

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Vaccinating against *Clostridioides difficile* InfectionVincent B. Young, M.D., Ph.D.^{1,2}

Two key approaches to counter infectious diseases are prevention and treatment: the first by means of the administration of vaccines, and the second by means of the administration of antimicrobial drugs (antibiotic agents). Although the benefits of antibiotics to health are undeniable, consequences to their use exist. Infection with the nosocomial bacterial pathogen *Clostridioides difficile* after the administration of antibacterial agents leads to gastrointestinal disease of variable severity and increased health care costs. *C. difficile* infection occurs when the indigenous microbiota of the gut is altered, most commonly after antibiotic administration. This alteration disrupts the metabolic activity of the microbiota, allowing the expansion of *C. difficile* in the gut owing to the loss of resistance to colonization.¹ The pathogen produces toxins (see below), and eventually the infected person sheds the environmentally stable spore form, which can contaminate the environment and foster the spread of *C. difficile* to other persons. The current overall strategy to limit *C. difficile* infection is to prevent it by screening, contact isolation, and limiting the use of antibiotics, such as quinolones, that can trigger *C. difficile* infection.

Because the pathogenesis of *C. difficile* infection depends on the production of the potent toxins TcdA (toxin A) and TcdB (toxin B) by vegetative *C. difficile* cells (Fig. 1A), there is hope that the development of vaccines targeting these virulence factors, analogous to the development of vaccines against the diphtheria and tetanus toxins, will be successful in limiting the development of *C. difficile* infection in patients receiving antibiotic treatment.² However, results of clinical trials have suggested that standard vaccine approaches against *C. difficile* infection that target TcdA and TcdB need to be reconsidered.

A trial of a vaccine composed of formalin-inactivated TcdA and TcdB purified from a highly toxicogenic *C. difficile* strain was stopped at

**Adjuvant** ⓘ

A component of vaccine formulations that increases the quality and magnitude of immune responses to a vaccine. Traditional adjuvants include aluminum compounds and various lipid compounds. The lipid nanoparticle, used in mRNA vaccines as a means of delivering mRNA to host cells, is a potent adjuvant.

mRNA vaccine ⓘ

An approach for triggering immune responses against a pathogen. Traditional vaccines use inactivated or weakened forms of a pathogen, or purified antigens from the pathogen, to trigger protective immune responses. With mRNA vaccines, nucleic acid is administered within lipid nanoparticles to trigger the production of one or more pathogen proteins (or parts of these proteins) by host cells. This in turn stimulates the immune system to target pathogen proteins.



An illustrated glossary is available at NEJM.org



the first planned interim analysis on the basis of clinical futility,³ and the development of this vaccine was terminated. More recently, results were published from the *Clostridium difficile* Vaccine Efficacy Trial (CLOVER), a phase 3, randomized trial of a genetically detoxified *C. difficile* vaccine composed of recombinant TcdA and TcdB (containing targeted amino-acid substitutions to limit toxic activity) that were further detoxified by chemical means.⁴ Although the trial did not show a benefit with respect to the primary end point of preventing a first episode of *C. difficile* infection, vaccinated patients in whom *C. difficile* infection developed had a shorter duration of symptoms and were less likely to receive medical attention for their infection than patients who had received placebo.

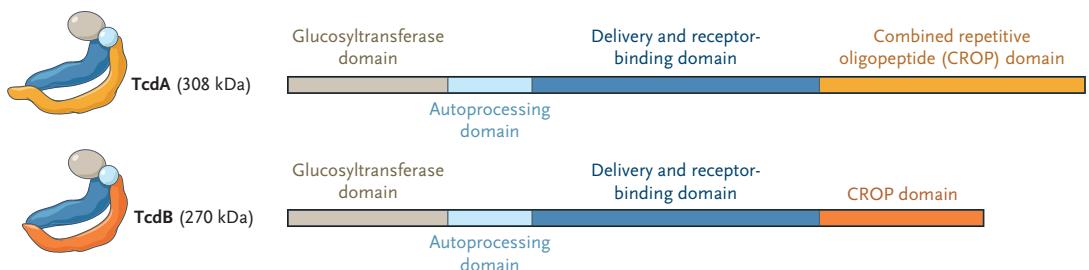
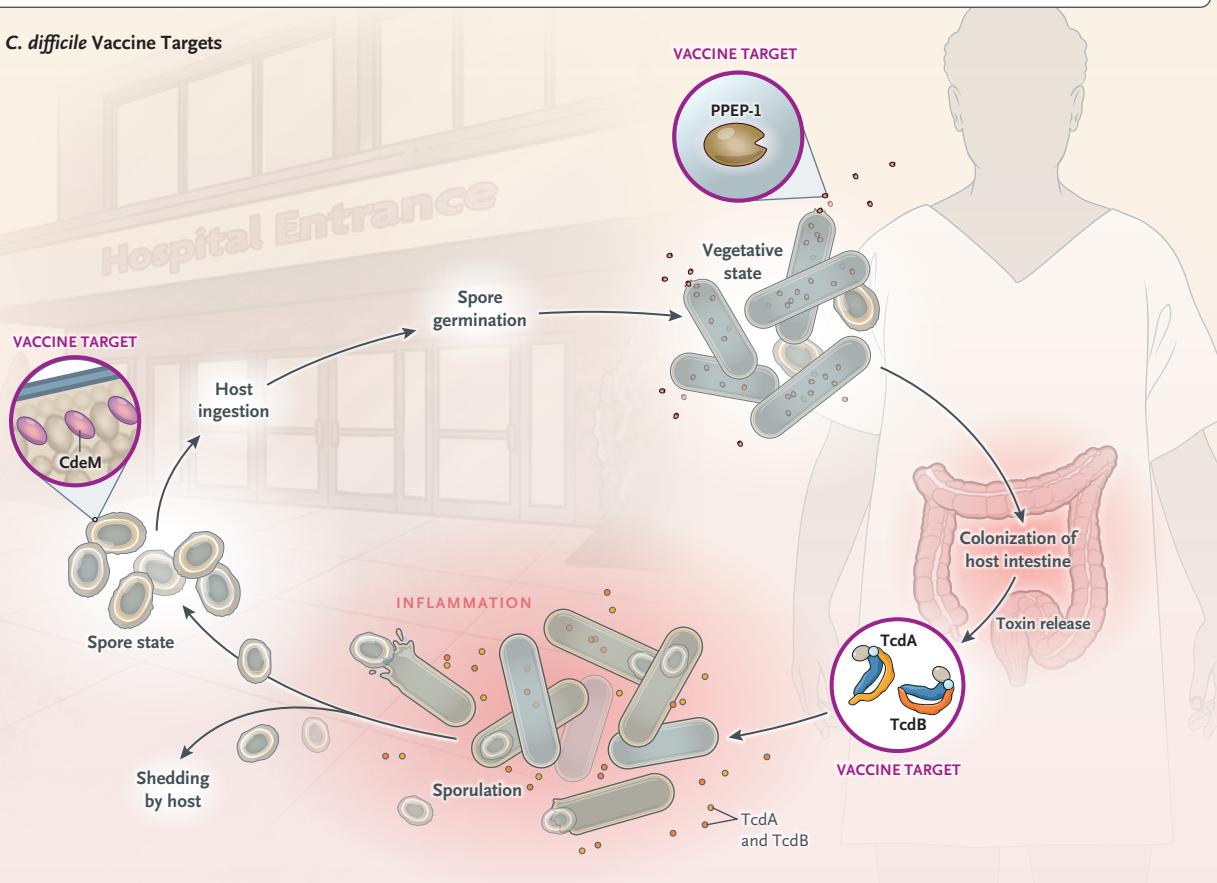
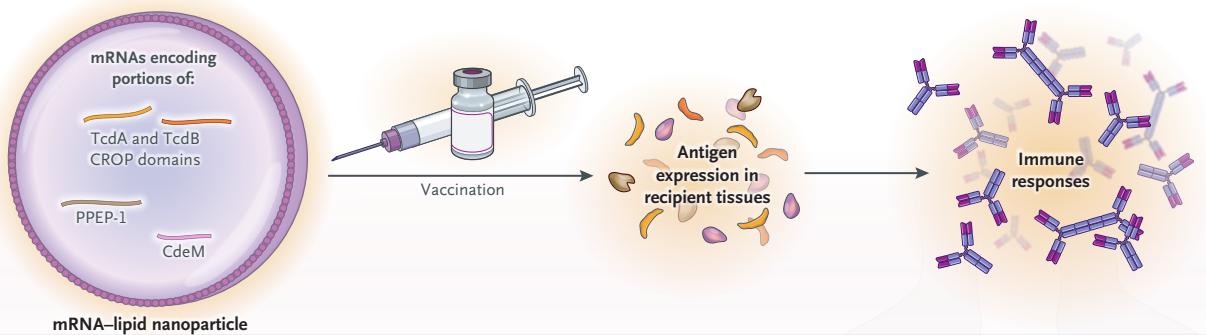
A Toxins Produced by *C. difficile***B *C. difficile* Vaccine Targets****C Immune Response against *C. difficile* Vaccine in Mice**

Figure 1 (facing page). Candidate Vaccine against *Clostridioides difficile* Infection.

The toxins TcdA (toxin A) and TcdB (toxin B) are the critical virulence factors leading to the intestinal damage and inflammation that characterize *C. difficile* infection. Binding to epithelial cells is mediated at least in part by the combined repetitive oligopeptide (CROP) domain at the C terminal of the toxin polypeptide, which is located separately from the glucosyltransferase domain that mediates cell toxicity (Panel A). The CROP domains of TcdA and TcdB, along with a secreted endopeptidase (Pro-Pro endopeptidase 1 [PPEP-1]) and a spore surface protein (CdeM), were chosen to target the key toxin virulence factors and surface molecules from vegetative bacteria and the spore form for a candidate vaccine (Panel B). The mRNAs encoding each of the vaccine targets were encapsulated in lipid nanoparticles (LNPs) to form an mRNA–LNP vaccine. Injection of the mRNA–LNP vaccine into mice resulted in antigen expression in recipient tissues that elicited an immune response to each of the antigens in the multivalent vaccine (Panel C). Injection of the quadrivalent vaccine into a rhesus macaque also elicited an immune response to each of the antigens.

In this setting, a new type of *C. difficile* vaccine candidate, described by Alameh, Semon, and colleagues,⁵ is of interest. These investigators developed a multivalent nucleoside-modified messenger RNA (mRNA) vaccine (see Key Concepts) delivered in lipid nanoparticles (LNPs). One mRNA encodes part of TcdA, and another mRNA encodes part of TcdB; each encodes two domains that are critical for the binding of each toxin to epithelial cells (Fig. 1A). In the initial vaccine formulation, Alameh and colleagues also included an mRNA encoding an additional *C. difficile* virulence factor, the metalloproteinase Pro–Pro endopeptidase 1 (PPEP-1), which enhances motility by degrading adhesion molecules on the surface of the pathogen (Fig. 1B). Encapsulated in LNPs, the mRNAs encoding TcdA, TcdB, and PPEP-1 (or portions thereof) represent a trivalent vaccine (Fig. 1C). The authors also made recombinant versions of the three vaccine targets and administered them with alum as an adjuvant. The trivalent mRNA–LNP vaccine and the recombinant vaccine were administered to mice.

The mRNA–LNP vaccine elicited higher antibody levels to all three vaccine targets than the recombinant vaccine with alum adjuvant. Furthermore, the mRNA–LNP vaccine provided complete protection against challenge with an intraperitoneally administered high dose of purified

TcdA or TcdB: all the vaccinated mice survived, whereas all the unvaccinated mice were moribund within 2 days. The recombinant–alum vaccines protected only 20% of the vaccinated animals.

Next, the investigators tested the ability of the multivalent mRNA–LNP vaccine to protect animals in a model of experimental *C. difficile* infection. Mice were pretreated with antibiotics and then challenged with spores of a virulent *C. difficile* strain. Unvaccinated control animals died from *C. difficile* infection within 2 days after the challenge; all the vaccinated animals survived. However, protection was not associated with the prevention of colonization: all the vaccinated animals shed high numbers of culturable *C. difficile* and had histopathological damage to intestinal tissue that was equivalent to that seen in unvaccinated animals according to analyses performed 2 days after infection. This finding suggests that protection was due to blocking of the systemic effects of the *C. difficile* toxins. However, additional data indicated that inclusion of the PPEP-1 antigen in the multivalent vaccine resulted in more rapid clearance of luminal toxin levels. A similar effect on the clearance of toxin was seen with an mRNA–LNP vaccine that was formulated with an antigen (CdeM) found on the surface of the *C. difficile* spore (Fig. 1B). These results suggest that the triggering of immune responses against the surface of the pathogen, in both the vegetative and spore states, limits toxin production. The immunogenicity of the quadrivalent vaccine in a nonhuman primate was tested by injection of the vaccine into a rhesus macaque. Two injections resulted in strong antibody responses to all four antigens.

The study by Alameh and colleagues is an advance not only for the development of vaccines against *C. difficile* but also for the development of vaccines against bacterial pathogens more generally. It is important to note, however, that the mRNA–LNP vaccine did not prevent colonization by *C. difficile* or intestinal inflammation in animals treated with antibiotics. The overall benefit of this new vaccine was in decreasing the severity of disease rather than in disease prevention. Perhaps the inclusion of mRNA encoding different antigens from the spore or vegetative forms of *C. difficile* would protect against colonization.

The rapid development of the SARS-CoV-2 vaccine and the quick refinement of subsequent versions that target emerging variants has al-

ready proved the power of the mRNA vaccine platform for viral infections. Alameh and colleagues have demonstrated that this platform can be used to target an important bacterial pathogen — a finding that should lead the way to future vaccine development.

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

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