Pseudomembranous colitis and *Clostridium difficile* infection

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**Introduction**

*Clostridium difficile* was first identified in 1935 as a commensal organism in the fecal flora of healthy neonates [1]. The organism was given its name because it grew very slowly in culture and was difficult to isolate. Although it produced cytoxins, and was pathogenic for guinea-pigs and rabbits, the organism was considered part of the normal neonatal gut flora that disappeared after weaning. *C. difficile* remained a laboratory curiosity until 1978, when Bartlett and colleagues [2] identified it as the source of a cytotoxin found in the stool of patients with antibiotic-associated pseudomembranous colitis. Since that time the incidence of *C. difficile* infection has increased dramatically and the organism is now recognized as the most frequent cause of nosocomial infectious diarrhea in developed countries [3–5]. Incidence rates of nosocomial infection range from 0.1 to 30 per 1000 hospital admissions in non-epidemic settings [6–11]. Over 30% of high-risk patients, such as those admitted to acute-care general medical wards and receiving antibiotics, may be colonized with *C. difficile* [12, 13]. In community populations the reported prevalence of *C. difficile*-associated diarrhea ranges from 8 to 12 per 100 000 person-years [14, 15].

As shown in Fig. 1, the sequence of events leading to *C. difficile* diarrhea and colitis in susceptible individuals comprises disturbance of the normal colonic microflora, exposure to and colonization by *C. difficile*, toxin production and toxin-mediated intestinal injury and inflammation. Depending on host factors, especially the immune response to *C. difficile* toxins, the outcome of colonization is either asymptomatic carriage or a spectrum of disease ranging from mild diarrhea to life-threatening pseudomembranous colitis.

During the two decades since its identification as a pathogen our understanding of the epidemiology, pathogenesis and management of disease caused by *C. difficile* has increased dramatically. Yet despite this increased knowledge there has been no substantial decline in the frequency of hospital-acquired *C. difficile* diarrhea and colitis.

**Clostridium difficile**

*Clostridium difficile* is a Gram-positive, obligate anaerobic rod that grows best in selective media containing cycloserine and cefoxitin and enriched with fructose and egg yolk [16]. This selective medium can detect as few as 2000 organisms in a stool sample. Individual strains of *C. difficile*, identified by agglutinating antisera [17], or DNA fingerprinting [18], may differ with regard to virulence [19]. Hospital outbreaks have been attributed to toxin-producing epidemic strains [20]. The organism forms spores, allowing it to survive in harsh environments and withstand antibiotic therapy.
Pathogenesis

*C. difficile* diarrhea is a toxin-mediated disease. Pathogenic strains of *C. difficile* produce two potent protein exotoxins, toxin A and toxin B. These toxins are encoded by two distinct genes in close proximity on the bacterial genome [21–23]. They are structurally similar and show 49% homology at the amino acid level [22]. These high molecular weight proteins are believed to bind receptors on the luminal aspect of the colonic epithelium and are then transported into the cytoplasm. However, specific cell surface receptors for toxin A or toxin B have not yet been characterized. In rabbit ileum the brush-border ectoenzyme, sucrase-isomaltase, binds *C. difficile* toxin A and functions as a cell surface receptor [24]. Since this enzyme is not present in human colonic mucosa, other membrane surface glycoproteins presumably serve as toxin receptors. Both toxins potently activate cell signaling molecules including NF-κB and MAP kinases in human monocytes, leading to the production and release of proinflammatory cytokines including IL-1β, TNF-α, and IL-8. These proinflammatory effects appear to precede toxin internalization and may be mediated by cell surface receptor binding [24, 25].

The aminoterminal regions of both toxins carry a series of repeated protein sequences that are believed to mediate toxin binding to the host cell membrane, while the carboxyterminal regions of both toxins possess similar glucosyltransferase activity. Once internalized, both toxins inactivate Rho proteins, a family of small GTP-binding proteins. The critical enzymatic action is the glycosylation of a specific, conserved threonine amino acid on Rho [26, 27]. The Rho protein targets of toxins A and B are rhoA, rac and cdc42, key cell signaling molecules that direct gene expression and are essential to maintain the actin cytoskeleton. Consequently, toxin-mediated Rho inactivation results in depolymerization of actin filaments, disruption of the cytoskeleton, cell rounding and cell death [24, 28]. In contrast to cholera toxin or *E. coli* heat-stable toxin, *C. difficile* toxins have no effects on intracellular levels of cyclic AMP or GMP. However, a number of other bacterial toxins target Rho proteins in a similar manner [29]. For example the cytotoxins from *C. sordellii* and *C. novyi* add a glucose to Rho and toxins from *Bacillus cereus* and *Staphylococcus aureus* also modify Rho family proteins. Thus it appears that *C. difficile* toxins and other structurally unrelated bacterial cytotoxins modify host cell structure and function by attacking Rho family proteins that are vital for maintenance of normal cell architecture and function.

Toxin A is an inflammatory enterotoxin that induces fluid secretion, increased mucosal permeability and marked enteritis and colitis when injected into the intestinal lumen of animals [24]. Toxin A also possesses weak cytotoxic activity against cultured cells [30, 31]. Although toxin B is an extremely potent cytotoxin, it has no enterotoxic activity in animal intestine *in vivo* [28, 30, 32, 33]. This led to the widely held belief that toxin B did not participate in the pathogenesis of *C. difficile* diarrhea and colitis in humans. However, recent evidence appears to contradict this hypothesis and suggests that toxin B may indeed be pathogenic in humans. First, toxin A and toxin B both cause injury and electrophysiological changes in human colonic strips *in vitro*. Toxin B is 10 times more potent than toxin A in inducing these changes [34]. Second, there have been reports of the isolation of toxin A-negative/toxin B-positive strains of *C. difficile* from patients with antibiotic-associated diarrhea and colitis [19, 35–38]. Toxin A-negative/toxin B-positive strains accounted for 3% of clinical isolates referred for typing to the Public Health Laboratory Service Anaerobic Reference Unit in England and Wales [19].

Both toxins of *C. difficile* bind to and damage human colonic epithelial cells [34]. *C. difficile* toxins produce colonic injury as a result of damage to the enterocyte cytoskeleton and disruption of tight junction function [34, 39]. The toxins also cause a severe inflammatory reaction in the lamina propria with the formation of microulcerations of the colonic epithelium that are covered by an inflammatory pseudomembrane. A characteristic of *C. difficile* infection is the intense acute neutrophilic inflammation seen in pseudomembranous colitis patients and in animal models of the disease. In contrast to cholera toxin, which stimulates massive intestinal fluid secretion without a significant inflammatory response, *C. difficile* toxin A stimulates fluid secretion accompanied by considerable mucosal edema, inflammatory cell infiltration and necrosis.

Interactions between neuropeptides and inflammatory mediators released from inflammatory cells of the intestinal lamina propria and from epithelial cells are also critical initiators of the toxin A-induced inflammatory process (Fig. 2). Pothoulakis and colleagues reported the release of the neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) from sensory nerves, and degranulation of mast cells within 15 min of luminal application of