New Perspectives in *Clostridium difficile* Disease Pathogenesis

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KEYWORDS

• *Clostridium difficile* • Virulence factors • Spore • Host response • Microbiota

KEY POINTS

- *Clostridium difficile* infection pathogenesis is a multifactorial process involving a complex interplay between bacterial virulence factors, the intestinal microbiota and host immune factors.
- *C difficile* spores are the principal vehicle of transmission, infection, and persistence.
- Deficiencies in innate and adaptive immune defense mechanisms affect disease outcomes.
- Inhibiting the function of the toxins, targeting specific host inflammatory pathways, and/or manipulating the intestinal microbiota may offer adjunctive treatments to current antimicrobials.

INTRODUCTION

Clostridium difficile is a gram-positive, endospore-forming, anaerobic, gastrointestinal pathogen that is the leading worldwide cause of hospital-acquired infective diarrhea.[1] *C difficile* exerts its major pathologic effects through the action of its 2 principal virulence factors, toxin A (TcdA) and toxin B (TcdB). The importance of these homologous exotoxins to *C difficile* pathogenesis is extensively supported by *in vitro* studies using epithelial cell lines derived from human colon cancer and small animal models,[2,3] as well as reports showing that *C difficile* clinical isolates lacking both toxin genes are nonpathogenic in humans and animals.[4–6] In addition to pathogenic toxin production, the composition and function of the intestinal microbiome and host immune factors have direct impacts on *C difficile* pathogenesis. This article highlights recent developments in the understanding of *C difficile* infection (CDI) pathogenesis.

Pathogenicity Locus

The genes encoding TcdA (*tcdA*) and TcdB (*tcdB*) are found on the pathogenicity locus (PaLoc), a 19.6-kb chromosomal region that also contains 3 further genes: *tcdR*,

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encoding an RNA polymerase sigma factor that positively regulates toxin expression[7]; *tcdC*, considered a corresponding negative regulator, although still a matter of current debate[8–11]; and *tcdE*, which is related to the bacteriophage holins.[12] In addition to *tcdR* and *tcdC*, factors outside the PaLoc, including CodY,[13] a common transcriptional regulator in gram-positive organisms, and the carbon catabolite repression system, involving the catabolite control protein, CcpA,[14] also participate in the regulation of toxin synthesis. Recent phylogenetic analyses reveal that the PaLoc resembles a mobile genetic element that has a complex evolutionary history with distinct PaLoc variants acquiring clade specificity after divergence.[15] These PacLoc variants, referred to as toxinotypes, include variants with intact genes but sequence changes, forms with truncated *tcdA*, variants of *tcdB*, and forms with *tcdC* encoding mutations and deletions.[16]

Mechanism of Action and Functional Domains of Toxin A and Toxin B

Similar to other members of the large clostridial family of toxins, TcdA and TcdB target the Rho/Ras superfamily of GTPases by irreversible modification through glucosylation at Thr-35/Th-37.[6] Because GTPases are key cellular regulatory proteins, their permanent inactivation within intoxicated epithelial cells leads to dysregulation of actin cytoskeleton and tight junction integrity, intestinal epithelial cell damage, and apoptosis by caspase activation.[6]

Both toxins are single-polypeptide chain, high-molecular-weight exotoxins arranged into large multidomain and functionally distinct structures represented schematically in **Fig. 1**. The molecular mode of action of the toxins is not completely understood. Based on current data, toxins seem to bind to an as-yet unidentified receptor and enter cells through receptor-mediated endocytosis.[17] Once inside the acidic endosomal compartment, a decrease in pH causes conformational changes within the toxin, allowing pore formation and subsequent translocation of the catalytic glucosyltransferase domain across the endosomal membrane. Knowledge of exactly how this process occurs and which regions of the translocation domain are critical for this process is starting to emerge. Recent findings have uncovered the pore-forming hotspot of the TcdB translocation domain, clustered between amino acid residues 1035 and 1107, which, when individually mutated, reduces cellular toxicity by greater than 1000-fold.[18] Release of the glucosyltransferase enzymatic moiety into the cytosol occurs by an autoproteolytic cleavage event, which is thought to involve exposure to the cysteine protease domain and requires inositol hexakisphosphate (InsP6).[19,20]

The relative importance of each toxin in disease pathogenesis is still a matter of debate. Both toxins seem to be lethal in animal challenge models, supported further by *C difficile* genetic manipulation studies reporting that $TcdA^{-}TcdB^{+}$ and $TcdA^{+}TcdB^{-}$ mutants of *C difficile* caused disease in hamsters.[21,22] Nevertheless, an earlier report generating equivalent mutants in the same *C difficile* strain found that only TcdB was essential for virulence, whereas TcdA was dispensable.[23] In support of the dominant role of TcdB, all naturally occurring pathogenic strains produce TcdB (but not necessarily TcdA), suggesting that TcdB may play the dominant role in human infection.[23–25]

Other Clostridium difficile Virulence Components

Some *C difficile* strains (eg, 027 and 078 ribotypes) also produce an adenosine diphosphate ribosyltransferase toxin, commonly referred to as *C difficile* binary toxin (CDT). This toxin is composed of an enzymatically active A component (CDTa), which causes ADP-ribosylation of G-actin, and a cell-binding and translocation B