little clinical utility except to track hospital outbreaks.[5]

Pathophysiology of *Clostridium difficile* Colitis

A critical chain of events is necessary to induce *C. difficile* colitis. Following antibiotic therapy the normal intestinal flora is disrupted allowing *C. difficile* to colonize the intestinal lumen. The fact that infection occurs only after the normal microbial intestinal flora is altered by administration of antibiotics suggests that certain organisms in the normal flora prevent colonization by *C. difficile*.[6] For example *Bacteroides* accounts for over 90% of the total fecal flora, and some of these species disappear in patients with *C. difficile* diarrhea and colitis, whereas recovery from the infection is accompanied by repopulation with *Bacteroides*.[7] Almost all antibiotics including vancomycin and metronidazole which are generally used to treat patients with active colitis[8] and even some cancer chemotherapeutic agents can predispose to colonization by *C. difficile*.[9] However some antibiotics are more likely to be associated with symptomatic *C. difficile* infection, probably due to differential effects on the normal colonic flora.[10] Clindamycin is the most common antibiotic associated with infection and diarrhea, however broad spectrum penicillins and cephalosporins are also commonly associated with *C. difficile* colitis.

When pathogenic strains of *C. difficile* colonize the colon they release two unique protein exotoxins, toxin A (308 kDa) and toxin B (275 kDa) (Table 1).[11] These toxins have no structural or functional similarities with the classic bacterial enterotoxins, such as cholera toxin, *E. coli* enterotoxin or shiga toxin.[12] *C. difficile* toxins posses potent cytotoxic activity against cultured fibroblast and other cells.[13] The cytotoxic effect results in cell rounding which is due to disaggregation of filamentous actin and dysfunction of tight junctions caused by inactivation of the small GTP-binding protein Rho, which is required to maintain actin integrity.[14] Both toxins bind to glycoprotein receptors on the human colonocyte brush border and cause necrosis and shedding of epithelial cells into the lumen.[15] Toxin A, but not toxin B, mediates inflammatory diarrhea in experimental animals.[11] *In vivo* studies in rodents showed that injection of purified toxin A into closed intestinal loops elicits an acute inflammatory diarrhea with massive fluid secretion, mucosal damage and a prominent neutrophils infiltration which is evident 2–4 hours after injection of the toxin.[8,11]

Table 1 *Clostridium difficile* Toxins A and B

	Toxin A	Toxin B
Molecular Weight	308 kDa	275 kDa
Receptor	Glycoprotein	Unknown
Cytotoxicity	+	100-fold more potent than Toxin A
Molecular Mechanisms	Rho-glucosylation	Rho-glucosylation
Neurotoxic	++	++
Enterotoxic:		
experimental animals	++	no
human colon	+	++

The pathogenesis of *C. difficile* toxin A inflammation involves interaction between epithelial cells, sensory nerves and inflammatory cells of the intestinal lamina propria. Sensory nerves appear to be involved in the early stages of the inflammatory cascade induced by *C. difficile* toxin A.[16] Several studies showed that the release of the peptide substance P from intestinal sensory nerves is a critical early step in the inflammatory cascade.[17,18] This peptide appears to activate macrophages and mast cells of the lamina propria, which in turn release inflammatory mediators that up-regulate the expression of adhesion molecules on endothelial cells and neutrophils, mediating neutrophil recruitment and migration into the intestinal mucosa. Neutrophil-derived inflammatory mediators are major elements in the pathogenesis of *C. difficile* colitis causing acute destruction and necrosis of enterocytes. The importance of neutrophils is supported by experiments showing that preventing neutrophil recruitment during toxin A-mediated colitis markedly reduced intestinal inflammation, epithelial damage and diarrhea.[19]

Pathology of *Clostridium difficile* Infection

On sigmoidoscopic inspection of the colonic or rectal mucosa of patients with *C. difficile* colitis, pseudomembranes appear as yellow or off-white raised plaques of 0.2 to 2 cm in diameter scattered over a fairly normal or hyperemic intervening mucosa. The exposure of human colon to *C. difficile* toxins is followed by shedding of cells from the basement membrane into the lumen leaving a shallow ulcer on the mucosal surface. Then serum protein, mucus and inflammatory cells flow outward from the ulcer, creating the typical colonic pseudomembrane. The spewing forth of the inflammatory exudate from the mucosal ulcerations produces the typical "volcano" or "summit" lesions of *C. difficile* colitis. The patchy distribution of the pseudomembranes is probably related to a toxin-dose response effect. Exposure *in vitro* of colonic mucosa to low toxin B concentrations causes patchy cellular damage, whereas at higher toxin concentrations the damaged area becomes nearly confluent.[20]