cells into memory cells or plasma cells (126). They also have an important role in inducing long-lasting and strong immunity. Albeit, nongerminal center Tfh cells present in the follicle or extrafollicular regions may also help in early B cell responses (127). In CDI, data showed that immunization with toxin B could produce toxin-specific memory, long-lived plasma, and plasma cells that can produce toxin-specific antibodies and are found to protect against disease. Upon infection, toxin-specific and nontoxin antibody responses were low, and the initial infection failed to expand the Tfh cell compartment enough to induce isotype-switched toxin-specific antibody responses compared with the expansion seen by the immunization with the C-terminal domain of the toxin. Further expansion of the memory B cell compartment also failed to mount a protective adaptive immune response due to lower Tfh cell expansion (32).

Both toxin A and toxin B can decrease the motility of human T cells (128), which is correlated with a reduction in the Tfh and Bmem cell compartment expansion (32). In accordance, CDI patients could be distinguished from healthy individuals by the T cell responses and not the antibody responses, as the frequency of TcdB-specific CD4$^+$ cells increased with repeated or severe CDI (129). In addition, disease severity in rCDI is

correlated directly with T cell responses, with effective T cell immunity depending on the development of the Th17 cell (129). In summary, it is suggested that in the generation of the protective immune response, Tfh cells play a crucial role. rCDI may occur in the absence of efficient T and memory B cell responses.

There are many conflicts found in different studies related to $CD4^+$ cell responses in CDI. One study showed that the ratio of Th1 to Th17 and Th1 to Th2 shifted in severe patients (130); in contrast, higher Th1/Th2 and Th1/Th17 ratios were associated with disease severity (131). Another study on CDI described the pathogenic role of Th17 cells (132). These functional dichotomies of Th17 response could be due to the timing of the blood collection of infected individuals and influenced by the progression of the disease. Hence, more studies are required to further elucidate the protective versus pathogenic effects of Th17 cells in CDI and rCDI.

Along with the role of Tfh, Th1, Th2, and Th17 cells in CDI, mucosal-associated invariant T (MAIT) cells, T regulatory (Treg), and $\gamma\delta$T cells are also shown to have a role in CDI. MAIT cells having antibacterial properties are present in lamina propria representing 10% of total T cells (133). MAIT cells are activated by *C. difficile* and do produce IFN-γ, perforins, and granzyme B. $\gamma\delta$T cells were found to protect from CDI by producing IL-17A/F in the mouse model and found to have a role in neonatal resistance to CDI (134). Treg cells likely have a critical role in maintaining intestinal homeostasis by checking innate and adaptive immune responses (135). In the case of CDI, there is not much information describing the role of Treg cells; however, the deletion of Treg cells from mice resulted in severe intestinal inflammation following CDI. Furthermore, these mice also failed to resolve infection even after FMT engraftment, suggesting that the Treg cells population is critical for FMT response (136). Immunoprofiling of peripheral blood mononuclear cell (PBMCs) of rCDI-infected patients showed a higher $CD3^+$ $CD4^+$ $Foxp3^+$ Treg cells population (137); however, due to the small number of test subjects in the study, it was hard to draw firm conclusions, and more studies are required with a larger cohort. Further research on $CD8^+$ T cell contributions to bacterial killing and clearance are also warranted. Additionally, more studies are needed to see if inhibition in the cellular mechanism of B and T cells is responsible for the failed humoral response.

ROLE OF B CELLS IN ANTIBODY PRODUCTION (IgG, IgA) AND MEMORY B CELLS IN DISEASE RECURRENCE

In CDI, the B cell response against toxins and nontoxin antigens likely regulates the disease outcome to a great extent. There are many research studies describing the importance of toxin B in disease pathology, which is already discussed in the Introduction. Targeting TcdB may play a critical role in protection against rCDI (30). However, for complete protection from rCDI, enough Bmem cell expansion, longevity, induction, and reactivation are also required. Albeit, immunization with the C-terminal domain of toxin B in mice showed protection against infection with *C. difficile* (55, 138). But this protection is due to immunization-induced plasma cell-secreted antibody and ongoing plasma cell response not because of the memory cells (32). However, this immunization could not expand enough memory B cell compartments due to failed Tfh expansion (32). Thus, an effective vaccine is required that can expand enough Tfh cells to induce B cells for protection against infection. Moreover, toxin immunization does not clear colonization and hence can spread disease. Along with this information, with age, the affinity of antibodies and survival of plasma cells decrease due to inadequate germinal center responses (139). In this case, booster vaccination is required to maintain long-lived plasma cell responses to protect from initial or rCDI as Bmem cell compartment expansion may not be sufficient in old age (139). So far, all the work related to rCDI is done by rechallenging with the same strain, although in the real world relapse and reinfection with the same or different strains occur (140). There is a need to replicate the relapse study on mice to see if the expansion of the Bmem cell and Tfh cell compartment differ with a different rechallenge, for example with the hypervirulent

B1/NAPI.027. Of note, a cystic fibrosis patient with previous CDI had enhanced and stable antibodies and circulating memory B cells (125). This result suggests that the increased intestinal permeability in cystic fibrosis enabled a better induction of B cells or Tfh cell expansion. In correlation with this suggestion, it was shown as shown that NAPI.027/B1 induces systemic anti-TcdB IgG and a non-027 strain induced a more prominent anti-TcdA IgA mucosal response (141). One must not ignore that asymptomatic CDI patients have higher toxin-specific antibodies (142). The severity of the disease is inversely correlated with toxin-specific systemic IgG and mucosal IgA (142). Studies on patients revealed that those with higher antitoxin serum IgG had less rCDI (143). In rCDI patients, anti-TcdB-Bmem cells did not undergo isotype switching (30). Furthermore, the role of serum IgG in rCDI prevention was demonstrated (30). Further work is required on recurrent versus nonrecurrent patients to compare the Bmem repertoire to provide critical insights into their functions. It is also important to note that CDI can be prevented by colonizing the hamster model with nontoxigenic strains of *C. difficile* (144). This result suggests that the host can respond better when the toxin is inactive or absent (32).

THERAPEUTIC APPROACH TO *C. difficile* INFECTION

The current approved treatments for CDI are the antibiotics metronidazole, vancomycin, and fidaxomicin (145). The first-line drug is fidaxomicin (146). Metronidazole was found to be inferior to vancomycin in clinical trials (147). Fidaxomicin is used as an alternative drug to metronidazole and vancomycin as its treatment gives a lower recurrence rate of *C. difficle* in patients (148). The recurrence rate of fidaxomicin is 15% and that of vancomycin is 25% approximately (145). The major drawback of these antibiotics, especially metronidazole and vancomycin, is they kill a good portion of indigenous flora along with *C. difficile*, but the narrower antimicrobial spectrum of fidaxomicin leads to less dysbiosis (148). For the treatment of rCDI, an oral microbiome therapy, SER-109, has come into the picture most recently (discussed in section "*C. difficile* Infection and the Microbiome" too). Essentially, SER-109 is made up of firmicutes spores which are given orally after finishing the standard-of-care of antibiotics to cure disease recurrence (81). Some newer and untested approaches to combat CDI are available, including the use of nanotechnology. For instance, gold nanoparticles showed great importance in targeting the entire spore coat, denaturing the surface protein, and forming holes (149). The gold nanoclusters can effectively kill *C. difficile in vitro*, but its role in CDI *in vivo* is still not clear. Since it is not toxic to humans (150), a clinical trial could be anticipated to check the efficacy of Ag or gold nanoparticles against CDI in humans.

ACTIVE AND PASSIVE IMMUNIZATION STATUS

To induce in an individual to produce antitoxin when exposed to CDI, active immunization can be used (e.g., a vaccine). Passive immunization is to give neutralizing antitoxin antibodies to the patient directly. Passive immunization or other therapeutic approaches are used to prevent rCDI. The U.S. Food and Drug Administration (FDA) approved two human monoclonal antibodies, namely, one against toxin A (actoxumab; MK-3415/GS-CDA1/CDA1) and the other against toxin B (bezlotoxumab; MK-6072/MDX-1388/CDB1) (52). Bezlotoxumab showed approximately 26% more protection against rCDI than actoxumab (151). Vaccines against *C. difficile* that failed in clinical trials are by Sanofi and Pfizer. Also, vaccines by Valneva (NCT01296386) and Shire (NCT01259726) are still in clinical trials (Fig. 3). Vaccines prepared by Sanofi contain formalin-inactivated preparations of toxin A and toxin B that were purified from the VPI 10463 strain of *C. difficile* mixed with alum adjuvant. The final immunization was 100 μg total antigen given on days 0, 7, and 30 (152). However, prevention of the initial infection failed to result in the halting of this vaccine program at phase III trials (153). Pfizer vaccines had genetically and chemically detoxified TcdA and TcdB with alum adjuvant (154). Pfizer vaccines was terminated recently at phase III trials. Valneva vaccine has a recombinant chimeric protein containing the C-terminal binding domain of both toxins (TcdA, 15 of 31 repeats; TcdB, 23 of 24 repeats) linked with 12 amino acids (155).

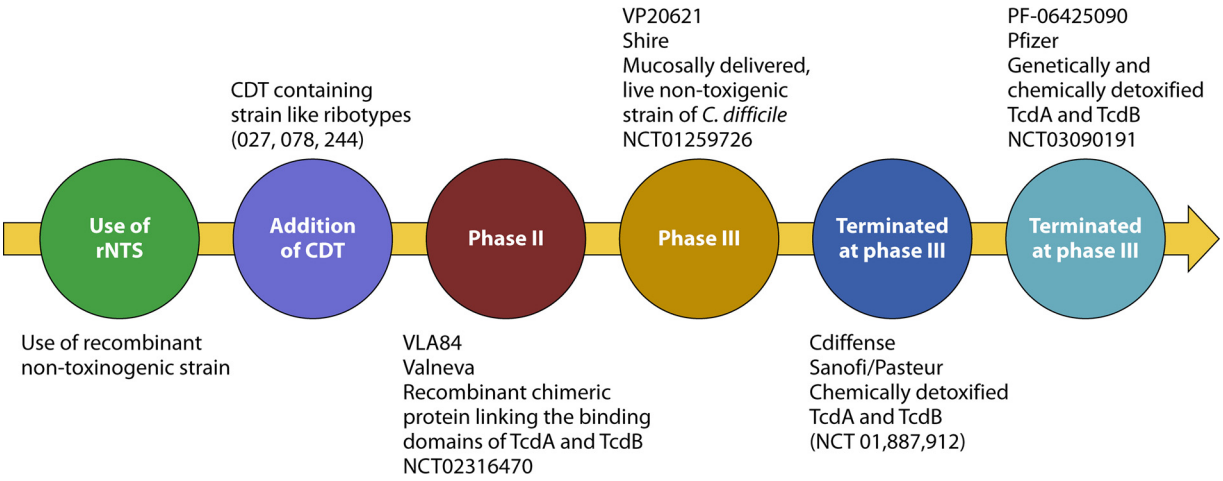

FIG 3 Past, present, and future of *C. difficile* vaccine in development. Two major phase III trials for *C. difficile* vaccines from Sanofi and Pfizer were determined futile and halted. The vaccines from Shire are in a phase III trial and one from Valneva was phase II terminated. Many vaccines have been tested at the murine model level by adding a third CDT toxin of *C. difficile*. Even nontoxinogenic strains with a recombinant vector containing domains of toxin A and toxin B were tested as potent vaccine candidates.

This vaccine had some drawbacks as it lacked several neutralizing epitopes like the epitopes present in the glucosyltransferase domain and binding regions present on TcdB (88, 156). This vaccine may not be effective against different clinical isolates as different TcdB subtypes may have amino acid substitution in the binding domain (157, 158). Although, it is not clear if the substitution has some role in neutralizing or not. The Shire vaccine has a live nontoxin strain of *C. difficile* that is delivered mucosally (159). Albeit, there are several studies where a plasmid expressing the receptor-binding domain (RBD) of both TcdA and TcdB was tested in cells and animal models and was shown to induce a B cell response and neutralizing antibody formation (160, 161), but previously, we have already discussed how domain immunization does not expand Tfh cell expansion. Details of the roadmap of vaccine strategies are illustrated in Fig. 3.

Along with the toxin-based active immunization studies, many studies also demonstrated nontoxin immunizations that can help to prevent colonization, which cannot be achieved by using toxin-based vaccines. Several spore core proteins like CotA, CotE, CdeC, and CdeH have been targeted (162) for the vaccine. Moreover, polysaccharides PSI, PSII, and PSIII have also been identified as potent vaccine candidates (163). Furthermore, Flagellin FliC is also described as a potent candidate (164). Many other antigens like SlpA, Cwp66, and Cwp84 have been tested preclinically (165) and showed immunogenicity but failed to protect against colonization or CDI (164, 165). One more direction for *C. difficile* vaccination is to make a genetically modified nontoxic *C. difficile* strain with a chimeric protein that has a glucosyltransferese domain, a cystein protease domain, and the receptor binding domain of both TcdA and TcdB (166–168). This modified bacterial vaccine was found to protect from infection and colonization. Nevertheless, a potential problem with this kind of vaccine is the possibility of genetic recombination with exogenous *C. difficile*. Another approach taken has been to express TcdA as an antigen in *Bacillus subtilis* to generate a mucosal toxin A-specific antibody that cross-reacted with the coat of *C. difficile* spores and the cell surface of vegetative cells (169).

To summarize, an effective vaccine against CDI must have both toxin-neutralizing capacities and protecting colonization abilities. It will be important that the vaccine induce enough B cell expansion to get the class switched, somatic hypermutated, clonally selected antibodies that should neutralize toxins.

AN OBSTACLE IN THE GENERATION OF VACCINES; KNOWLEDGE GAP

An ideal vaccine should protect against primary infection, intestinal colonization, and toxicity from secreted toxins; must be able to work against different pathogenic

strains; should be able to protect the aged individual; should be long-lived by the T cell and Bmem cell response; provide rapid activation of cellular and humoral response; and possess the therapeutic potential to prevent rCDI. Upon infection, vaccines should be able to provide immediate protection through toxin-specific antibodies and should be able to stimulate Bmem and Tfh cells to make Ab-secreting plasma cells. Achieving this goal is still warranted. The obstacle is selecting the appropriate antigen for the vaccine to get a proper host immune response and the selection of appropriate vaccine adjuvants, targeting aging populations with waning immunity and comorbidities. Also, knowing the role of the microbiome in host immunity, the role of host immune response toward bacteria, and the role of systemic and mucosal immunity in CDI will give us a guide for developing an ideal vaccine.

Many antigens have been screened until now, namely, tcdA, tcdB, peptidoglycans, cell wall proteins, polysaccharides, and even complete nonpathogenic *C. difficile* strains, to develop a vaccine. All of them were found to be potent antigens (170). Among them, the vaccines based on the toxin antigens TcdA and TcdB were found to be more potent (170). Moreover, TcdB is the most potent antigen candidate as it is responsible for the pathology in animal models and less severe disease was found to correlate with high TcdB-specific serum antibodies in CDI patients (33, 54). Additionally, in clinical trials, reduction in rCDI was shown by using a monoclonal antibody against TcdB (52). However, we cannot ignore the colonization of bacteria. Hence, toxin B plus a colonizing factor with an appropriate adjuvant might be the best vaccine candidate to cure CDI and rCDI. An appropriate route of vaccine administration is of concern to maximize the immune response.

The other most important point is the existence of different isotypes of toxin B. Until now, 8 to 12 subtypes of toxin B have been identified (44, 47). The hypervirulent strain 027 has a different toxin B, namely, TcdB2, than the classical strain TcdB1 with 92% similarity. A TcdB1-specific antibody failed to neutralize TcdB2 (171). In contrast, both toxin B1 and B2 produce similar antibody subclass profiles (55), but long-term Bmem cell response, neutralization, and resistance to *in vivo* toxin challenge were found to be superior for TcdB1 (55). The 027 ribotype produces a third toxin CDT, and concerning this topic, Secore et al. (172) showed that a tetravalent vaccine, taking TcdA, TcdB, and both CDTa and CDTb, provided better protection than a bivalent vaccine (TcdA and TcdB).

Screening and selecting adjuvants are also essential ingredients of a vaccine. Alum was used in both the failed clinical trials by Pfizer and Sanofi in the *C. difficile* vaccine. Alum can provide good humoral immunity but may fail to stimulate enough cellular immunity (173). In addition, the main knowledge gap from the previous clinical trials on failed vaccines is the lack of knowledge of mucosal IgA, the role of Tfh in local and systemic adaptive immune response, and the impact of the gut microbiome in shaping the vaccine-mediated response. In this regard, it is suggested that new generation vaccines against *C. difficile* may fill up these shortcomings.

CONCLUSIONS

In this review, we provided critical insight into the factors that influence CDI severity. While there is a growing understanding that the host immune response may play an important role in skewing the balance during the infection, other factors, such as the host microbiome and *C. difficile* toxins, can further determine the degree of susceptibility of the host. The role of IL-33 and IL-25 in shaping type 2 immunity and protecting from CDI is an additional key factor. Toxin B, the major virulence factor of *C. difficile*, and its association with the disease were also described. We also defined the role of microbiota in shaping host immunity in CDI. The current status of the *C. difficile* vaccine and the obstacle in its generation was debated. We conclude that recurrent *C. difficile* infection (CDI) is due to a microbiota-induced failure of innate priming of the acquired immune response, leading to failure to protect from colonization and intoxication.

The contribution of CD8 T cell responses in *C. difficile* killing and clearance is poorly understood. In addition, very fewer studies have focused on the role of Tfh cells that

are essential for the Bmem cells and antibody-producing plasma cell response. Hence, emphasis on studying T cell differentiation and activation against *C. difficile* is required. In this regard, a single-cell analysis of Tfh and Bmem cells may offer critical insights for developing an ideal vaccine for CDI and rCDI. Hence, more studies are needed to understand the cross talk and interplay between microbiota, host immune system, *C. difficile*, and disease outcome. Taken together, understanding both the adaptive and humoral immune response to *C. difficile* is needed to develop an efficacious vaccine. We believe our review will help researchers understand the key findings in the *C. difficile* field in recent years and point out knowledge gaps.

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REFERENCES

1. Guh AY, Mu Y, Winston LG, Johnston H, Olson D, Farley MM, Wilson LE, Holzbauer SM, Phipps EC, Dumyati GK, Beldavs ZG, Kainer MA, Karlsson M, Gerding DN, McDonald LC, Infection CD. Emerging Infections Program Clostridioides difficile Infection Working Group. 2020. Trends in US burden of Clostridioides difficile infection and outcomes. N Engl J Med 382:1320–1330. https://doi.org/10.1056/NEJMoa1910215.
2. De Roo AC, Regenbogen SE. 2020. Clostridium difficile infection: an epidemiology update. Clin Colon Rectal Surg 33:49–57. https://doi.org/10.1055/s-0040-1701229.
3. Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, Farley MM, Holzbauer SM, Meek JI, Phipps EC, Wilson LE, Winston LG, Cohen JA, Limbago BM, Fridkin SK, Gerding DN, McDonald LC. 2015. Burden of Clostridium difficile infection in the United States. N Engl J Med 372:825–834. https://doi.org/10.1056/NEJMoa1408913.
4. Johnson S, Gerding DN. 1998. Clostridium difficile-associated diarrhea. Clin Infect Dis 26:1027–1034. https://doi.org/10.1086/520276.
5. Mitty RD, LaMont JT. 1994. Clostridium difficile diarrhea: pathogenesis, epidemiology, and treatment. Gastroenterologist 2:61–69.
6. Dulęba K, Pawłowska M, Wietlicka-Piszcz M. 2014. Clostridium difficile infection in children hospitalized due to diarrhea. Eur J Clin Microbiol Infect Dis 33:201–209. https://doi.org/10.1007/s10096-013-1946-1.
7. Marra AR, Perencevich EN, Nelson RE, Samore M, Khader K, Chiang HY, Chorazy ML, Herwaldt LA, Diekema DJ, Kuxhausen MF, Blevins A, Ward MA, McDanel JS, Nair R, Balkenende E, Schweizer ML. 2020. Incidence and outcomes associated with Clostridium difficile infections: a systematic review and meta-analysis. JAMA Netw Open 3:e1917597. https://doi.org/10.1001/jamanetworkopen.2019.17597.
8. Lessa FC, Gould CV, McDonald LC. 2012. Current status of Clostridium difficile infection epidemiology. Clin Infect Dis 55:S65–S70. https://doi.org/10.1093/cid/cis319.
9. Wiegand PN, Nathwani D, Wilcox MH, Stephens J, Shelbaya A, Haider S. 2012. Clinical and economic burden of Clostridium difficile infection in Europe: a systematic review of healthcare-facility-acquired infection. J Hosp Infect 81:1–14. https://doi.org/10.1016/j.jhin.2012.02.004.
10. Loo VG, Bourgault AM, Poirier L, Lamothe F, Michaud S, Turgeon N, Toye B, Beaudoin A, Frost EH, Gilca R, Brassard P, Dendukuri N, Beliveau C, Oughton M, Brukner I, Dascal A. 2011. Host and pathogen factors for Clostridium difficile infection and colonization. N Engl J Med 365:1693–1703. https://doi.org/10.1056/NEJMoa1012413.
11. Kelly CP, Pothoulakis C, Lamont JT. 1994. Clostridium difficile colitis. N Engl J Med 330:257–262. https://doi.org/10.1056/NEJM199401273300406.
12. Brown KA, Khanafer N, Daneman N, Fisman DN. 2013. Meta-analysis of antibiotics and the risk of community-associated Clostridium difficile infection. Antimicrob Agents Chemother 57:2326–2332. https://doi.org/10.1128/AAC.02176-12.
13. Dial S, Kezouh A, Dascal A, Barkun A, Suissa S. 2008. Patterns of antibiotic use and risk of hospital admission because of Clostridium difficile infection. CMAJ 179:767–772. https://doi.org/10.1503/cmaj.071812.
14. VanInsberghe D, Elsherbini JA, Varian B, Poutahidis T, Erdman S, Polz MF. 2020. Diarrhoeal events can trigger long-term Clostridium difficile colonization with recurrent blooms. Nat Microbiol 5:642–650. https://doi.org/10.1038/s41564-020-0668-2.
15. Crobach MJT, Vernon JJ, Loo VG, Kong LY, Pechine S, Wilcox MH, Kuijper EJ. 2018. Understanding Clostridium difficile colonization. Clin Microbiol Rev 31:e00021-17. https://doi.org/10.1128/CMR.00021-17.
16. Smits WK, Lyras D, Lacy DB, Wilcox MH, Kuijper EJ. 2016. Clostridium difficile infection. Nat Rev Dis Primers 2:16020. https://doi.org/10.1038/nrdp.2016.20.
17. Zhu D, Sorg JA, Sun X. 2018. Clostridioides difficile biology: sporulation, germination, and corresponding therapies for C. difficile infection. Front Cell Infect Microbiol 8:29. https://doi.org/10.3389/fcimb.2018.00029.
18. Knoop FC, Owens M, Crocker IC. 1993. Clostridium difficile: clinical disease and diagnosis. Clin Microbiol Rev 6:251–265. https://doi.org/10.1128/CMR.6.3.251.
19. Aktories K, Schwan C, Jank T. 2017. Clostridium difficile toxin biology. Annu Rev Microbiol 71:281–307. https://doi.org/10.1146/annurev-micro-090816-093458.
20. Cowardin CA, Buonomo EL, Saleh MM, Wilson MG, Burgess SL, Kuehne SA, Schwan C, Eichhoff AM, Koch-Nolte F, Lyras D, Aktories K, Minton NP, Petri WA. 2016. The binary toxin CDT enhances Clostridium difficile virulence by suppressing protective colonic eosinophilia. Nat Microbiol 1:16108. https://doi.org/10.1038/nmicrobiol.2016.108.
21. Fletcher JR, Pike CM, Parsons RJ, Rivera AJ, Foley MH, McLaren MR, Montgomery SA, Theriot CM. 2021. Clostridioides difficile exploits toxin-mediated inflammation to alter the host nutritional landscape and exclude competitors from the gut microbiota. Nat Commun 12:462. https://doi.org/10.1038/s41467-020-20746-4.
22. Pruss KM, Sonnenburg JL. 2021. C. difficile exploits a host metabolite produced during toxin-mediated disease. Nature 593:261–265. https://doi.org/10.1038/s41586-021-03502-6.
23. Xu H, Yang JL, Gao WQ, Li L, Li P, Zhang L, Gong YN, Peng XL, Xi JZJ, Chen S, Wang FC, Shao F. 2014. Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. Nature 513:237–241. https://doi.org/10.1038/nature13449.
24. Jose S, Madan R. 2016. Neutrophil-mediated inflammation in the pathogenesis of Clostridium difficile infections. Anaerobe 41:85–90. https://doi.org/10.1016/j.anaerobe.2016.04.001.
25. Meyer GKA, Neetz A, Brandes G, Tsikas D, Butterfield JH, Just I, Gerhard R. 2007. Clostridium difficile toxins A and B directly stimulate human mast cells. Infect Immun 75:3868–3876. https://doi.org/10.1128/IAI.00195-07.
26. Leffler DA, Lamont JT. 2015. Clostridium difficile infection. N Engl J Med 373:287–288. https://doi.org/10.1056/NEJMc1506004.
27. Johal SS, Lambert CP, Hammond J, James PD, Borriello SP, Mahida YR. 2004. Colonic IgA producing cells and macrophages are reduced in recurrent and non-recurrent Clostridium difficile associated diarrhoea. J Clin Pathol 57:973–979. https://doi.org/10.1136/jcp.2003.015875.
28. Singh T, Bedi P, Bumrah K, Singh J, Rai M, Seelam S. 2019. Updates in treatment of recurrent Clostridium difficile infection. J Clin Med Res 11:465–471. https://doi.org/10.14740/jocmr3854.

29. Pizarro-Guajardo M, Chamorro-Veloso N, Vidal RM, Paredes-Sabja D. 2019. New insights for vaccine development against Clostridium difficile infections. Anaerobe 58:73–79. https://doi.org/10.1016/j.anaerobe.2019.04.009.
30. Shah HB, Smith K, Scott EJ, Larabee JL, James JA, Ballard JD, Lang ML. 2020. Human C. difficile toxin-specific memory B cell repertoires encode poorly neutralizing antibodies. JCI Insight 5:e138137. https://doi.org/10.1172/jci.insight.138137.
31. Del Pino REH, Barbero AM, Espanol LA, Morro LS, Pasquinelli V. 2021. The adaptive immune response to Clostridioides difficile: a tricky balance between immunoprotection and immunopathogenesis. J Leukoc Biol 109:195–210. https://doi.org/10.1002/JLB.4VMR0720-201R.
32. Amani SA, Shadid T, Ballard JD, Lang ML. 2020. Clostridioides difficile infection induces an inferior IgG response to that induced by immunization and is associated with a lack of T follicular helper cell and memory B cell expansion. Infect Immun 88:e00829-19. https://doi.org/10.1128/IAI.00829-19.
33. Leav BA, Blair B, Leney M, Knauber M, Reilly C, Lowy I, Gerding DN, Kelly CP, Katchar K, Baxter R, Ambrosino D, Molrine D. 2010. Serum anti-toxin B antibody correlates with protection from recurrent Clostridium difficile infection (CDI). Vaccine 28:965–969. https://doi.org/10.1016/j.vaccine.2009.10.144.
34. Haddad NS, Nozick S, Kim G, Ohanian S, Kraft CS, Rebolledo PA, Wang Y, Wu H, Bressler A, Le SNT, Kuruvilla M, Runnstrom MC, Ramonell RP, Cannon LE, Lee FE, Daiss JL. 2022. Detection of newly secreted antibodies predicts nonrecurrence in primary Clostridioides difficile infection. J Clin Microbiol 60:e0220121. https://doi.org/10.1128/jcm.02201-21.
35. Ghose C, Kelly CP. 2015. The prospect for vaccines to prevent Clostridium difficile infection. Infect Dis Clin North Am 29:145–162. https://doi.org/10.1016/j.idc.2014.11.013.
36. Papatheodorou P, Barth H, Minton N, Aktories K. 2018. Cellular uptake and mode-of-action of Clostridium difficile toxins. Adv Exp Med Biol 1050:77–96. https://doi.org/10.1007/978-3-319-72799-8_6.
37. Lyerly DM, Krivan HC, Wilkins TD. 1988. Clostridium difficile: its disease and toxins. Clin Microbiol Rev 1:1–18. https://doi.org/10.1128/CMR.1.1.1.
38. Burnham CA, Carroll KC. 2013. Diagnosis of Clostridium difficile infection: an ongoing conundrum for clinicians and for clinical laboratories. Clin Microbiol Rev 26:604–630. https://doi.org/10.1128/CMR.00016-13.
39. Knight DR, Elliott B, Chang BJ, Perkins TT, Riley TV. 2015. Diversity and evolution in the genome of Clostridium difficile. Clin Microbiol Rev 28:721–741. https://doi.org/10.1128/CMR.00127-14.
40. Kuehne SA, Cartman ST, Heap JT, Kelly ML, Cockayne A, Minton NP. 2010. The role of toxin A and toxin B in Clostridium difficile infection. Nature 467:711–713. https://doi.org/10.1038/nature09397.
41. Kuehne SA, Collery MM, Kelly ML, Cartman ST, Cockayne A, Minton NP. 2014. Importance of toxin A, toxin B, and CDT in virulence of an epidemic Clostridium difficile strain. J Infect Dis 209:83–86. https://doi.org/10.1093/infdis/jit426.
42. Freeman J, Bauer MP, Baines SD, Corver J, Fawley WN, Goorhuis B, Kuijper EJ, Wilcox MH. 2010. The changing epidemiology of Clostridium difficile infections. Clin Microbiol Rev 23:529–549. https://doi.org/10.1128/CMR.00082-09.
43. Voth DE, Ballard JD. 2005. Clostridium difficile toxins: mechanism of action and role in disease. Clin Microbiol Rev 18:247–263. https://doi.org/10.1128/CMR.18.2.247-263.2005.
44. Shen EH, Zhu KL, Li DY, Pan ZR, Luo Y, Bian Q, He LQ, Song XJ, Zhen Y, Jin DZ, Tao L. 2020. Subtyping analysis reveals new variants and accelerated evolution of Clostridioides difficile toxin B. Commun Biol 3:347. https://doi.org/10.1038/s42003-020-1078-y.
45. Stabler RA, Dawson LF, Phua LTH, Wren BW. 2008. Comparative analysis of BI/NAP1/027 hypervirulent strains reveals novel toxin B-encoding gene (tcdB) sequences. J Med Microbiol 57:771–775. https://doi.org/10.1099/jmm.0.47743-0.
46. Quesada-Gomez C, Lopez-Urena D, Chumbler N, Kroh HK, Castro-Pena C, Rodriguez C, Orozco-Aguilar J, Gonzalez-Camacho S, Rucavado A, Guzman-Verri C, Lawley TD, Lacy DB, Chaves-Olarte E. 2016. Analysis of TcdB proteins within the hypervirulent clade 2 reveals an impact of RhoA glucosylation on Clostridium difficile proinflammatory activities. Infect Immun 84:856–865. https://doi.org/10.1128/IAI.01291-15.
47. Luo JH, Yang Q, Zhang XF, Zhang YY, Wan L, Zhan XC, Zhou Y, He LQ, Li DY, Jin DZ, Zhen Y, Huang J, Li YY, Tao L. 2022. TFPI is a colonic crypt receptor for TcdB from hypervirulent clade 2 C. difficile. Cell 185:980–994.e15. https://doi.org/10.1016/j.cell.2022.02.010.
48. Pan ZR, Zhang YY, Luo JH, Li DY, Zhou Y, He LQ, Yang Q, Dong M, Tao L. 2021. Functional analyses of epidemic Clostridioides difficile toxin B variants reveal their divergence in utilizing receptors and inducing pathology. PLoS Pathog 17:e1009197. https://doi.org/10.1371/journal.ppat.1009197.
49. Bacci S, Molbak K, Kjeldsen MK, Olsen KE. 2011. Binary toxin and death after Clostridium difficile infection. Emerg Infect Dis 17:976–982. https://doi.org/10.3201/eid/1706.101483.
50. Young MK, Leslie JL, Madden GR, Lyerly DM, Carman RJ, Lyerly MW, Stewart DB, Abhyankar MM, Petri WA, Jr. 2022. Binary toxin expression by Clostridioides difficile is associated with worse disease. Open Forum Infect Dis 9:ofac001. https://doi.org/10.1093/ofid/ofac001.
51. Gerding DN, Johnson S. 2013. Does infection with specific Clostridium difficile strains or clades influence clinical outcome? Clin Infect Dis 56:1601–1603. https://doi.org/10.1093/cid/cit133.
52. Wilcox MH, Gerding DN, Poxton IR, Kelly C, Nathan R, Birch T, Cornely OA, Rahav G, Bouza E, Lee C, Jenkin G, Jensen W, Kim YS, Yoshida J, Gabryelski L, Pedley A, Eves K, Tipping R, Guris D, Kartsonis N, Dorr MB. Modify I and Modify II Investigators. 2017. Bezlotoxumab for prevention of recurrent Clostridium difficile infection. N Engl J Med 376:305–317. https://doi.org/10.1056/NEJMoa1602615.
53. Carter GP, Chakravorty A, Nguyen TAP, Mileto S, Schreiber F, Li L, Howarth P, Clare S, Cunningham B, Sambol SP, Cheknis A, Figueroa I, Johnson S, Gerding D, Rood JI, Dougan G, Lawley TD, Lyras D. 2015. Defining the roles of TcdA and TcdB in localized gastrointestinal disease, systemic organ damage, and the host response during Clostridium difficile infections. mBio 6:e00551-15. https://doi.org/10.1128/mBio.00551-15.
54. Lyras D, O'Connor JR, Howarth PM, Sambol SP, Carter GP, Phumoonna T, Poon R, Adams V, Vedantam G, Johnson S, Gerding DN, Rood JI. 2009. Toxin B is essential for virulence of Clostridium difficile. Nature 458:1176–1179. https://doi.org/10.1038/nature07822.
55. Devera TS, Lang GA, Lanis JM, Rampuria P, Gilmore CL, James JA, Ballard JD, Lang ML. 2016. Memory B cells encode neutralizing antibody specific for toxin B from the Clostridium difficile strains VPI 10463 and NAP1/BI/027 but with superior neutralization of VPI 10463 toxin B. Infect Immun 84:194–204. https://doi.org/10.1128/IAI.00011-15.
56. Buonomo EL, Cowardin CA, Wilson MG, Saleh MM, Pramoonjago P, Petri WA, Jr. 2016. Microbiota-regulated IL-25 increases eosinophil number to provide protection during Clostridium difficile infection. Cell Rep 16:432–443. https://doi.org/10.1016/j.celrep.2016.06.007.
57. Frisbee AL, Saleh MM, Young MK, Leslie JL, Simpson ME, Abhyankar MM, Cowardin CA, Ma JZ, Pramoonjago P, Turner SD, Liou AP, Buonomo EL, Petri WA, Jr. 2019. IL-33 drives group 2 innate lymphoid cell-mediated protection during Clostridium difficile infection. Nat Commun 10:2712. https://doi.org/10.1038/s41467-019-10733-9.
58. Liu R, Suarez JM, Weisblum B, Gellman SH, McBride SM. 2014. Synthetic polymers active against Clostridium difficile vegetative cell growth and spore outgrowth. J Am Chem Soc 136:14498–14504. https://doi.org/10.1021/ja506798e.
59. McDonald JAK, Mullish BH, Pechlivanis A, Liu Z, Brignardello J, Kao D, Holmes E, Li JV, Clarke TB, Thursz MR, Marchesi JR. 2018. Inhibiting growth of Clostridioides difficile by restoring valerate, produced by the intestinal microbiota. Gastroenterology 155:1495–1507.e15. https://doi.org/10.1053/j.gastro.2018.07.014.
60. Staley C, Weingarden AR, Khoruts A, Sadowsky MJ. 2017. Interaction of gut microbiota with bile acid metabolism and its influence on disease states. Appl Microbiol Biotechnol 101:47–64. https://doi.org/10.1007/s00253-016-8006-6.
61. Tinoco-Veras CM, Santos A, Stipursky J, Meloni M, Araujo APB, Foschetti DA, Lopez-Urena D, Quesada-Gomez C, Leitao RFC, Gomes FCA, Brito GAC. 2017. Transforming growth factor beta1/SMAD signaling pathway activation protects the intestinal epithelium from Clostridium difficile toxin A-induced damage. Infect Immun 85:e00430-17. https://doi.org/10.1128/IAI.00430-17.
62. Nagao-Kitamoto H, Leslie JL, Kitamoto S, Jin C, Thomsson KA, Gillilland MG, III, Kuffa P, Goto Y, Jenq RR, Ishii C, Hirayama A, Seekatz AM, Martens EC, Eaton KA, Kao JY, Fukuda S, Higgins PDR, Karlsson NG, Young VB, Kamada N. 2020. Interleukin-22-mediated host glycosylation prevents Clostridioides difficile infection by modulating the metabolic activity of the gut microbiota. Nat Med 26:608–617. https://doi.org/10.1038/s41591-020-0764-0.
63. Mullish BH, Quraishi MN, Segal JP, McCune VL, Baxter M, Marsden GL, Moore DJ, Colville A, Bhala N, Iqbal TH, Settle C, Kontkowski G, Hart AL, Hawkey PM, Goldenberg SD, Williams HRT. 2018. The use of faecal microbiota transplant as treatment for recurrent or refractory Clostridium difficile infection and other potential indications: joint British Society of

Gastroenterology (BSG) and Healthcare Infection Society (HIS) guidelines. Gut 67:1920–1941. https://doi.org/10.1136/gutjnl-2018-316818.
64. Gupta A, Cifu AS, Khanna S. 2018. Diagnosis and treatment of Clostridium difficile infection. JAMA 320:1031–1032. https://doi.org/10.1001/jama.2018.12194.
65. Kassam Z, Lee CH, Yuan Y, Hunt RH. 2013. Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. Am J Gastroenterol 108:500–508. https://doi.org/10.1038/ajg.2013.59.
66. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JF, Tijssen JG, Speelman P, Dijkgraaf MG, Keller JJ. 2013. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med 368:407–415. https://doi.org/10.1056/NEJMoa1205037.
67. Youngster I, Russell GH, Pindar C, Ziv-Baran T, Sauk J, Hohmann EL. 2014. Oral, capsulized, frozen fecal microbiota transplantation for relapsing Clostridium difficile infection. JAMA 312:1772–1778. https://doi.org/10.1001/jama.2014.13875.
68. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, Dubberke ER, Garey KW, Gould CV, Kelly C, Loo V, Shaklee Sammons J, Sandora TJ, Wilcox MH. 2018. Clinical practice guidelines for Clostridium difficile infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis 66:987–994. https://doi.org/10.1093/cid/ciy149.
69. Zheng D, Liwinski T, Elinav E. 2020. Interaction between microbiota and immunity in health and disease. Cell Res 30:492–506. https://doi.org/10.1038/s41422-020-0332-7.
70. Greenberg SA, Youngster I, Cohen NA, Livovsky DM, Strahilevitz J, Israeli E, Melzer E, Paz K, Fliss-Isakov N, Maharshak N. 2018. Five years of fecal microbiota transplantation—an update of the Israeli experience. World J Gastroenterol 24:5403–5414. https://doi.org/10.3748/wjg.v24.i47.5403.
71. Iqbal U, Siddique O, Ovalle A, Anwar H, Moss SF. 2018. Safety and efficacy of a minimally invasive cell sampling device ("Cytosponge") in the diagnosis of esophageal pathology: a systematic review. Eur J Gastroenterol Hepatol 30:1261–1269. https://doi.org/10.1097/MEG.0000000000001210.
72. Krajicek E, Fischer M, Allegretti JR, Kelly CR. 2019. Nuts and bolts of fecal microbiota transplantation. Clin Gastroenterol Hepatol 17:345–352. https://doi.org/10.1016/j.cgh.2018.09.029.
73. De Leon LM, Watson JB, Kelly CR. 2013. Transient flare of ulcerative colitis after fecal microbiota transplantation for recurrent Clostridium difficile infection. Clin Gastroenterol Hepatol 11:1036–1038. https://doi.org/10.1016/j.cgh.2013.04.045.
74. Shogbesan O, Poudel DR, Victor S, Jehangir A, Fadahunsi O, Shogbesan G, Donato A. 2018. A systematic review of the efficacy and safety of fecal microbiota transplant for Clostridium difficile infection in immunocompromised patients. Can J Gastroenterol Hepatol 2018:1394379. https://doi.org/10.1155/2018/1394379.
75. DeFilipp Z, Bloom PP, Torres Soto M, Mansour MK, Sater MRA, Huntley MH, Turbett S, Chung RT, Chen YB, Hohmann EL. 2019. Drug-resistant E. coli bacteremia transmitted by fecal microbiota transplant. N Engl J Med 381:2043–2050. https://doi.org/10.1056/NEJMoa1910437.
76. Quera R, Espinoza R, Estay C, Rivera D. 2014. Bacteremia as an adverse event of fecal microbiota transplantation in a patient with Crohn's disease and recurrent Clostridium difficile infection. J Crohns Colitis 8:252–253. https://doi.org/10.1016/j.crohns.2013.10.002.
77. Trubiano JA, Gardiner B, Kwong JC, Ward P, Testro AG, Charles PG. 2013. Faecal microbiota transplantation for severe Clostridium difficile infection in the intensive care unit. Eur J Gastroenterol Hepatol 25:255–257. https://doi.org/10.1097/MEG.0b013e32835b2da9.
78. Solari PR, Fairchild PG, Noa LJ, Wallace MR. 2014. Tempered enthusiasm for fecal transplant. Clin Infect Dis 59:319. https://doi.org/10.1093/cid/ciu278.
79. Baxter M, Ahmad T, Colville A, Sheridan R. 2015. Fatal aspiration pneumonia as a complication of fecal microbiota transplant. Clin Infect Dis 61:136–137. https://doi.org/10.1093/cid/civ247.
80. McGovern BH, Ford CB, Henn MR, Pardi DS, Khanna S, Hohmann EL, O'Brien EJ, Desjardins CA, Bernardo P, Wortman JR, Lombardo MJ, Litcofsky KD, Winkler JA, McChalicher CWJ, Li SS, Tomlinson AD, Nandakumar M, Cook DN, Pomerantz RJ, Aunins JG, Trucksis M. 2021. SER-109, an investigational microbiome drug to reduce recurrence after Clostridioides difficile infection: lessons learned from a phase 2 trial. Clin Infect Dis 72:2132–2140. https://doi.org/10.1093/cid/ciaa387.
81. Feuerstadt P, Louie TJ, Lashner B, Wang EEL, Diao L, Bryant JA, Sims M, Kraft CS, Cohen SH, Berenson CS, Korman LY, Ford CB, Litcofsky KD, Lombardo M-J, Wortman JR, Wu H, Auniņš JG, McChalicher CWJ, Winkler JA, McGovern BH, Trucksis M, Henn MR, von Moltke L. 2022. SER-109, an oral microbiome therapy for recurrent Clostridioides difficile infection. N Engl J Med 386:220–229. https://doi.org/10.1056/NEJMoa2106516.
82. Frisbee AL, Petri WA, Jr. 2020. Considering the immune system during fecal microbiota transplantation for Clostridioides difficile infection. Trends Mol Med 26:496–507. https://doi.org/10.1016/j.molmed.2020.01.009.
83. Abt MC, Lewis BB, Caballero S, Xiong H, Carter RA, Susac B, Ling L, Leiner I, Pamer EG. 2015. Innate immune defenses mediated by two ILC subsets are critical for protection against acute Clostridium difficile infection. Cell Host Microbe 18:27–37. https://doi.org/10.1016/j.chom.2015.06.011.
84. Fachi JL, Secca C, Rodrigues PB, Mato FCPD, Luccia B, Felipe JS, Pral LP, Rungue M, Rocha VM, Sato FT, Sampaio U, Clerici M, Rodrigues HG, Camara NOS, Consonni SR, Vieira AT, Oliveira SC, Mackay CR, Layden BT, Bortoluci KR, Colonna M, Vinolo MAR. 2020. Acetate coordinates neutrophil and ILC3 responses against C. difficile through FFAR2. J Exp Med 217:jem.20190489. https://doi.org/10.1084/jem.20190489.
85. Rea MC, Dobson A, O'Sullivan O, Crispie F, Fouhy F, Cotter PD, Shanahan F, Kiely B, Hill C, Ross RP. 2011. Effect of broad- and narrow-spectrum antimicrobials on Clostridium difficile and microbial diversity in a model of the distal colon. Proc Natl Acad Sci U S A 108:4639–4644. https://doi.org/10.1073/pnas.1001224107.
86. Farrow MA, Chumbler NM, Lapierre LA, Franklin JL, Rutherford SA, Goldenring JR, Lacy DB. 2013. Clostridium difficile toxin B-induced necrosis is mediated by the host epithelial cell NADPH oxidase complex. Proc Natl Acad Sci U S A 110:18674–18679. https://doi.org/10.1073/pnas.1313658110.
87. Di Bella S, Ascenzi P, Siarakas S, Petrosillo N, di Masi A. 2016. Clostridium difficile toxins A and B: insights into pathogenic properties and extraintestinal effects. Toxins 8:134. https://doi.org/10.3390/toxins8050134.
88. Chandrasekaran R, Lacy DB. 2017. The role of toxins in Clostridium difficile infection. FEMS Microbiol Rev 41:723–750. https://doi.org/10.1093/femsre/fux048.
89. Orrell KE, Melnyk RA. 2021. Large clostridial toxins: mechanisms and roles in disease. Microbiol Mol Biol Rev 85:e0006421. https://doi.org/10.1128/MMBR.00064-21.
90. Warny M, Keates AC, Keates S, Castagliuolo I, Zacks JK, Aboudola S, Qamar A, Pothoulakis C, LaMont JT, Kelly CP. 2000. p38 MAP kinase activation by Clostridium difficile toxin A mediates monocyte necrosis, IL-8 production, and enteritis. J Clin Invest 105:1147–1156. https://doi.org/10.1172/JCI7545.
91. Cowardin CA, Kuehne SA, Buonomo EL, Marie CS, Minton NP, Petri WA, Jr. 2015. Inflammasome activation contributes to interleukin-23 production in response to Clostridium difficile. mBio 6:e02386-14. https://doi.org/10.1128/mBio.02386-14.
92. Liu YH, Chang YC, Chen LK, Su PA, Ko WC, Tsai YS, Chen YH, Lai HC, Wu CY, Hung YP, Tsai PJ. 2018. The ATP-P2X7 signaling axis is an essential sentinel for intracellular Clostridium difficile pathogen-induced inflammasome activation. Front Cell Infect Microbiol 8:84. https://doi.org/10.3389/fcimb.2018.00084.
93. Awad MM, Hutton ML, Quek AJ, Klare WP, Mileto SJ, Mackin K, Ly D, Oorschot V, Bosnjak M, Jenkin G, Conroy PJ, West N, Fulcher A, Costin A, Day CJ, Jennings MP, Medcalf RL, Sanderson-Smith M, Cordwell SJ, Law RHP, Whisstock JC, Lyras D. 2020. Human plasminogen exacerbates Clostridioides difficile enteric disease and alters the spore surface. Gastroenterology 159:1431–1443.e6. https://doi.org/10.1053/j.gastro.2020.06.032.
94. Savidge TC, Urvil P, Oezguen N, Ali K, Choudhury A, Acharya V, Pinchuk I, Torres AG, English RD, Wiktorowicz JE, Loeffelholz M, Kumar R, Shi L, Nie W, Braun W, Herman B, Hausladen A, Feng H, Stamler JS, Pothoulakis C. 2011. Host S-nitrosylation inhibits clostridial small molecule-activated glucosylating toxins. Nat Med 17:1136–1141. https://doi.org/10.1038/nm.2405.
95. Engevik MA, Danhof HA, Shrestha R, Chang-Graham AL, Hyser JM, Haag AM, Mohammad MA, Britton RA, Versalovic J, Sorg JA, Spinler JK. 2020. Reuterin disrupts Clostridioides difficile metabolism and pathogenicity through reactive oxygen species generation. Gut Microbes 12:1788898. https://doi.org/10.1080/19490976.2020.1795388.
96. Huang AM, Marini BL, Frame D, Aronoff DM, Nagel JL. 2014. Risk factors for recurrent Clostridium difficile infection in hematopoietic stem cell transplant recipients. Transpl Infect Dis 16:744–750. https://doi.org/10.1111/tid.12267.
97. Luo R, Greenberg A, Stone CD. 2015. Outcomes of Clostridium difficile infection in hospitalized leukemia patients: a nationwide analysis. Infect Control Hosp Epidemiol 36:794–801. https://doi.org/10.1017/ice.2015.54.
98. Paredes-Sabja D, Cofre-Araneda G, Brito-Silva C, Pizarro-Guajardo M, Sarker MR. 2012. Clostridium difficile spore-macrophage interactions:

spore survival. PLoS One 7:e43635. https://doi.org/10.1371/journal.pone.0043635.
99. Wang J, Ortiz C, Fontenot L, Mukhopadhyay R, Xie Y, Chen X, Feng H, Pothoulakis C, Koon HW. 2020. Therapeutic mechanism of macrophage inflammatory protein 1 alpha neutralizing antibody (CCL3) in Clostridium difficile infection in mice. J Infect Dis 221:1623–1635. https://doi.org/10.1093/infdis/jiz640.
100. Saavedra PHV, Huang L, Ghazavi F, Kourula S, Vanden Berghe T, Takahashi N, Vandenabeele P, Lamkanfi M. 2018. Apoptosis of intestinal epithelial cells restricts Clostridium difficile infection in a model of pseudomembranous colitis. Nat Commun 9:4846. https://doi.org/10.1038/s41467-018-07386-5.
101. Kulaylat AS, Buonomo EL, Scully KW, Hollenbeak CS, Cook H, Petri WA, Jr, Stewart DB, Sr. 2018. Development and validation of a prediction model for mortality and adverse outcomes among patients with peripheral eosinopenia on admission for Clostridium difficile infection. JAMA Surg 153:1127–1133. https://doi.org/10.1001/jamasurg.2018.3174.
102. Gieseck RL, III, Wilson MS, Wynn TA. 2018. Type 2 immunity in tissue repair and fibrosis. Nat Rev Immunol 18:62–76. https://doi.org/10.1038/nri.2017.90.
103. Donlan A, Petri WA, Jr. 2020. The inflammasome and type-2 immunity in Clostridium difficile infection. Clin Colon Rectal Surg 33:67–72. https://doi.org/10.1055/s-0040-1701231.
104. Buonomo EL, Madan R, Pramoonjago P, Li L, Okusa MD, Petri WA, Jr. 2013. Role of interleukin 23 signaling in Clostridium difficile colitis. J Infect Dis 208:917–920. https://doi.org/10.1093/infdis/jit277.
105. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, Furusawa J, Ohtani M, Fujii H, Koyasu S. 2010. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. Nature 463:540–544. https://doi.org/10.1038/nature08636.
106. Sasaki T, Moro K, Kubota T, Kubota N, Kato T, Ohno H, Nakae S, Saito H, Koyasu S. 2019. Innate lymphoid cells in the induction of obesity. Cell Rep 28:202–217.e7. https://doi.org/10.1016/j.celrep.2019.06.016.
107. Sonnenberg GF, Artis D. 2012. Innate lymphoid cell interactions with microbiota: implications for intestinal health and disease. Immunity 37:601–610. https://doi.org/10.1016/j.immuni.2012.10.003.
108. Ganal-Vonarburg SC, Duerr CU. 2020. The interaction of intestinal microbiota and innate lymphoid cells in health and disease throughout life. Immunology 159:39–51. https://doi.org/10.1111/imm.13138.
109. Geiger TL, Abt MC, Gasteiger G, Firth MA, O'Connor MH, Geary CD, O'Sullivan TE, van den Brink MR, Pamer EG, Hanash AM, Sun JC. 2014. Nfil3 is crucial for development of innate lymphoid cells and host protection against intestinal pathogens. J Exp Med 211:1723–1731. https://doi.org/10.1084/jem.20140212.
110. Chen W, Shu Q, Fan J. 2020. Neural regulation of interactions between group 2 innate lymphoid cells and pulmonary immune cells. Front Immunol 11:576929. https://doi.org/10.3389/fimmu.2020.576929.
111. Oliphant CJ, Hwang YY, Walker JA, Salimi M, Wong SH, Brewer JM, Englezakis A, Barlow JL, Hams E, Scanlon ST, Ogg GS, Fallon PG, McKenzie AN. 2014. MHCII-mediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. Immunity 41:283–295. https://doi.org/10.1016/j.immuni.2014.06.016.
112. von Burg N, Chappaz S, Baerenwaldt A, Horvath E, Bose Dasgupta S, Ashok D, Pieters J, Tacchini-Cottier F, Rolink A, Acha-Orbea H, Finke D. 2014. Activated group 3 innate lymphoid cells promote T-cell-mediated immune responses. Proc Natl Acad Sci U S A 111:12835–12840. https://doi.org/10.1073/pnas.1406908111.
113. Hepworth MR, Monticelli LA, Fung TC, Ziegler CG, Grunberg S, Sinha R, Mantegazza AR, Ma HL, Crawford A, Angelosanto JM, Wherry EJ, Koni PA, Bushman FD, Elson CO, Eberl G, Artis D, Sonnenberg GF. 2013. Innate lymphoid cells regulate CD4+ T-cell responses to intestinal commensal bacteria. Nature 498:113–117. https://doi.org/10.1038/nature12240.
114. Halim TY, Steer CA, Matha L, Gold MJ, Martinez-Gonzalez I, McNagny KM, McKenzie AN, Takei F. 2014. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. Immunity 40:425–435. https://doi.org/10.1016/j.immuni.2014.01.011.
115. Maizels RM, Withers DR. 2014. MHC-II: a mutual support system for ILCs and T cells? Immunity 41:174–176. https://doi.org/10.1016/j.immuni.2014.07.006.
116. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, Bucks C, Kane CM, Fallon PG, Pannell R, Jolin HE, McKenzie AN. 2010. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature 464:1367–1370. https://doi.org/10.1038/nature08900.
117. Licona-Limon P, Kim LK, Palm NW, Flavell RA. 2013. TH2, allergy and group 2 innate lymphoid cells. Nat Immunol 14:536–542. https://doi.org/10.1038/ni.2617.
118. Fukuoka A, Futatsugi-Yumikura S, Takahashi S, Kazama H, Iyoda T, Yoshimoto T, Inaba K, Nakanishi K, Yonehara S. 2013. Identification of a novel type 2 innate immunocyte with the ability to enhance IgE production. Int Immunol 25:373–382. https://doi.org/10.1093/intimm/dxs160.
119. Mattiola I, Diefenbach A. 2020. Enabling anti-tumor immunity by unleashing ILC2. Cell Res 30:461–462. https://doi.org/10.1038/s41422-020-0330-9.
120. Donlan AN, Simpson ME, Petri WA, Jr. 2020. Type 2 cytokines IL-4 and IL-5 reduce severe outcomes from Clostridiodes difficile infection. Anaerobe 66:102275. https://doi.org/10.1016/j.anaerobe.2020.102275.
121. Hasegawa M, Yada S, Liu MZ, Kamada N, Munoz-Planillo R, Do N, Nunez G, Inohara N. 2014. Interleukin-22 regulates the complement system to promote resistance against pathobionts after pathogen-induced intestinal damage. Immunity 41:620–632. https://doi.org/10.1016/j.immuni.2014.09.010.
122. McDonald LC, Coignard B, Dubberke E, Song X, Horan T, Kutty PK. Ad Hoc Clostridium difficile Surveillance Working Group. 2007. Recommendations for surveillance of Clostridium difficile-associated disease. Infect Control Hosp Epidemiol 28:140–145. https://doi.org/10.1086/511798.
123. Johnston PF, Gerding DN, Knight KL. 2014. Protection from Clostridium difficile infection in CD4 T cell- and polymeric immunoglobulin receptor-deficient mice. Infect Immun 82:522–531. https://doi.org/10.1128/IAI.01273-13.
124. Bauer MP, Nibbering PH, Poxton IR, Kuijper EJ, van Dissel JT. 2014. Humoral immune response as predictor of recurrence in Clostridium difficile infection. Clin Microbiol Infect 20:1323–1328. https://doi.org/10.1111/1469-0691.12769.
125. Monaghan TM, Robins A, Knox A, Sewell HF, Mahida YR. 2013. Circulating antibody and memory B-cell responses to C. difficile toxins A and B in patients with C. difficile-associated diarrhoea, inflammatory bowel disease and cystic fibrosis. PLoS One 8:e74452. https://doi.org/10.1371/journal.pone.0074452.
126. Rampuria P, Lang GA, Devera TS, Gilmore C, Ballard JD, Lang ML. 2017. Coordination between T helper cells, iNKT cells, and their follicular helper subsets in the humoral immune response against Clostridium difficile toxin B. J Leukoc Biol 101:567–576. https://doi.org/10.1189/jlb.4A0616-271R.
127. Crotty S. 2011. Follicular helper CD4 T cells (TFH). Annu Rev Immunol 29:621–663. https://doi.org/10.1146/annurev-immunol-031210-101400.
128. Wu D, Joyee AG, Nandagopal S, Lopez M, Ma X, Berry J, Lin F. 2013. Effects of Clostridium difficile toxin A and B on human T lymphocyte migration. Toxins (Basel) 5:926–938. https://doi.org/10.3390/toxins5050926.
129. Cook L, Rees WD, Wong MQ, Kwok WW, Levings MK, Steiner TS. 2021. Recurrent Clostridioides difficile infection is associated with impaired T helper type 17 immunity to C difficile toxin B. Gastroenterology 160:1410–1413.e4. https://doi.org/10.1053/j.gastro.2020.11.043.
130. Hamo Z, Azrad M, Nitzan O, Peretz A. 2019. Characterization of the immune response during infection caused by Clostridioides difficile. Microorganisms 7:435. https://doi.org/10.3390/microorganisms7100435.
131. Yu H, Chen K, Sun Y, Carter M, Garey KW, Savidge TC, Devaraj S, Tessier ME, von Rosenvinge EC, Kelly CP, Pasetti MF, Feng H. 2017. Cytokines are markers of the Clostridium difficile-induced inflammatory response and predict disease severity. Clin Vaccine Immunol 24:e00037-17. https://doi.org/10.1128/CVI.00037-17.
132. Saleh MM, Frisbee AL, Leslie JL, Buonomo EL, Cowardin CA, Ma JZ, Simpson ME, Scully KW, Abhyankar MM, Petri WA, Jr. 2019. Colitis-induced Th17 cells increase the risk for severe subsequent Clostridium difficile infection. Cell Host Microbe 25:756–765.e5. https://doi.org/10.1016/j.chom.2019.03.003.
133. Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, Affaticati P, Gilfillan S, Lantz O. 2003. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. Nature 422:164–169. https://doi.org/10.1038/nature01433.
134. Chen YS, Chen IB, Pham G, Shao TY, Bangar H, Way SS, Haslam DB. 2020. IL-17-producing gammadelta T cells protect against Clostridium difficile infection. J Clin Invest 130:2377–2390. https://doi.org/10.1172/JCI127242.
135. Barnes MJ, Powrie F. 2009. Regulatory T cells reinforce intestinal homeostasis. Immunity 31:401–411. https://doi.org/10.1016/j.immuni.2009.08.011.
136. Littmann ER, Lee JJ, Denny JE, Alam Z, Maslanka JR, Zarin I, Matsuda R, Carter RA, Susac B, Saffern MS, Fett B, Mattei LM, Bittinger K, Abt MC. 2021. Host immunity modulates the efficacy of microbiota transplantation

for treatment of Clostridioides difficile infection. Nat Commun 12:755. https://doi.org/10.1038/s41467-020-20793-x.
137. Yacyshyn MB, Reddy TN, Plageman LR, Wu J, Hollar AR, Yacyshyn BR. 2014. Clostridium difficile recurrence is characterized by pro-inflammatory peripheral blood mononuclear cell (PBMC) phenotype. J Med Microbiol 63:1260–1273. https://doi.org/10.1099/jmm.0.075382-0.
138. Liu YW, Chen YH, Chen JW, Tsai PJ, Huang IH. 2017. Immunization with recombinant TcdB-encapsulated nanocomplex induces protection against Clostridium difficile challenge in a mouse model. Front Microbiol 8:1411. https://doi.org/10.3389/fmicb.2017.01411.
139. Pritz T, Lair J, Ban M, Keller M, Weinberger B, Krismer M, Grubeck-Loebenstein B. 2015. Plasma cell numbers decrease in bone marrow of old patients. Eur J Immunol 45:738–746. https://doi.org/10.1002/eji.201444878.
140. Figueroa I, Johnson S, Sambol SP, Goldstein EJ, Citron DM, Gerding DN. 2012. Relapse versus reinfection: recurrent Clostridium difficile infection following treatment with fidaxomicin or vancomycin. Clin Infect Dis 55: S104–S109. https://doi.org/10.1093/cid/cis357.
141. von Eichel-Streiber A, Paik W, Knight K, Gisch K, Nadjafi K, Decker C, Bosnjak O, Cheknis A, Johnson S, von Eichel-Streiber C. 2016. Induction of antitoxin responses in Clostridium-difficile-infected patients compared to healthy blood donors. Anaerobe 41:91–103. https://doi.org/10.1016/j.anaerobe.2016.07.001.
142. Kyne L, Warny M, Qamar A, Kelly CP. 2000. Asymptomatic carriage of Clostridium difficile and serum levels of IgG antibody against toxin A. N Engl J Med 342:390–397. https://doi.org/10.1056/NEJM200002103420604.
143. Gupta SB, Mehta V, Dubberke ER, Zhao X, Dorr MB, Guris D, Molrine D, Leney M, Miller M, Dupin M, Mast TC. 2016. Antibodies to toxin B are protective against Clostridium difficile infection recurrence. Clin Infect Dis 63:730–734. https://doi.org/10.1093/cid/ciw364.
144. Sambol SP, Merrigan MM, Tang JK, Johnson S, Gerding DN. 2002. Colonization for the prevention of Clostridium difficile disease in hamsters. J Infect Dis 186:1781–1789. https://doi.org/10.1086/345676.
145. Louie TJ, Miller MA, Mullane KM, Weiss K, Lentnek A, Golan Y, Gorbach S, Sears P, Shue YK. OPT-80-003 Clinical Study Group. 2011. Fidaxomicin versus vancomycin for Clostridium difficile infection. N Engl J Med 364: 422–431. https://doi.org/10.1056/NEJMoa0910812.
146. Debast SB, Bauer MP, Kuijper EJ, European S, Of CM, Infectious D. European Society of Clinical Microbiology and Infectious Diseases. 2014. European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for Clostridium difficile infection. Clin Microbiol Infect 20:1–26. https://doi.org/10.1111/1469-0691.12418.
147. Johnson S, Louie TJ, Gerding DN, Cornely OA, Chasan-Taber S, Fitts D, Gelone SP, Broom C, Davidson DM. Polymer Alternative for CDI Treatment Investigators. 2014. Vancomycin, metronidazole, or tolevamer for Clostridium difficile infection: results from two multinational, randomized, controlled trials. Clin Infect Dis 59:345–354. https://doi.org/10.1093/cid/ciu313.
148. Polivkova S, Krutova M, Capek V, Sykorova B, Benes J. 2021. Fidaxomicin versus metronidazole, vancomycin and their combination for initial episode, first recurrence and severe Clostridioides difficile infection—an observational cohort study. Int J Infect Dis 103:226–233. https://doi.org/10.1016/j.ijid.2020.11.004.
149. Gopinath PM, Ranjani A, Dhanasekaran D, Thajuddin N, Archunan G, Akbarsha MA, Gulyas B, Padmanabhan P. 2016. Multi-functional nano silver: a novel disruptive and theranostic agent for pathogenic organisms in real-time. Sci Rep 6:34058. https://doi.org/10.1038/srep34058.
150. Yang H, Cai R, Zhang Y, Chen Y, Gu B. 2020. Gold nanoclusters as an antibacterial alternative against Clostridium difficile. Int J Nanomed 15: 6401–6408. https://doi.org/10.2147/IJN.S268758.
151. Lowy I, Molrine DC, Leav BA, Blair BM, Baxter R, Gerding DN, Nichol G, Thomas WD, Jr, Leney M, Sloan S, Hay CA, Ambrosino DM. 2010. Treatment with monoclonal antibodies against Clostridium difficile toxins. N Engl J Med 362:197–205. https://doi.org/10.1056/NEJMoa0907635.
152. de Bruyn G, Saleh J, Workman D, Pollak R, Elinoff V, Fraser NJ, Lefebvre G, Martens M, Mills RE, Nathan R, Trevino M, van Cleeff M, Foglia G, Ozol-Godfrey A, Patel DM, Pietrobon PJ, Gesser R, Team HCIS. H-030–012 Clinical Investigator Study Team. 2016. Defining the optimal formulation and schedule of a candidate toxoid vaccine against Clostridium difficile infection: a randomized Phase 2 clinical trial. Vaccine 34:2170–2178. https://doi.org/10.1016/j.vaccine.2016.03.028.
153. de Bruyn G, Gordon DL, Steiner T, Tambyah P, Cosgrove C, Martens M, Bassily E, Chan ES, Patel D, Chen J, Torre-Cisneros J, Fernando De Magalhaes Francesconi C, Gesser R, Jeanfreau R, Launay O, Laot T, Morfin-Otero R, Oviedo-Orta E, Park YS, Piazza FM, Rehm C, Rivas E, Self S, Gurunathan S. 2021. Safety, immunogenicity, and efficacy of a Clostridioides difficile toxoid vaccine candidate: a phase 3 multicentre, observer-blind, randomised, controlled trial. Lancet Infect Dis 21:252–262. https://doi.org/10.1016/S1473-3099(20)30331-5.
154. Donald RGK, Flint M, Kalyan N, Johnson E, Witko SE, Kotash C, Zhao P, Megati S, Yurgelonis I, Lee PK, Matsuka YV, Severina E, Deatly A, Sidhu M, Jansen KU, Minton NP, Anderson AS. 2013. A novel approach to generate a recombinant toxoid vaccine against Clostridium difficile. Microbiology (Reading) 159:1254–1266. https://doi.org/10.1099/mic.0.066712-0.
155. Bezay N, Ayad A, Dubischar K, Firbas C, Hochreiter R, Kiermayr S, Kiss I, Pinl F, Jilma B, Westritschnig K. 2016. Safety, immunogenicity and dose response of VLA84, a new vaccine candidate against Clostridium difficile, in healthy volunteers. Vaccine 34:2585–2592. https://doi.org/10.1016/j.vaccine.2016.03.098.
156. Wang H, Sun X, Zhang Y, Li S, Chen K, Shi L, Nie W, Kumar R, Tzipori S, Wang J, Savidge T, Feng H. 2012. A chimeric toxin vaccine protects against primary and recurrent Clostridium difficile infection. Infect Immun 80:2678–2688. https://doi.org/10.1128/IAI.00215-12.
157. Monot M, Eckert C, Lemire A, Hamiot A, Dubois T, Tessier C, Dumoulard B, Hamel B, Petit A, Lalande V, Ma L, Bouchier C, Barbut F, Dupuy B. 2015. Clostridium difficile: new insights into the evolution of the pathogenicity locus. Sci Rep 5:15023. https://doi.org/10.1038/srep15023.
158. May M, Wang T, Muller M, Genth H. 2013. Difference in F-actin depolymerization induced by toxin B from the Clostridium difficile strain VPI 10463 and toxin B from the variant Clostridium difficile serotype F strain 1470. Toxins (Basel) 5:106–119. https://doi.org/10.3390/toxins5010106.
159. Gerding DN, Meyer T, Lee C, Cohen SH, Murthy UK, Poirier A, Van Schooneveld TC, Pardi DS, Ramos A, Barron MA, Chen H, Villano S. 2015. Administration of spores of nontoxigenic Clostridium difficile strain M3 for prevention of recurrent C. difficile infection: a randomized clinical trial. JAMA 313:1719–1727. https://doi.org/10.1001/jama.2015.3725.
160. Zhang BZ, Cai J, Yu B, Hua Y, Lau CC, Kao RY, Sze KH, Yuen KY, Huang JD. 2016. A DNA vaccine targeting TcdA and TcdB induces protective immunity against Clostridium difficile. BMC Infect Dis 16:596. https://doi.org/10.1186/s12879-016-1924-1.
161. Luo D, Liu X, Xing L, Sun Y, Huang J, Zhang L, Li J, Wang H. 2019. Immunogenicity and protection from receptor-binding domains of toxins as potential vaccine candidates for Clostridium difficile. Vaccines 7:180. https://doi.org/10.3390/vaccines7040180.
162. Permpoonpattana P, Phetcharaburanin J, Mikelsone A, Dembek M, Tan S, Brisson MC, La Ragione R, Brisson AR, Fairweather N, Hong HA, Cutting SM. 2013. Functional characterization of Clostridium difficile spore coat proteins. J Bacteriol 195:1492–1503. https://doi.org/10.1128/JB.02104-12.
163. Ganeshapillai J, Vinogradov E, Rousseau J, Weese JS, Monteiro MA. 2008. Clostridium difficile cell-surface polysaccharides composed of pentaglycosyl and hexaglycosyl phosphate repeating units. Carbohydr Res 343: 703–710. https://doi.org/10.1016/j.carres.2008.01.002.
164. Ghose C, Eugenis I, Sun X, Edwards AN, McBride SM, Pride DT, Kelly CP, Ho DD. 2016. Immunogenicity and protective efficacy of recombinant Clostridium difficile flagellar protein FliC. Emerg Microbes Infect 5:e8. https://doi.org/10.1038/emi.2016.8.
165. Bruxelle JF, Mizrahi A, Hoys S, Collignon A, Janoir C, Pechine S. 2016. Immunogenic properties of the surface layer precursor of Clostridium difficile and vaccination assays in animal models. Anaerobe 37:78–84. https://doi.org/10.1016/j.anaerobe.2015.10.010.
166. Hughes J, Aston C, Kelly ML, Griffin R. 2022. Towards development of a nontoxigenic Clostridioides difficile oral spore vaccine against toxigenic C. difficile. Pharmaceutics 14:1086. https://doi.org/10.3390/pharmaceutics14051086.
167. Wang Y, Wang S, Bouillaut L, Li C, Duan Z, Zhang K, Ju X, Tzipori S, Sonenshein AL, Sun X. 2018. Oral immunization with nontoxigenic Clostridium difficile strains expressing chimeric fragments of TcdA and TcdB elicits protective immunity against C. difficile infection in both mice and hamsters. Infect Immun 86:e00489-18. https://doi.org/10.1128/IAI.00489-18.
168. Wang S, Zhu D, Sun X. 2022. Development of an effective nontoxigenic Clostridioides difficile-based oral vaccine against C. difficile infection. Microbiol Spectr 10:e0026322. https://doi.org/10.1128/spectrum.00263-22.
169. Hong HA, Hitri K, Hosseini S, Kotowicz N, Bryan D, Mawas F, Wilkinson AJ, van Broekhoven A, Kearsey J, Cutting SM. 2017. Mucosal antibodies to the C terminus of toxin A prevent colonization of Clostridium difficile. Infect Immun 85:e01060-16. https://doi.org/10.1128/IAI.01060-16.
170. Leuzzi R, Adamo R, Scarselli M. 2014. Vaccines against Clostridium difficile. Hum Vaccin Immunother 10:1466–1477. https://doi.org/10.4161/hv.28428.
171. Larabee JL, Krumholz A, Hunt JJ, Lanis JM, Ballard JD. 2015. Exposure of neutralizing epitopes in the carboxyl-terminal domain of TcdB is altered by a proximal hypervariable region. J Biol Chem 290:6975–6985. https://doi.org/10.1074/jbc.M114.612184.

172. Secore S, Wang S, Doughtry J, Xie J, Miezeiewski M, Rustandi RR, Horton M, Xoconostle R, Wang B, Lancaster C, Kristopeit A, Wang SC, Christanti S, Vitelli S, Gentile MP, Goerke A, Skinner J, Strable E, Thiriot DS, Bodmer JL, Heinrichs JH. 2017. Development of a novel vaccine containing binary toxin for the prevention of Clostridium difficile disease with enhanced efficacy against NAP1 strains. PLoS One 12:e0170640. https://doi.org/10.1371/journal.pone.0170640.
173. Oleszycka E, Lavelle EC. 2014. Immunomodulatory properties of the vaccine adjuvant alum. Curr Opin Immunol 28:1–5. https://doi.org/10.1016/j.coi.2013.12.007.
174. Shaw HA, Preston MD, Vendrik KEW, Cairns MD, Browne HP, Stabler RA, Crobach MJT, Corver J, Pituch H, Ingebretsen A, Pirmohamed M, Faulds-Pain A, Valiente E, Lawley TD, Fairweather NF, Kuijper EJ, Wren BW. 2020. The recent emergence of a highly related virulent Clostridium difficile clade with unique characteristics. Clin Microbiol Infect 26:492–498. https://doi.org/10.1016/j.cmi.2019.09.004.
175. Walker AS, Eyre DW, Wyllie DH, Dingle KE, Griffiths D, Shine B, Oakley S, O'Connor L, Finney J, Vaughan A, Crook DW, Wilcox MH, Peto TE. Infections in Oxfordshire Research Database. 2013. Relationship between bacterial strain type, host biomarkers, and mortality in Clostridium difficile infection. Clin Infect Dis 56:1589–1600. https://doi.org/10.1093/cid/cit127.
176. Cairns MD, Preston MD, Lawley TD, Clark TG, Stabler RA, Wren BW. 2015. Genomic epidemiology of a protracted hospital outbreak caused by a toxin A-negative Clostridium difficile sublineage PCR ribotype 017 strain in London, England. J Clin Microbiol 53:3141–3147. https://doi.org/10.1128/JCM.00648-15.
177. Kullin B, Wojno J, Abratt V, Reid SJ. 2017. Toxin A-negative toxin B-positive ribotype 017 Clostridium difficile is the dominant strain type in patients with diarrhoea attending tuberculosis hospitals in Cape Town, South Africa. Eur J Clin Microbiol Infect Dis 36:163–175. https://doi.org/10.1007/s10096-016-2790-x.

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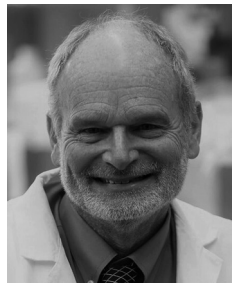

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