JOURNAL OF CLINICAL MICROBIOLOGY June 1982, p. 1157–1158
0095-1137/82/061157-02$02.00/0

Vol. 15, No. 6

Differential Effects of *Clostridium difficile* Toxins on Tissue-Cultured Cells

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Received 7 December 1981/Accepted 22 February 1982

Two immunologically distinct *Clostridium difficile* toxins elicited similar morphological changes on cultured cells, although there were differences in both toxin potency and cell sensitivities.

Strains of *Clostridium difficile* have been associated with the majority of cases of antibiotic-associated colitis (2). The pathogenesis of the disease is thought to be mediated by one or more toxins elaborated by the organism (1, 10). Recently, evidence has been presented in support of the existence of two immunologically distinct *C. difficile* toxins (8, 12). One of the toxins (toxin A) produces an intense fluid response, whereas the other toxin (toxin B) is more active on tissue-cultured cells (9, 12). In the study reported here, the effects of the two toxins on different cultured cells are compared.

Toxins A and B were purified as previously reported (11). The toxin A preparation was homogeneous by polyacrylamide gel electrophoresis and crossed immunoelectrophoresis. The toxin B preparation contained two to four contaminants when examined by these procedures, but was free of detectable amounts of toxin A. Protein concentrations of the toxins A and B preparations were 0.8 and 0.2 mg/ml, respectively. The toxins were stored at 4°C in 0.05 M phosphate–0.15 M NaCl buffer, pH 7.4 (phosphate-buffered saline), and dilutions were made in phosphate-buffered saline at the time of assay. An antiserum specific for each toxin was prepared as previously described (8). Dilutions of antitoxin were made in phosphate-buffered saline.

Cells derived from mouse adrenal tumors (Y1), rat hepatoma (MHC), Chinese hamster ovary (CHO), and human cervical epithelium (HeLa) were propagated and maintained in a Charity Waymouth medium supplemented with 10% fetal calf serum at 37°C in a humidified atmosphere of 95% air–5% CO_2 (4, 6). Cells grown to confluence in 24-well plates (Linbro) were used for the toxin assays and neutralization studies.

For the neutralization studies, toxin was incubated in the presence of antitoxin or phosphate-buffered saline at 37°C for 30 min before transfer to cell-containing plates. Morphological changes (rounding) were assessed at 18 h after exposure of cells to toxin (6). The toxin titer was defined as the greatest dilution of toxin that elicited obvious morphological changes. The morphological changes were scored as ++ when all cells were rounded; + when many, but not all, cells were rounded; and − when cells were no different from controls. The antitoxin titer was defined as the greatest dilution that was capable of neutralizing the undiluted toxin's effects.

Toxins A and B both caused rounding of each cell type. The effects of each toxin could be neutralized by homologous, but not by heterologous, antitoxin, thus confirming the specificity of each toxin and antitoxin. Both antitoxins exhibited neutralizing titers of 1:64.

There were insignificant differences in cellular sensitivities to toxin B (Table 1). This is in agreement with previous work (3) that used fecal filtrates (mainly toxin B activity) or partially purified toxin (toxin B). Greater differences in sensitivity to toxin A were noted among the four cell lines, with differences as great as 64-fold between HeLa and Y1 cells. Regardless of cell type, toxin A was uniformly much less active than toxin B.

Although the two toxins appear to induce similar morphological effects in different cells, the biochemical basis for the morphological changes remains to be delineated. Despite immunochemical differences, the two toxins could share ligand determinants; alternatively, the toxins might bind to different receptors, but induce common functions. The two toxins' effects are not similar on all cells, however, since they produce different effects in the intestine. Toxin A elicits a positive fluid response in rabbit ileal loops (9, 12) whereas toxin B does not. This fluid response is hemorrhagic in nature and more closely resembles the response produced by shigella enterotoxin than that by either *Vibrio cholerae* or *Escherichia coli* (LT) enterotoxins

TABLE 1. Differential effects of *C. difficile* A and B toxins on cultured cells

Toxin	Concn[a]	Morphological changes for cell type[b]:			
		Y1	CHO	Hela	MHC
A	20.0 μg/ml	++	++	++	++
	5.0 μg/ml	++	++	+	++
	1.25 μg/ml	++	+	−	++
	0.31 μg/ml	++	−	−	+
	0.08 μg/ml	+	−	−	−
	0.02 μg/ml	−	−	−	−
B	20.0 ng/ml	++	++	++	++
	5.0 ng/ml	++	++	++	++
	1.25 ng/ml	++	++	++	++
	0.31 ng/ml	++	++	++	++
	0.08 ng/ml	++	+	+	+
	0.02 ng/ml	+	−	−	−

[a] Final concentration of toxin in tissue-culture medium. All cells were tested at the same time with identical toxin preparations.

[b] ++, All cells rounded; +, many but not all cells rounded; −, cells same as controls.

(7). Conversely, the effects of both toxins A and B on cultured cells more closely resemble those of cholera and *E. coli* toxins than those of the cytotoxins of *Shigella, Aeromonas, Clostridium perfringens*, and non-O-1 *V. cholerae* (5, 7).

If toxin A is more important in the pathogenesis of *C. difficile*-associated colitis and diarrhea, then assays directed towards the detection of this toxin might result in tests of greater specificity than those currently available. In preliminary studies, however, we could not detect toxin A by Y1 cell toxin neutralization assay in the toxin-containing stools of asymptomatic neonates or symptomatic adults. It may be that toxin A is indeed present in these specimens in amounts smaller than can be detected by tissue culture assay, yet amounts that are sufficient to cause disease.

These studies were supported in part by grants from the Veterans Administration Research and Education Service and grants AI 11416, AI 11794, and AI 15749 from the National Institutes for Health.

We are grateful for the expert technical assistance of Carol Haverkamp and for the toxin-specific antisera supplied by Jeffrey Libby.

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