A Potential New Tool for Managing *Clostridium difficile* Infection

Glen D. Armstrong,1 Dylan R. Pillai,1,2,3,6 Thomas J. Louie,3,5 Justin A. MacDonald,4 and Paul L. Beck2

1Department of Microbiology, Immunology, and Infectious Diseases, 2Department of Pathology and Laboratory Medicine, 3Department of Medicine, and 4Department of Biochemistry and Molecular Biology, University of Calgary, 5Alberta Health Services Infection Control Program, and 6Calgary Laboratory Services, Calgary, Canada

(See the major article by Howerton et al on pages 1498–504.)

*Clostridium difficile*, a strictly anaerobic, motile, gram-positive, spore-forming bacterium, is responsible for 15%–20% of hospital-acquired antibiotic-associated colitis cases and nearly all cases of pseudomembranous colitis, a more serious condition [1, 2]. *C. difficile* infection (CDI) occurs when the normal microflora populating the human gastrointestinal (GI) tract is altered in composition and total numbers, a condition referred to as dysbiosis [3]. Antibiotics, particularly clindamycin, the amino-penicillins, or the cephalosporins, to which *C. difficile* is inherently resistant [4], render a host species vulnerable to opportunistic colonization by *C. difficile* because the normal microflora, or microbiome, competitively excludes these organisms from the human GI tract.

Once established in the lower bowel, *C. difficile* produces 2 large exotoxins (TcdA and TcdB), which are primarily responsible for the clinical symptoms of CDI [5]. For example, *C. difficile* toxins trigger the inflammasome-dependent release of interleukin 1β (IL-1β), which contributes to inflammation and intestinal injury. The effect of the toxins on the inflammasome is entirely consistent with the elevated levels of IL-1β and correlates with disease severity in patients with CDI [6]. In addition to exotoxins, *C. difficile* may express additional toxins and important virulence factors, such as fimbrial adhesins (type IV pili) and other cell wall-associated proteins, but their role in the *C. difficile* pathogenic program is less well understood.

Death from CDI is primarily due to systemic toxicity, shock, and multiorgan failure as a result of perforation of an edematous, friable, and leaky bowel. In a piglet CDI model [7], measurable amounts of lethal exotoxins are detected in the circulation of moribund animals. If toxin were to be similarly detected in severely ill CDI patients, such a finding would correct the classical notion that CDI is a benign mucosal disease.

Outbreaks of hypervirulent “epidemic” strains of *C. difficile* are now contributing to a worsening problem, with an aging population and diminishing health care resources [8, 9]. This epidemic strain is classified as toxinotype III, REA type BI, and North American pulsed-field gel electrophoresis type 1 (NAP1) [10]. It is less responsive to conventional CDI therapeutic strategies and contains 234 additional genes, compared with the nonepidemic genome sequence clinical 630 strain [11–13]. These additional genes may contribute to phenotypic differences relating to cell wall structures, motility, antibiotic resistance, and toxicity between epidemic and nonepidemic strains [13]. Newer diagnostic tests using molecular methods are more sensitive and capable of identifying the NAP1 strain earlier, which may affect management and infection control [14]. The additional genes may also be related to the ability of *C. difficile* to spread in the environment and contribute to an increased incidence of non–hospital-associated community-acquired CDI.

For individuals with relatively mild symptoms, the first step in present treatment is to discontinue the implicated antibiotic, if possible, and administer fluids and electrolytes to maintain a balanced state of hydration until the patient recovers. In many cases, however, antibiotic therapy, typically metronidazole or vancomycin, is started to treat CDI. Although metronidazole or vancomycin treatment results in the complete recovery of approximately 80% of subjects without further complications, the remainder may experience multiple episodes of recurring CDI. At present, the risk factors for recurring CDI are not well defined; however, clinical recurrence may happen when the normal GI microbiome fails to become reestablished in time to prevent reinfection with *C. difficile*. Recurring CDI is often treated with tapering regimens of vancomycin, a strategy of unproven efficacy. Both metronidazole and vancomycin are nonspecific
Drugs, resulting in further disruption of the already impaired normal microbiome. In the posttreatment period, the combination of an impaired host microbiome, which is slow to return to normal, and pathogen persistence, primarily as spores, is a leading cause of disease recurrence. Moreover, the importance of prudent antibiotic use has become paramount, but it is difficult to implement in settings where empirical treatment requires broad-spectrum antibiotics. This issue was recently highlighted in a study demonstrating that resistance to fluoroquinolones, especially the newer-generation drugs in this class, may be the defining selective advantage of the epidemic NAP1/O27 strain [15].

In addition to standard clinical practice, many experimental approaches have been and continue to be investigated [16, 17]. Examples include (1) C. difficile–targeted antimicrobial agents, such as fidaxomicin (Optimer Pharma) [18], REP3123 (Replidyne; now off study) [19], and CB-183,315 (Cubist Pharmaceuticals) [20], that are more sparing of the normal GI microbiome; (2) exotoxin-neutralizing agents such as tolevamer (Genzyme) [21], passive systemic immunization that uses humanized mouse monoclonal exotoxin-neutralizing antibodies (Medarex/Merck) [22] in addition to C. difficile cell wall-associated components; (3) oral immunotherapeutic C. difficile–specific egg yolk (IgY) antibodies [23]; (4) active immunization with C. difficile toxoid preparations [24, 25]; and (5) ingestion of a nontoxicogenic C. difficile strain [26] or various other bacterial or fungal probiotic preparations. The role of passive immunization by oral ingestion of binary toxin–specific bovine colostrum antibodies or milk whey containing C. difficile–specific antibodies has also been investigated in animal CDI models [27, 28].

For several decades, clinicians have also resorted to fecal bacteriotherapy, or fecal microbiome transplantation (FMT), as a means of arresting multiple recurrent CDI. This approach represents a paradigm shift in treating infections, with the objective of restoring the normal gut microbiome, rather than eradicating the pathogen by using a conventional antibiotic. Anecdotal reports, as well as meta-analyses, suggest a very high success rate for FMT in patients who do not respond to conventional therapy. Furthermore, the American Gastroenterological Association has provided guidelines on how best to conduct FMT [29]. In formal polls of medical centers across North America report that several dozen now conduct FMT. Unfortunately, use and regulatory acceptance has lagged behind the clinical need, and research has been hampered by an imperfect understanding of the role played by the complex GI microbiome in sickness and health. This lack of understanding represents a significant barrier to universal acceptance of FMT. However, with the advent of rapid DNA sequencing technologies, it is becoming possible to obtain a higher-resolution picture of the human GI microbiome. Combined with studies in mice and humans, microbiome interrogation techniques are beginning to reveal that successful FMT results in a radical shift in phylogenetic diversity of the GI microbiome and that this change is associated with clinical cure from CDI [30, 31].

The article by Abel-Santos et al in this issue of the Journal presents another very promising strategy for preventing or treating CDI. The authors predicate their study on the observation that current management practices focus on the vegetative cell and treating established illness. In contrast, their report is based on the understanding that C. difficile spore germination is required for symptomatic infection and, consequently, that antigermination tactics could represent an approach to preventing CDI. It is also founded on earlier studies demonstrating that a bile salt, taurocholate, and the amino acid glycine activate the germination program in C. difficile spores [32, 33].

In a more recent publication, Howerton et al used chemical analogs to map the interactions between germination promoters, such as glycine and taurocholate, and C. difficile spores [34]. These studies revealed that both the carboxy and amino functions of glycine are important for initiating the germination program in C. difficile spores and that the glycine-binding site on the spore is permissive for more widely separated carboxy and amino termini in addition to other hydrophobic amino acids. They also demonstrated that C. difficile spores are able to detect taurocholate analogs with shorter, but not longer, alkyl amino sulfonic acid side chains and that the sulfonic acid group can be partially substituted with other acidic functions. Importantly, the mapping studies demonstrated that a cholate meta-benzene sulfonic (CamSA) derivative was a strong inhibitor of C. difficile spore germination.

In the present report, the authors convincingly demonstrate that CamSA, when orally delivered 24 hours prior to challenge, protected antibiotic-treated mice from 10^9 C. difficile 630 or VPI 10463 spores in a dose-dependent manner. Furthermore, CamSA at the highest dose tested (50 mg/Kg body weight) completely protected mice from CDI when administered at the same time as infection, whereas the same dose delivered 24 hours prior to infection was only partially effective. Given the compound’s demonstrated antigermination activity in vitro, in addition to its dose-dependent efficacy profile in vivo, it is perhaps not surprising that CamSA demonstrated greater potency when it was administered concurrently with the challenge dose of C. difficile spores. Moreover, recovered mice in the dose-ranging study failed to relapse when treated with a second course of antibiotics, indicating that most, if not all, C. difficile organisms from the challenge dose had been eliminated from the intestines of mice over the course of the experiment. Also consistent with the
primary hypothesis, CamSA failed to protect mice from a challenge dose of vegetative C. difficile bacteria, and mice challenged with spores and then treated with the highest dose of CamSA shed spores almost exclusively in their feces for up to 96 hours after challenge. All these observations are consistent with the hypothesis that CamSA protected mice from CDI by preventing the administered C. difficile spores from germinating in their intestines.

These results presented by Abel-Santos et al therefore support another paradigm shift in CDI management strategies, one involving a prophylactic approach that uses the antigermination effect of taurocholate-based drugs such as CamSA. This novel approach could represent a significant advance in treating CDI or recurring CDI, particularly in at-risk individuals, if the encouraging results eventually translate to use in human subjects. It also represents another welcome departure from the use of antibiotics, thereby lessening the problems associated with organisms acquiring and transmitting resistance determinants to these drugs.

Note

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References