

***Clostridium difficile* Toxin B Is More Potent than Toxin A in Damaging Human Colonic Epithelium In Vitro**

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Abstract

Toxin A but not toxin B, appears to mediate intestinal damage in animal models of *Clostridium difficile* enteritis. The purpose of this study was to investigate the electrophysiologic and morphologic effects of purified *C. difficile* toxins A and B on human colonic mucosa in Ussing chambers. Luminal exposure of tissues to 16–65 nM of toxin A and 0.2–29 nM of toxin B for 5 h caused dose-dependent epithelial damage. Potential difference, short-circuit current and resistance decreased by 76, 58, and 46%, respectively, with 32 nM of toxin A and by 76, 55, and 47%, respectively, with 3 nM of toxin B, when compared with baseline ($P < 0.05$). 3 nM of toxin A did not cause electrophysiologic changes. Permeability to [³H]mannitol increased 16-fold after exposure to 32 nM of toxin A and to 3 nM of toxin B when compared with controls ($P < 0.05$). Light and scanning electron microscopy after exposure to either toxin revealed patchy damage and exfoliation of superficial epithelial cells, while crypt epithelium remained intact. Fluorescent microscopy of phalloidin-stained sections showed that both toxins caused disruption and condensation of cellular F-actin. Our results demonstrate that the human colon is ~10 times more sensitive to the damaging effects of toxin B than toxin A, suggesting that toxin B may be more important than toxin A in the pathogenesis of *C. difficile* colitis in man. (*J. Clin. Invest.* 1995. 95:2004–2011.) Key words: *C. difficile* toxins • toxin-mediated enteritis • actin filaments • transepithelial resistance • antibiotic-associated colitis

Introduction

Clostridium difficile, the causative agent of antibiotic-associated colitis in animals (1, 2) and humans (3, 4), releases two high molecular weight exotoxins: toxin A and toxin B (5, 6). Although numerous animal studies have shown that *C. difficile* colitis is toxin-mediated, the exact role of toxin A and B in pathogenesis, especially in humans, remains unclear. Both toxins possess cytotoxic activity against a variety of cultured cells (6–9) and cause death when injected parenterally in laboratory

animals (10). However, experiments in rabbits (11, 12), rats (13), mice (14), and hamsters (15) suggest that only toxin A is capable of damaging the intestinal mucosa in vivo. For example, previous in vivo studies demonstrated that toxin A, but not toxin B, caused severe inflammation, epithelial damage and fluid secretion when injected into rabbit ileal loops in vivo (11, 12). Mitchell et al. showed that toxin A caused epithelial damage in rabbit colon, while toxin B had no effect (11). Lyerly et al. also reported that intragastric administration of toxin A, but not toxin B, to hamsters and mice caused hemorrhagic secretion in the small intestine unless the bowel was first damaged before toxin B exposure (15). Moreover, hamsters immunized against toxin B alone were not protected from clindamycin colitis, whereas animals immunized against toxin A, or a combination of toxins A and B were protected (16). Taken together, these results indicate that toxin A causes intestinal damage in animal models, while toxin B is innocuous when placed in the lumen.

While lacking an enterotoxic effect in experimental animals, toxin B is extremely cytotoxic to cultured mammalian cells (6, 17), including those derived from the human intestine (19). Recently Hecht et al. demonstrated, that toxin B (19), like toxin A (20), perturbs the cytoskeleton of human colonic cancer T84 cell monolayers causing an increase in permeability and a decrease in transepithelial resistance. However, the possibility remains that the T84 cell-line derived from a colonic adenocarcinoma does not accurately reflect the response of the normal human colon to toxin B.

The purpose of this study was to compare the effects of highly purified toxins A and B on human colonic mucosa mounted in Ussing chambers. We report here that toxins A and B disrupt the epithelial barrier and cause epithelial cell necrosis and electrophysiologic changes of the human colon in vitro, but that toxin B is more effective than toxin A in producing these effects.

Methods

Toxin preparation. Toxins A and B were purified to homogeneity as previously described (6, 21). Enterotoxic activity of toxin A was assayed in rat ileal loops (22–24) and cytotoxic activity of both toxins was tested against IMR-90 fibroblasts as previously (6, 21). Purity of toxins A and B were determined by SDS-PAGE (6, 21). Molar concentrations of toxins A and B were based on molecular weights of 308 kD for toxin A (25) and 279 kD for toxin B (26).

Experimental design. Human colonic mucosal sheets were first equilibrated in Ussing chamber with buffer alone for 60 min. Luminal solution was then replaced with buffer containing 16–65 nM of toxin A, or 0.2–29 nM of toxin B for 5 h. Control tissues were incubated for 5 h in buffer only. In an additional series, colonic explants from a single individual were paired and exposed to buffer alone, 3 nM of toxin A and 3 nM of toxin B, respectively, for 5 h ($n = 4$, paired tissues).

Ussing chamber measurements. Colonic mucosae were obtained from patients who underwent surgery for colorectal cancer. In this study

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a total of 50 individual specimens of histologic tumor-free sigmoid colon was used. After removal of the seromuscular layer by blunt dissection 1–5 mucosal sheets from each specimen measuring 4–10 cm² were mounted horizontally in custom-made incubation chambers with the luminal surface (1 cm² surface area) facing upwards as previously described (27). Luminal and serosal side were bathed at 37°C with a nutrient buffer containing (mM/liter): 122.0 NaCl, 2.0 CaCl₂, 1.3 MgSO₄, 5.0 KCl, 20.0 glucose, 25.0 NaHCO₃ (pH = 7.51 when gassed with 95% O₂/5% CO₂; temperature 37°C). The upper level of fluids in both luminal and serosal reservoirs was identical. Potential difference (PD)¹ and short-circuit current (Isc) were continuously recorded every 10 min. Luminal and serosal solutions were connected via Ag-AgCl electrodes and Ringer-agar bridges to a voltmeter (Model DVC-1000 Voltage/Current Clamp; World Precision Instruments, Inc., New Haven, CT). Resistance (R) was calculated using Ohm's law from the open-circuit PD and the Isc. PD values were given in mV, Isc in $\mu\text{A}/\text{cm}^2$, and R in Ohm.cm². PD values were corrected for the junction potentials (< 0.1 mV) between the luminal and serosal solutions.

Epithelial permeability to [³H]mannitol was determined under short circuit conditions. After an equilibration period of 60 min [³H]mannitol (26.4 Ci/mmol; Dupont-NEN, Boston, MA) was added to the serosal side at a final concentration of 0.32 nM. After toxin exposure, luminal aliquots of 100 μl were taken for liquid scintillation-counting using 5 ml of "Quicksafe A" scintillation fluid (Zinsser, Maidenhead, UK). The radioactivity of [³H]mannitol in the luminal fluid was measured in counts per minute (cpm) and was assessed at 1, 3, and 5 h in the presence or absence of 16 and 32 nM of toxin A or 1.5 and 3 nM of toxin B in the luminal chamber.

Morphometry. After Ussing chamber experiments tissues were processed for light microscopy. Mucosal damage was assessed by an histopathologist (R. Sedivy), who performed morphometry on coded, paraffin embedded, H&E stained slides as previously described (27), and was "blinded" as to the experimental conditions to which a particular tissue had been exposed. The surface area of each tissue mounted into chambers was 1 × 1 cm. Morphometry was performed on nine vertical sections from different locations each of which representing the total height and length (1 cm) of the mucosal preparation. Thus a total length of 8–9 cm of mucosal surface was examined for each tissue. We used a Leitz Diaplan research microscope (Wild Leitz Ltd, Heerbrugg, Switzerland; objective magnification, ×4, ×6.3, ×16, ×25, ×40; ocular magnification, ×12.5), a Panasonic color CCD video camera (model WV-CD 130/G; Matsushita Com. Ind. Co. Ltd., Japan) an analog/digital monitor screen (model PVM-1271Q, superfine pitch; Sony, Japan), a personal computer (IBM-PS/2, model PS2; IBM, Armonk, NY), extended by insertion of a frame grabber (ITI PCVision plus board; Imaging Technology Corp., Woburn, MA) and a graphic tablet (Summasketch plus 12" × 12" MM 1201; Summagraphics Corp., Fairfield, CT). Downloading "MIPSY" (The Micro-based Image Processing System[®]) on the personal computer, real-time morphometry was performed. The extent of epithelial damage was measured in micrometers and expressed as percent (%) of total mucosal surface.

Histological criteria for epithelial damage were: reduced staining intensity of epithelial cells, karyopyknosis, karyolysis and karyorrhexis, cell disruption, formation of subepithelial blebs and lifting off of cells from the basal lamina (27).

Fluorescent microscopy. Fluorescent staining of F actin was performed using rhodamine-labeled phalloidin (Molecular Probes, Inc., Eugene, OR) on fresh-frozen tissue. Sections (4 μm) were fixed in 4% paraformaldehyde (pH 8.0), washed in phosphate buffered saline solution (pH 8.0; PBS) and incubated for 45 min. Subsequently, slides were mounted with Glycerol:PBS/1:9 and examined and photographed using a Zeiss Axiophot fluorescence microscope.

Processing for scanning electron microscopy (SEM). Tissues were washed in 0.1 M cacodylate buffer pH 7.3 and postfixed for 90 min in the same buffer containing 1.0% buffered osmium tetroxide. Samples

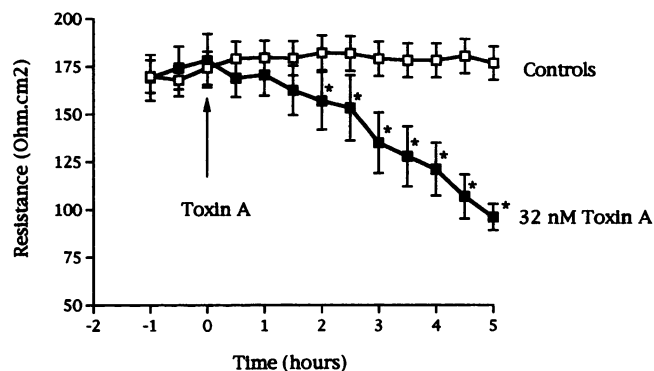


Figure 1. Time course of toxin A on colonic resistance. Human colonic mucosal sheets were incubated continuously with either buffer alone (control) or toxin A for 5 h ($n = 6$ per group). Values represent mean \pm SEM per group. (* $P < 0.05$, when compared with controls).

were washed again in the buffer and dehydrated in graded series of ethanol. Samples were dried by means of the critical point method (Polaron) and coated with gold up to a thickness of 400 Å in a sputter coating unit (Hummer Jr. Technics). Observations were performed in a Jeol JSM-35CF SEM.

Statistics. All data were expressed as mean \pm SEM and probabilities were regarded as significant if they reached a 95% level of confidence ($P < 0.05$). Statistical analyses were performed by the test for independent samples by Mann and Whitney using the exact permutational significance levels. The statistical test compared the baseline values for morphometric changes, PD, Isc and R to the values obtained 5 h after exposure to toxins A and B; the values obtained from permeability studies after 1, 3, and 5 h of toxin exposure were also compared by the same test. For paired observations we performed the test for paired samples by Mann and Whitney comparing the electrophysiological values of 3 nM of toxin A or B to controls.

Results

Toxin effects on electrophysiology. The effects of toxins A and B on colonic electrophysiology were compared using potential difference (PD), short-circuit current (Isc), and electrical resistance (R). Both toxins caused a time-dependent decrease of electrophysiological parameters. The time course effect of 32 nM of toxin A is shown in Fig. 1. Luminal toxin exposure caused a rapid decline of resistance that became statistically significant after 2 h of toxin exposure ($P < 0.05$), and continued to decline until the end of the 5-h observation period ($n = 6$). A similar progressive decline of resistance was observed in tissues exposed to 16 nM of toxin A, which became significantly different from controls 3 h ($P < 0.05$) after toxin administration (data not shown).

We next compared the effects of different doses of toxins A and B on electrophysiological parameters. As shown in Table I, PD, Isc and R remained stable for up to 5 h in the control strips incubated with buffer alone. In contrast, luminal exposure of mucosal strips to 65, 32, and 16 nM of toxin A or 15, 3, and 1.5 nM of toxin B for 5 h caused a significant dose-dependent decrease in all parameters tested as compared to baseline values. For each data set, we compared the effects of toxins to the baseline value for the same colonic strip derived prior to addition of toxins. As shown in Table I, considerable variability in baseline values for all parameters was observed in tissues from different patients. Toxin B was ~ 10 times more potent than toxin A on a molar basis in producing a comparable drop in resistance (Table I, Fig. 2).

1. **Abbreviations used in this paper:** Isc, short circuit current; PD, potential difference; R, resistance.

Table 1. Effect of Various Concentrations of *C. difficile* Toxins A and B on Electrophysiology of Human Colonic Mucosa

	Toxin A				Toxin B		
	Control (n = 6)	65 nM (n = 3)	32 nM (n = 6)	16 nM (n = 4)	15 nM (n = 3)	3 nM (n = 5)	1.5 nM (n = 6)
PD (mV)							
BL	-22.7±3.4	-24.5±4.5	-27.3±2.8	-23.5±5.3	-38.4±13.6	-28.5±3.5	-22.7±2.6
5 h	-22.3±2.6	-4.7±1.9*	-6.4±1.4*	-6.3±3.8*	-2.6±1.1*	-5.3±0.9*	-5.5±1.5*
Isc (μA/cm ²)							
BL	129.3±16.2	126.2±16.9	152.0±15.0	153±18.3	166±19.0	157.6±18.6	126.8±9.2
5 h	124.8±8.5	58.0±8.0*	63.2±1.0*	54±13.6*	42.5±10.2*	59.2±7.5*	56.6±9.9*
R (Ω·cm ²)							
BL	174.2±12.6	216.8±9.7	178.2±14.0	156.5±3.7	225±16.3	180.4±5.1	179.4±12.5
5 h	176.7±11.4	81.3±12.8*	96.0±7.0*	98.5±8.1*	56±13.0*	86.4±6.8*	94.0±11.3*

Mucosal sheets were incubated in Ussing chambers in luminal presence or absence (controls) of various concentrations of toxins A and B for 5 h. Values are mean±SEM. BL, baseline, 5 h, after 5 h. PD, potential difference; Isc, short-circuit current; R, resistance. (*P < 0.05, when compared to baseline obtained in the same tissue before toxin exposure; n equals the number of experiments using colonic mucosa from separate patients.)

Because, as noted above, tissues from individual patients exhibited considerable variation in baseline electrophysiologic parameters, we carried out experiments where mucosal strips from the same patient were exposed to toxins A and B. As shown in Fig. 3, 3 nM of toxin B caused an immediate decline of resistance, which became statistically significant after 1 h (P < 0.05), while 3 nM of toxin A failed to show an effect (n = 4, paired tissues). Resistance further declined in the 3 nM toxin B group until the end of the experiments (Fig. 3).

Permeability to [³H]mannitol. We next investigated the effect of various doses of toxins A and B on serosal to mucosal permeability to [³H]mannitol (Fig. 4). A small increase was noted at 3 and 5 h in control tissues incubated only with buffer. Permeability was increased by 6- and 11-fold in tissues exposed to 16 or 32 nM of toxin A or 1.5 or 3 nM of toxin B, respectively, after 3 h of continuous toxin administration. A similar trend in increased [³H]mannitol permeability was measured after 5 h exposure of tissues to the same doses of toxins (Fig.

4). Thus toxin B elicited an equivalent increase of [³H]mannitol permeability at a 10-fold lower dose than toxin A.

Histology. No tissue strips used in these experiments showed histological criteria of malignancy. Histologic examination of colonic strips incubated with buffer alone for 5 h showed excellent preservation of normal colonic architecture (Fig. 5 A). Toxins A and B exerted similar histologic changes depending on the concentration. The primary effect of both toxins was exerted on superficial epithelial cells whereas the epithelium of the crypts remained intact (Fig. 5 B, toxin A, 32 nM and 5C, toxin B, 3 nM). A typical histologic finding was a patchy distribution of the lesions, resulting in appearance of intact epithelium adjacent to severely damaged areas (Fig. 5 D). After toxin A or toxin B exposure, histologic examination revealed flat epithelial cells with regular staining intensity covering the basal lamina propria in the damaged areas (Fig. 5 D, arrow). These colonocytes had flattened nuclei which were oriented parallel to the basal lamina propria (Fig. 5 D).

Toxin-mediated morphologic damage expressed as the percent of damaged mucosa was dose-dependent (Fig. 6). Again

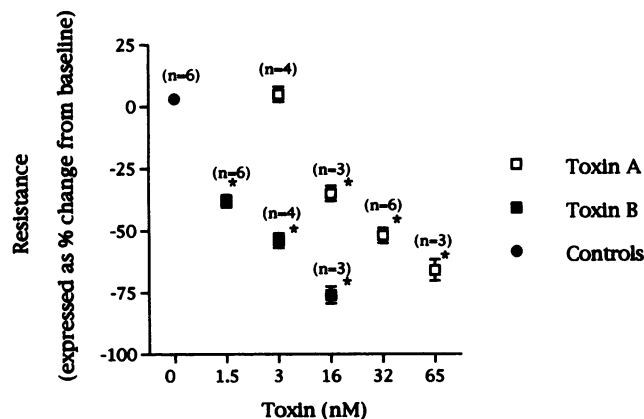


Figure 2. Dose-dependent effect of toxin A and B on colonic resistance. Human colonic tissues were placed in Ussing chambers and incubated with various doses of toxins A or B for 5 h. Resistance was calculated from potential difference and short-circuit current and results are presented as % change from baseline. Values represent mean±SEM. The number of experiments is given in brackets. (*P < 0.05 vs. controls).

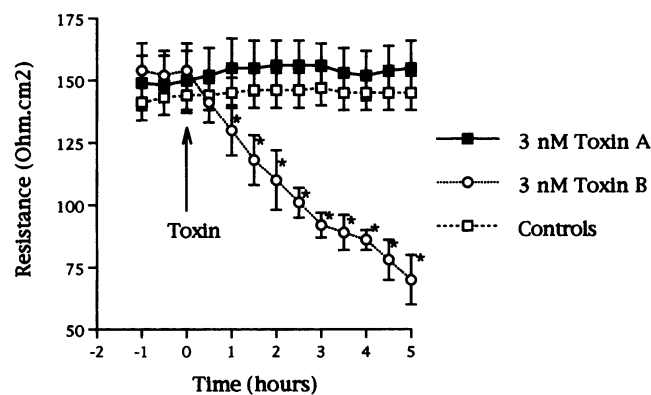


Figure 3. Time course of toxin A and B on resistance of paired colonic tissues. Paired human colonic mucosal sheets were continuously incubated with buffer alone (controls) or 3 nM of toxin A or B for 5 h (n = 4 per group). Values represent mean±SEM per group. (*P < 0.05, when compared with controls).

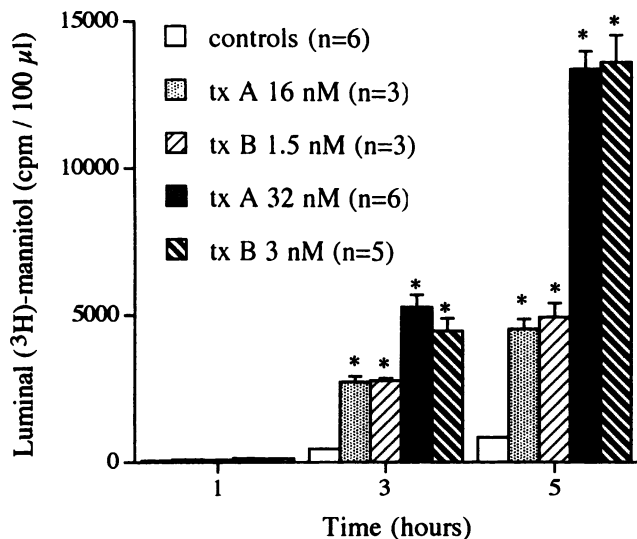


Figure 4. Effect of toxins A and B on colonic [^3H]mannitol permeability. Human colonic mucosal sheets were incubated with either buffer alone (control), or buffer containing toxin A (tx A) or toxin B (tx B). Toxin B elicited an equivalent increase of luminal [^3H]mannitol at a 10-fold lower dose than toxin A. Results are expressed as mean \pm SEM. The number of experiments is given in brackets. Luminal [^3H]mannitol was determined as described in Methods. (* $P < 0.05$ vs. control).

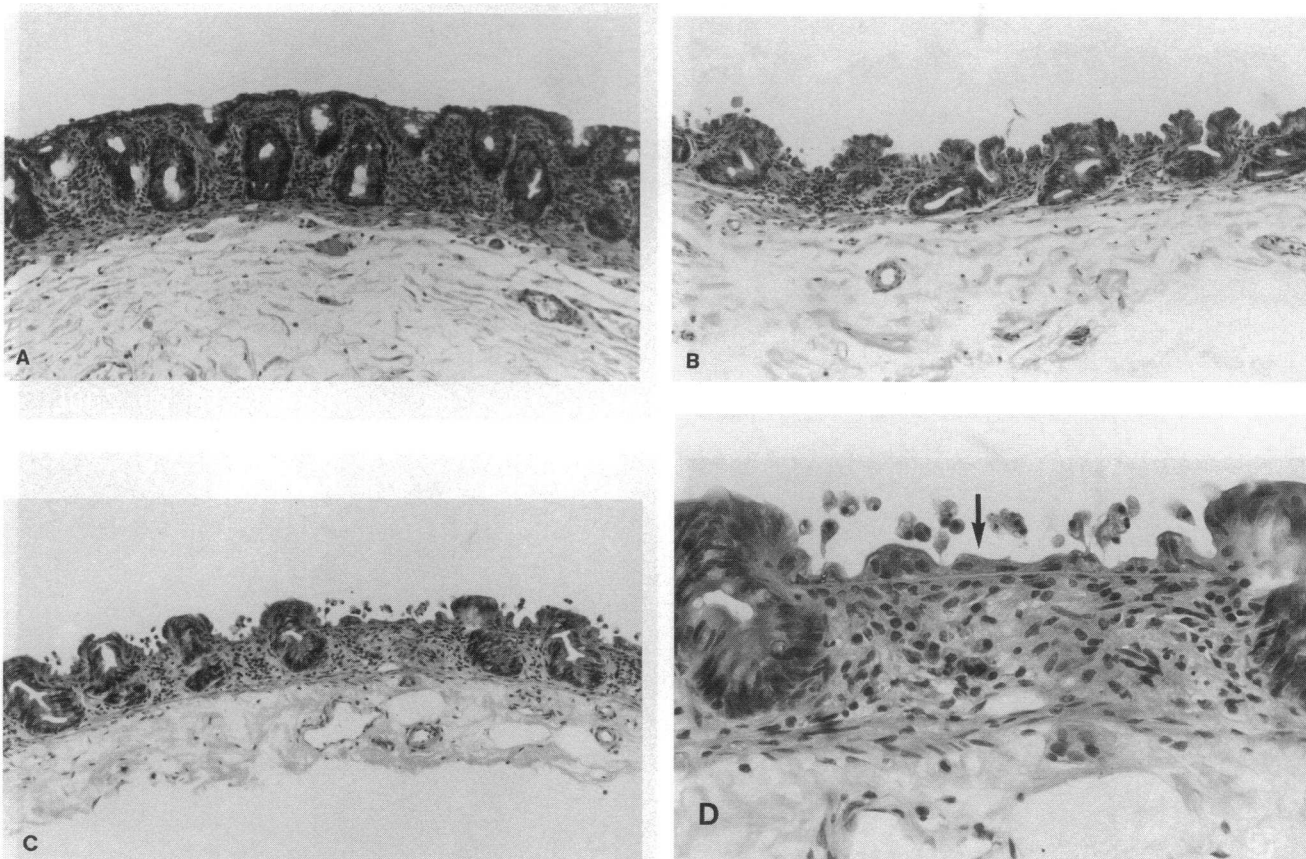


Figure 5. Morphologic effect of toxin A and B on human colonic mucosa. Human colonic mucosal sheets were incubated with either buffer alone (A) or buffer containing 32 nM of toxin A (B) or 3 nM of toxin B (C and D) for 5 h. At the end of the incubation period sheets were fixed in formalin and processed for light microscopy. Control tissue (A) appears normal and epithelial cells form a continuous sheet of cells over the lamina propria. In contrast, mucosal sheets exposed to toxin A (B) or B (C) show disruptions of the superficial epithelium whereas the crypt epithelium remains intact. D shows higher magnification of human colonic mucosa exposed to buffer containing 3 nM of toxin B for 5 h. Damaged epithelial cells are rounded up and unattached to the basal lamina whereas viable epithelial cells flatten to reseal the epithelial discontinuity (arrow). Undamaged epithelium is present at both sides of the damaged area in the center of the slide. A–C, $\times 50$; in D, $\times 200$.

toxin B was ~ 8 – 10 times more potent than toxin A on a molar basis. While 3 nM of toxin B caused mucosal damage of 31%, 3 nM of toxin A had no effect (Fig. 6). At the lowest concentration of toxin B used in these studies (0.2 nM) we observed morphologic damage to single cells (Fig. 7, A and C). At 3 nM of toxin B scanning electron microscopy revealed widespread damage of superficial colonic epithelial cells (Fig. 7 D).

Distribution of F-Actin. In previous studies we reported that purified toxins A and B caused a marked decrease in fluorescent staining for F-actin in monolayers of the T84 colonic adenocarcinoma cell line (19, 20). We therefore assessed the effect of toxins A and B on F-actin distribution in human colonic strips. Control cells had a polygonal shape with F-actin distributed in the peripheral actinomyosin ring associated with the cell membrane (Fig. 8 A). In contrast, tissues treated with toxin B for 5 h showed complete disorganization of the normal F-actin pattern with clumping of actin within cells (Fig. 8 B). Similar alterations of intracellular F-actin were seen in tissues treated with toxin A (data not shown).

Discussion

In this in vitro study we provide evidence that toxins A and B of *Clostridium difficile* cause dose-dependent electrophysiologic alterations and morphologic damage in human colonic

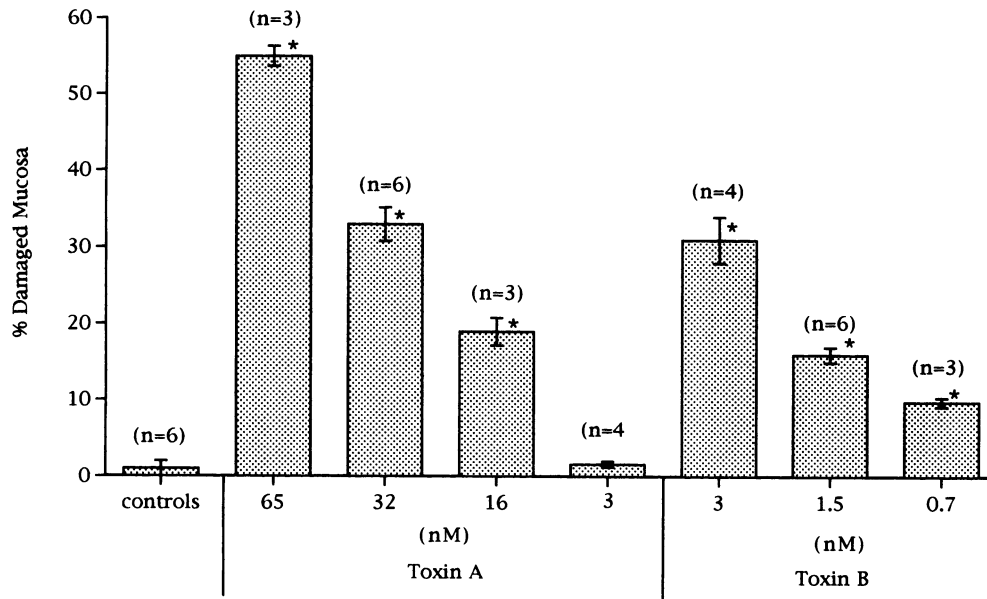


Figure 6. Morphometric analysis of colonic epithelial cell damage by toxins A and B. Human colonic mucosal sheets were incubated with buffer alone (control) or buffer containing either 65, 32, 16, or 3 nM of toxin A or 3, 1.5, or 0.7 nM of toxin B for 5 h. Mucosal sheets were fixed in formalin, processed for light microscopy and morphometric analysis was performed as described in Methods. Results are presented as mean \pm SEM. The number of experiments is given in brackets. (* $P < 0.05$ vs. controls).

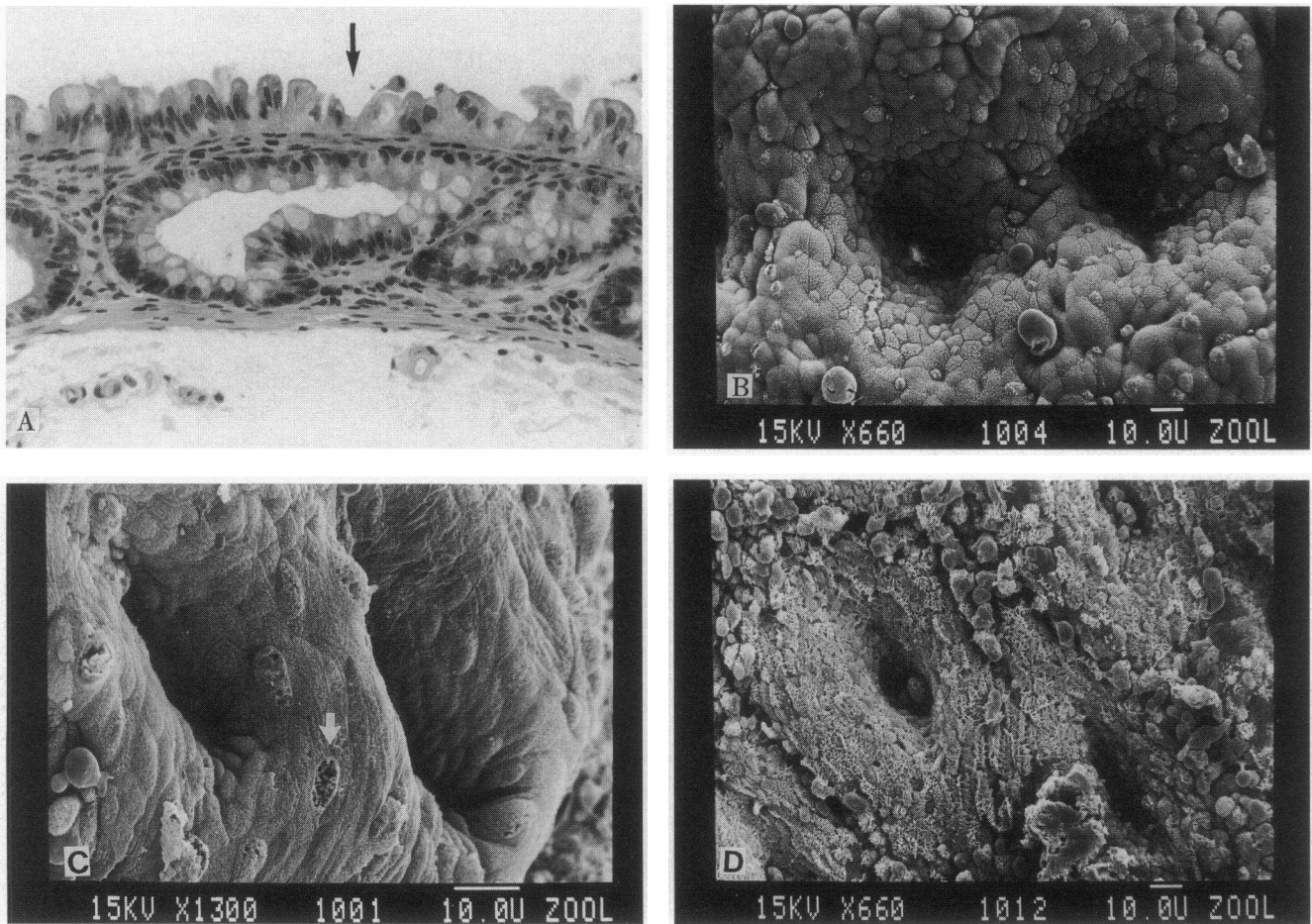


Figure 7. Light (A) and scanning electron microscopy (B–D) of toxin B-exposed human epithelial cells. Human colonic mucosal sheets were placed in Ussing chambers and incubated with either buffer alone (B) or buffer containing 0.2 nM (A and C) or 3 nM of toxin B for 5 h (D). Mucosa exposed to buffer alone (B) shows regular arrangement of polygonal epithelial cells. Low dose of toxin B causes single cell lesions (A and C, arrows). Exposure to higher concentration (3 nM) causes extensive destruction of the surface epithelium (D). Damaged epithelial cells round up and detach into the lumen. A: $\times 200$; B and D: $\times 330$; C: $\times 650$.

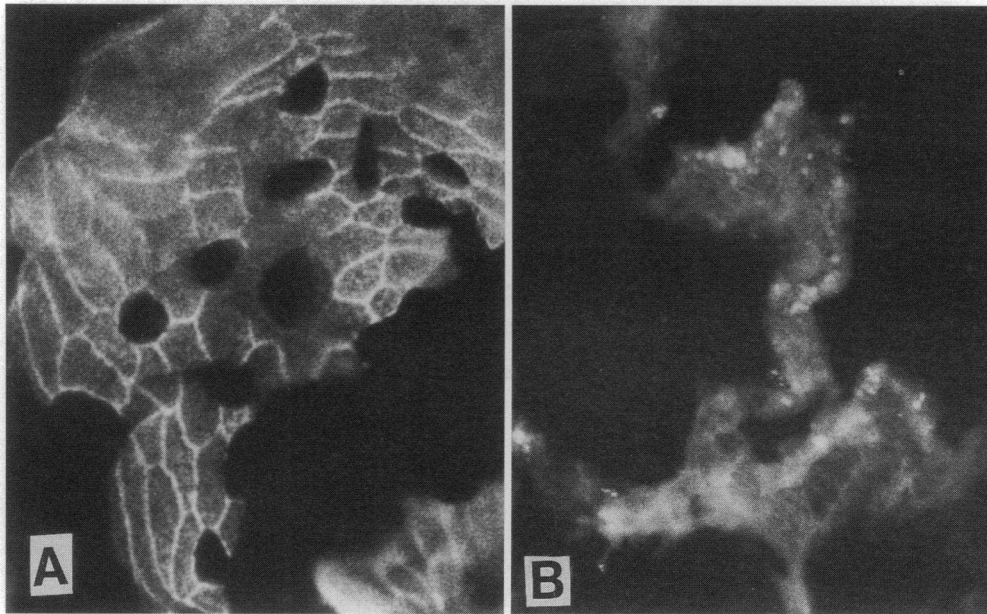


Figure 8. Effect of toxin B on cellular F-actin. Fluorescent photomicrograph of human colonic mucosal sheet incubated with buffer alone (A) or buffer containing 3 nM of toxin B (B) for 5 h. Samples were then fixed and fresh-frozen sections cut perpendicular to the long axis of surface cells were stained with the F actin specific rhodamine-labeled phalloidin. Note the intracellular condensation of F actin in toxin B exposed tissues (B), as opposed to the ring like distribution of F actin seen in controls (A). $\times 400$.

strips in Ussing chambers. Toxin exposure caused an immediate decrease of resistance (Figs. 1 and 2) which became statistically significant within 1–3 h of toxin exposure. This is in contrast to our previous results obtained with T84 cells (19) which showed that a latency period of 1–2 h occurred between toxin exposure and the onset of an electrophysiologic effect. While toxin A was more potent than toxin B in all previous studies in experimental animals, we report here for the first time that toxin B is ~ 10 -fold more potent on a molar basis than toxin A in human mucosa (Table I, Fig. 2). For example, we previously reported that in T84 cells toxin A at a concentration of 0.2 nM caused a resistance drop of 80% at 72 h (20), compared with toxin B which caused the same effect at a concentration of 2.5 nM (19). As reported here (Fig. 2) resistance was decreased by 46% at a toxin A concentration of 32 nM whereas only 3 nM toxin B was required to produce a similar decrease (Table I, Fig. 3). These differences in relative sensitivity to toxins probably reflect differences between neoplastic T84 cells vs. normal intestinal strips, as well as methodological differences between cell-lines and tissue strips in a chamber. One limitation of Ussing chamber experiments is that the maximum period of observation is 6 h after which time necrosis of tissue ensues. For this reason, higher concentrations of toxins are required to produce an effect within a few hours, while in the T84 cell system continuous observation for 2 or 3 d are possible, thus allowing lower toxin doses. In these experiments reported here we used concentrations of toxin A or B ranging between 0.2 and 65 nM. Similar doses of toxin A (6–30 nM) were required in previous experiments to produce an acute inflammatory and secretory response in rat ileal loops in vivo (12). However, since no detailed studies are available regarding the concentration of either toxin in stools from patients with *C. difficile* colitis, the relationship between our in vitro results and actual *C. difficile* infection in patients remains speculative.

Our previous results (20) on cultured human intestinal T84 cell monolayers indicate that electrical resistance changes in response to toxin A are a more sensitive indicator of barrier function than mannitol flux. In T84 cells, large changes in sero-

sal-to-mucosal fluxes in response to toxin A were observed only after resistance was maximally affected (e.g., 3–4-fold decrease). Similarly in this study, mannitol fluxes were not correlated directly with maximal resistance changes at 5 h. For example, exposure of colonic explants to 32 nM toxin A or 1.5 nM toxin B produced an equivalent drop in resistance to $95 \text{ Ohm} \times \text{cm}^2$ at 5 h (Table I). Despite these nearly identical resistance values, mannitol fluxes were nearly threefold higher in the explants exposed to 32 nM toxin A vs. 1.5 nM toxin B. Some of this discrepancy possibly relates to a difference in the time of onset and rate of decline of resistance changes at varying toxin concentrations. Although the final resistance might be identical at 5 h, mannitol fluxes over the entire 5-h period might vary considerably. However, measurement of mannitol fluxes over consecutive 15- or 30-min periods might reveal closer correlation with resistance changes.

In contrast to earlier animal studies where toxin A was reported to cause diffuse mucosal damage (12, 13, 23, 24), we report here that toxins A and B cause patchy or even discrete single cell lesions in human colon depending on the toxin concentration. At lower toxin doses epithelial cell damage is patchy, but becomes more confluent at higher doses (Figs. 5–7). This finding may correlate with the variability of colonic pseudomembrane observed in patients with antibiotic-associated colitis secondary to *C. difficile*. In some patients with *C. difficile* colitis pseudomembranes are discrete, while in others the membrane and subjacent inflammatory response may cover the entire mucosal surface. Our in vitro results suggest that the extent of colonic damage is related to the toxin concentrations and the duration of exposure before diagnosis. We observed in our study that both toxins caused damage of superficial epithelial cells while crypt epithelium remained intact (Fig. 5 and 7). This finding could be explained by a different distribution of toxin A and B receptors on mucosal epithelium. For example, we have previously reported that binding of biotinylated toxin A to rabbit ileum was limited to the brush border of villus tip cells with no binding on the crypt epithelial cells (28). Conceivably, a similar distribution of toxin A or B receptors in superficial vs. crypt colonocytes may explain the absence of damage

to crypt cells compared with the extensive necrosis observed in superficial epithelial cells.

A possible explanation for the lack of intestinal effects of toxin B in animal models (11, 12, 15) is absence of receptor binding sites for this toxin in rodent intestine. Previous studies have suggested a strong correlation between presence of membrane receptors for either toxin A or toxin B and biologic responsiveness to these toxins. For example Rolfe (29), using radiolabeled toxin B, failed to show any toxin B specific binding sites for this toxin in hamster intestine, a tissue known to be insensitive to toxin B (15). Conversely, hamster and rabbit intestine possess specific toxin A brush border receptors and both tissues are sensitive to the intestinal effects of toxin A (11, 12, 15, 29). In addition, diminished toxin A receptor binding activity in newborn rabbit ileum was associated with a substantial reduction in biologic effect, while developmental increase in receptor activity from birth to age 24 d was associated with acquisition of toxin sensitivity (28). Although binding activity was not measured in the present study, our results imply that receptors for both toxins are present in the luminal aspect of colonocytes.

We observed disruption and clumping of actin in colonocytes exposed to toxin B (Fig. 8) similar to changes reported previously by us in guinea pig ileum (30) and in human T 84 colonic cells (19, 20). In human fibroblast cell lines the level of filamentous actin declined prior to cell rounding (6). The action of these toxins on filamentous actin in target cells is most likely related to the catalytic action of toxins on Rho A and B, small GTP-binding proteins known to be involved in actin filament assembly (31). The similarity of action of toxins A and B in the human colon is not surprising based on their striking amino acid homology (25, 26).

The mechanism by which toxins A and B damage the human intestine can be categorized as direct and indirect. Direct toxicity occurs when cultured cells are exposed to toxins A and B, and presumably occurs via toxin binding, internalization (17) and disruption of the cytoskeleton with eventual inhibition of protein synthesis (18) and cell division. Part of the intestinal effects of toxin A described in ileal loop studies results from indirect activation of lamina propria neuroimmune cells (23, 24, 32–35) and infiltration of the ileal submucosa by neutrophils (23, 24, 35, 36). In the experiments reported here we assume that the immediate onset of electrophysiological changes after luminal toxin exposure suggests direct damage, although we can not exclude the release of cytokines, prostanooids or other pro-inflammatory mediators from lamina propria immune cells present in these mucosal strips. Studies are underway to define the possible contribution of epithelial and non-epithelial cells to the toxin effects described here.

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