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Interference by Trypsin in the Interaction of Staphylococcal Enterotoxin B and Cell Cultures of Human Embryonic Intestine[1]

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The inhibitory effect of trypsin on the cytotoxicity of staphylococcal enterotoxin B acting with human embryonic intestine cell cultures was examined. Trypsin treatment of the cells rendered them resistant to enterotoxin for a period of 48 hr. The resistance increased proportionally with increased time of exposure of the cells to trypsin. Neither ethylenediaminetetraacetic acid nor scraping, which were used as alternate means of cell suspension, caused any resistance to the toxin. The effect is enzymatic and appears to be similar to the inhibitory action of trypsin and chymotrypsin on the attachment of polioviruses and coxsackieviruses to HeLa cells.

In a previous report (4), we described the cytotoxicity in cell cultures of human embryonic intestine (Henle strain) when acted upon by staphylococcal enterotoxin B. The data on changes in cell susceptibility showed that the cells were temporarily resistant to the lethal action of enterotoxin B when challenged with the toxin immediately after planting of the cultures, that is, after the ordinary trypsinization procedure for dispersing cell monolayers in stock cultures. The acquired resistance was maintained up to 48 hr after trypsinization. However, if the same cell cultures were grown for 48 hr after trypsinization in the absence of toxin and were then challenged with toxin, full susceptibility was evident. At that time we attributed this phenomenon to the age of the cultures.

We have continued to investigate this phenomenon. The present report describes the nature of the interaction of enterotoxin and trypsinized cells.

MATERIALS AND METHODS

Cell culture. The heteroploid cell culture used in this study was human embryonic intestine derived from ileum and jejunum tissue (Microbiological Associates, Inc., Bethesda, Md.). The maintenance of stock cultures, the enterotoxin B preparation, and the experimental procedures were the same as have been described previously (4). Test cultures were prepared in screw-capped culture tubes by trypsinizing stock cultures and suspending the cells in medium at a concentration of 8.5×10^4 to 9.0×10^4 cells per milliliter. The cultures were incubated at 37 C until monolayers were established, which generally occurred within 2 to 3 days.

To determine the effects of toxin, the following tests were performed.

Trypsin tests. Cell monolayers in screw-capped test tubes were dispersed with 0.042% trypsin (Grand Island Biological Co., Grand Island, N.Y.) in calcium- and magnesium-free phosphate-buffered saline solution. Bottle cultures were trypsinized with 0.125% trypsin solution. In studies with heat-inactivated trypsin, a concentration of 0.125% trypsin was inactivated at 56 C for 30 min.

To determine the kinetics of the effect of trypsin on the response of human embryonic intestine cell cultures to staphylococcal enterotoxin B, 1 ml of the trypsin solution was added to each of three tube cultures. The tubes were rotated to bring the entire monolayer in contact with the trypsin solution. The fluid was decanted and the tubes, containing only the residual trypsin solution, were incubated in a 37 C water bath for varying lengths of time.

At the end of each incubation period, 1 ml of complete medium was added, the cells were dispersed by trituration, and 0.1 ml of enterotoxin (100 μg) was added. The cultures were incubated in a stationary position at 37 C to effect reattachment of the cells to the glass. The cultures were observed for 48 hr, after which cell protein was determined by use of the Lowry modification of the Folin-Ciocalteau test (1) to measure the extent of cytotoxicity.

Ethylenediaminetetraacetic acid (EDTA) tests. Cell

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monolayers were first washed with calcium- and magnesium-free phosphate-buffered saline solution. A 5-ml amount of EDTA solution (from Grand Island Biological Co.) was added to each culture bottle and remained in contact with the cell monolayer for 1 min. The monolayers were incubated at 37 C with the residual EDTA until they detached from the glass. The cells were then suspended in complete medium, counted, and planted at the desired concentration in screw-capped tubes.

To test the effect of toxin on EDTA-detached cells at the time of planting, toxin was added immediately to the newly planted cultures. The cultures were incubated at 37 C and were examined for cytotoxicity.

Scraping tests. After removal of the spent growth medium, the cultures received fresh growth medium. The cell monolayers were suspended by scraping with a rubber policeman to eliminate the use of any chemical dispersing agent (trypsin and EDTA). After trituration of the cell suspension, 100 μg of toxin per ml was added. The cultures were reincubated for 48 hr.

RESULTS

Figure 1 illustrates the results obtained when trypsinized cells were planted in the presence of 100 μg of enterotoxin per ml. The growth of cultures during an incubation period of 4 days did not indicate any cell destruction or growth inhibition, although this concentration of toxin normally destroys nontrypsinized cells completely in 2 days.

To ascertain whether cultures exposed to enterotoxin at the time of planting were permanently resistant to 100 μg of enterotoxin per ml, toxin was added to cultures at the time of planting and again 48 hr later. Control cultures for the second dose of toxin were grown for 48 hr in the absence of toxin.

After 48 hr of growth in the presence of toxin, the cultures were as susceptible as those grown for 48 hr in the absence of toxin (Fig. 2). Therefore, it was apparent that no permanent resistance to the enterotoxin was engendered by growth of the cell cultures, after trypsinization, in the presence of the enterotoxin.

At this stage of our investigation, we examined the enzymatic action of trypsin to determine whether it or the physical removal of the cells from the growing surface induced the temporary resistance to the enterotoxin. To determine this, both EDTA and scraping were used as alternate means of suspending the cells.

Table 1 summarizes the results of morphological cytotoxicity. It is evident that scraping and EDTA treatment as means of cell suspension did not interfere with the cytotoxic manifestations of enterotoxin. However, no cytotoxicity was evident in the cultures which were trypsinized immediately prior to the addition of toxin.

If it is assumed that the inhibitory effect of trypsin is enzymatic in nature, it should be possible to demonstrate an increase in resistance of the cell cultures with increased time of exposure to trypsin. The results indicated that resistance to the enterotoxin did increase with the time of exposure to trypsin (Table 2).

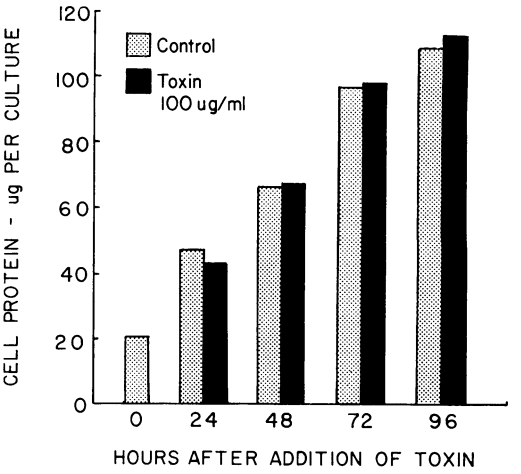

FIG. 1. *Interference of trypsin with the cytotoxic effects of staphylococcal enterotoxin B for human embryonic intestine cells. The values were obtained by protein determinations after incubation of trypsin-treated cell cultures in the presence and absence of enterotoxin for the indicated periods of time.*

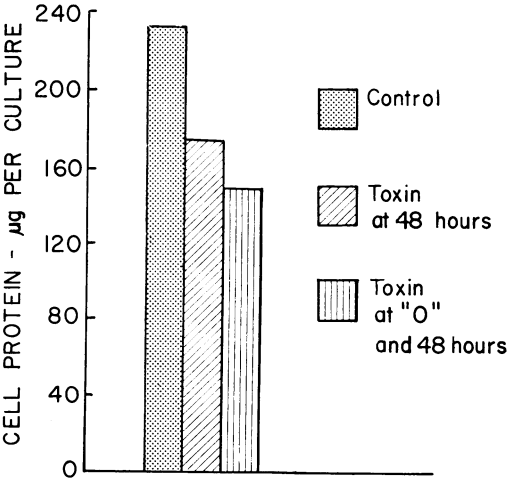

FIG. 2. *Cytotoxicity shown after challenge of cultures with 100 μg of enterotoxin B at the time of planting, and again after 48 hr of growth in the presence of toxin as well as after 48 hr of growth in the absence of toxin.*

TABLE 1. *Scraping and EDTA treatment versus trypsinization of human intestine cells prior to inoculation with staphylococcal enterotoxin B*

Treatment of cultures	Toxin, 100 μg/ml	Cytotoxicity[a]	
		24 hr	48 hr
Control	−	0	0
	+	2+	3+
Trypsinized	−	0	0
	+	0	0
Scraped	−	±	±
	+	3+	4+
EDTA-treated	−	0	±
	+	3+	4+

[a] Cytotoxicity was determined by microscopic examination and was classified as follows: ±, less than 10% destruction of cell monolayers; 1+, 10 to 25% destruction; 2+, 25 to 50% destruction; 3+, 50 to 75% destruction; and 4+, 75 to 100% destruction.

In additional experiments, the heat-inactivated trypsin did not remove the cells from the glass growing surface, nor did it engender any resistance to the enterotoxin.

Since the resistance was temporary, it was of interest to determine at what stage the susceptibility once again became manifest.

In a previous publication (4), data were presented which showed that, after trypsinization, cells are resistant to enterotoxin B. However, when the cultures were planted and grown in the absence of toxin and challenged after 1, 2, or 3 days with enterotoxin, full susceptibility became evident after only 48 hr. This is supported by the data in Fig. 2.

DISCUSSION

A number of possibilities were considered to explain the resistance of the cell cultures to enterotoxin B after trypsinization. The possibility that pinocytosis, the mechanism for the assimilation of larger molecular weight substances into the cell, might be inhibited by the trypsinization procedure is not a plausible one, because the evidence at present appears to favor an increased surface activity of pinocytosis in recently trypsinized cells (2).

The possibility of some residual trypsin activity remaining in recently trypsinized cells is eliminated by the data obtained with cells trypsinized until removed from the growing surface. These cells continued to retain some susceptibility to the enterotoxin. Full resistance was obtained only when cells remained in contact with the trypsin for extended lengths of time. Moreover, toxin was always added to the cultures only after complete medium with serum had first been added, thus inactivating any residual trypsin.

TABLE 2. *Effect of enterotoxin B (100 μg/ml) on human embryonic intestine cells exposed to trypsin*

Time of exposure to trypsin	Cell protein[a] (% of control)
sec	
0	50
60	56
120	81
200	86
260	97
Control[b]	100

[a] After 48-hr test.
[b] Without toxin.

An additional possibility is that some specific surface configuration of the cell required for the interaction with the toxin is destroyed during the incubation with trypsin. This may be considered for further investigations of the resistance. An analogous effect was recently reported by Zajac and Crowell (5, 6). They showed that the incubation of HeLa cells with chymotrypsin inhibited the attachment of coxsackievirus B_3, and that incubation of HeLa cells with trypsin inhibited the attachment of poliovirus T_1. In addition, these investigators showed that the concentration of trypsin for a given quantity of cells is critical for the receptor inactivation to take place and that the regeneration of the receptor sites for both viruses was effected only after 24 to 48 hr of incubation after the enzyme treatment.

Rapoport et al. (3) have recently published data supporting a "receptor-like" hypothesis with respect to staphylococcal enterotoxin B interaction with cells. Using Rhesus monkeys and ^{131}I-labeled enterotoxin, they have shown that intravenously injected enterotoxin is rapidly cleared from the circulation. However, if antiserum to the enterotoxin is injected after the enterotoxin, some of the toxin is actually returned to the circulation after clearance. These authors have postulated the presence of tissue-binding sites to explain this phenomenon. The antitoxin, they suggest, represents a more potent binding site so that the toxin is actually removed from the tissue sources when antitoxin is present and is returned to the circulation as antitoxin-toxin conglomerates.

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