ELECTRON-MICROSCOPIC STUDY OF INTERACTION BETWEEN ENTEROTOXIGENIC FORMS OF Escherichia coli AND THE INTESTINAL EPITHELIUM

Yu. E. Polotskii, E. S. Snigirevskaya, and E. M. Dragunskaya

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Enterotoxigenic forms of Escherichia coli (ETEC), the agents of chlorea-like diseases in man, if introduced into ligated loops of rabbit small intestine, induce hypersecretion of the epithelium with the accumulation of large volumes of fluid in the lumen of the loops. ETEC do not induce inflammation and do not penetrate into the epithelium but proliferate on its surface. Electron-microscopic studies showed that under these circumstances ETEC come into contact only with the supramembranous coat of the microvilli (the glycocalyx). Increased functional activity and the formation of autophagosomes were observed in the cytoplasm of the epithelial cells. Many cells with autophagosomes sloughed into the lumen, but the continuity of the epithelial cover was undisturbed. The results confirm the hypothesis of hypersecretion of the epithelium under the influence of ETEC and they also provide evidence of ultrastructural injuries to the cell cytoplasm not leading to destruction of the epithelium.

In recent years a new group of agents of acute intestinal infections in man has been discovered – the enterotoxigenic forms of Escherichia coli (ETEC) that produce cholera-like diseases [1, 5, 8]. The ETEC have been shown to secrete toxins similar to cholera enterotoxin. Injection of ETEC and their enterotoxins into ligated loops of rabbit small intestine leads to the accumulation of large quantities of fluid with a low protein and high salt content, just as in similar experiments with cholera vibrios or their enterotoxin. The results of biochemical and pathophysiological tests have shown that in both cases the accumulation of fluid in the intestinal lumen is caused by hypersecretion of the epithelial cells of the small intestine [3, 6, 9]. Like cholera vibrios, ETEC do not penetrate into the intestinal epithelium and do not induce inflammation [5]. The electron-microscopic character of interaction between ETEC and the intestinal epithelium has not been investigated.

An electron-microscopic study was made of interaction between the enterotoxigenic strain of Escherichia coli No. 3976, isolated and tested in experiments at the Leningrad Pasteur Institute [1], and the intestinal epithelium.

EXPERIMENTAL METHOD

Loops of small intestine 10 cm in length were ligated in rabbits under intravenous hexobarbital anesthesia and 5 ml of a 6-h broth culture of ETEC ($11\text{-}66 \cdot 10^9$ bacterial cells), grown with shaking (125 rpm), was injected into each loop. Small pieces were excised from the infected loops 30 min and 2 and 12 h later, fixed successively with glutaraldehyde with the addition of thiophosphamide and with osmium tetroxide to

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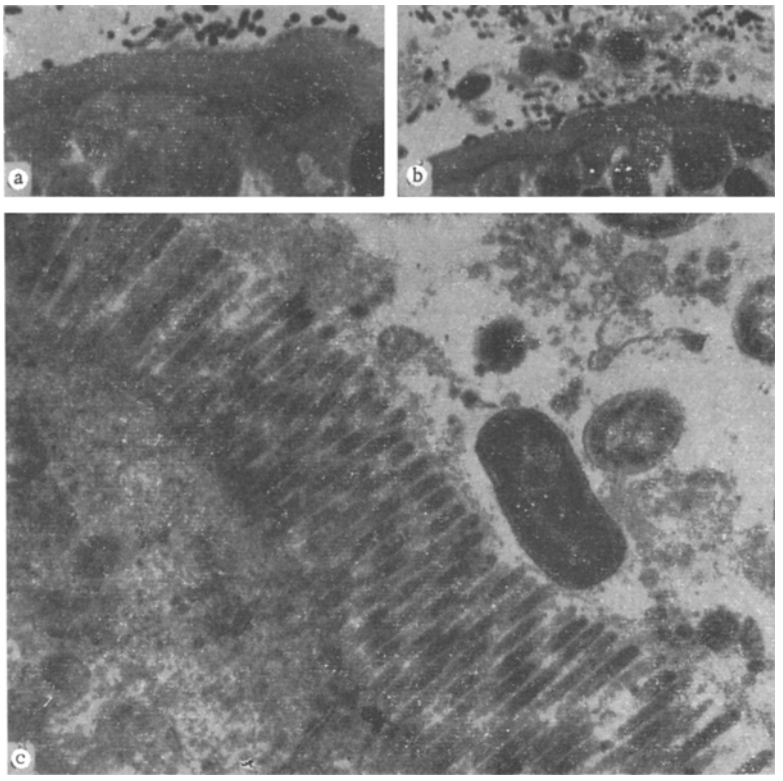

Fig. 1. Enterotoxigenic forms of E. coli on the surface of the epithelial cells: a and b) brush border unchanged. Toluidine blue; a) 1430 ×; b) 1070 ×; 3) bacteria in contact with glycocalyx only, 20,000 ×.

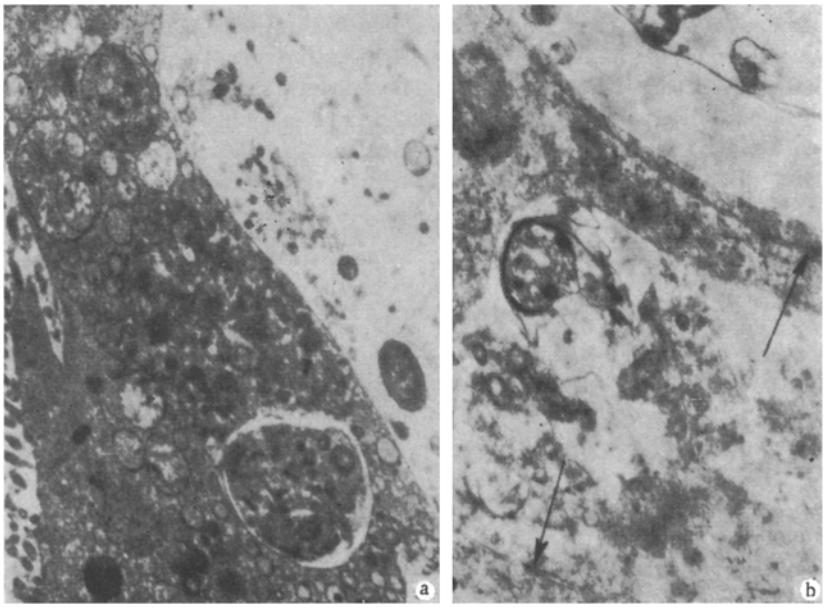

Fig. 2. Autophagosomes in epithelial cells: a) in desquamated cell, 17,000 ×; b) in cell of epithelial layer, desmosomes marked by arrows; 23,000 ×.

reveal the glycocalyx, by the method of Komissarchik and Snigirevskaya,* and embedded in Epon. Sections 1 μ in thickness were stained with toluidine blue for examination under the light microscope, whereas ultrathin sections were stained with lead citrate and uranyl acetate and studied in the JEM-5g or JEM-7 electron microscope.

EXPERIMENTAL RESULTS

Examination of the histological sections in the light microscope revealed no inflammatory changes in the intestinal loops infected with ETEC. Even after the accumulation of large quantities of fluid in the intestinal lumen (which was observed in about half of the experiments by 10-14 h after infection) only considerable edema and dilatation of the blood vessels of the mucous membrane and submucosa of the intestinal wall were observed. The epithelial layer was unchanged and only the goblet cells as a rule were emptied. Collections of bacteria, apparently fixed in some places to the brush border (Fig. 1a, b), but not penetrating into the epithelial cells, were found on the surface of the epithelium.

The results of the electron-microscopic investigations showed that the microorganisms were in contact only with the glycocalyx covering the microvilli of the epithelium (Fig. 1c), even when the intestinal loops were distended most strongly with fluid 12 h after infection. Close to the apical ends of the microvilli, between them, and nearby in the lumen of the intestine there were small vesicles with a wall consisting of a triple membrane (this is also found under normal conditions but much less frequently). Structureless, reticular, or finely granular masses, evidently consisting of serous fluid, numerous separate granules of secretion of the epithelial cells of the crypts, a few erythrocytes, and desquamated epithelial cells in various stages of destruction (loss of microvilli, pycnosis of nucleus, death of the organelles, and electron-transparency of the cytoplasm, disintegration of the cell) were found in the lumen. Neither leukocytes nor their granules were present, however, in the lumen.

Autophagosomes – vacuoles, bounded by a membrane, with areas of cytoplasm and its organelles (especially often with secretory granules) in different stages of destruction – were often found in the desquamated epithelial cells (Fig. 2a). Vacuoles of this type were often seen lying freely in the lumen. Autophagosomes usually containing secretory granules were also detected in the cytoplasm of the epithelial cells covering the villi and lining the crypts (Fig. 2b). Numerous large mitochondria as well as many bands of rough endoplasmic reticulum were observed in the epithelial cells and, in particular, in the apical cytoplasm. These changes are evidently signs of an increase in functional activity of the cells.

The results of the experiments thus showed that ETEC – the agent of chlorea-like diseases – when injected into isolated loops of rabbit small intestine, proliferate like cholera vibrios on the surface of the epithelial cells but do not penetrate into them and do not cause inflammation. A similar picture was observed in experiments on ligated loops of pig and rabbit small intestine with the agents of porcine colibacillosis, which also produce cholera-like enterotoxins [4, 8]. These bacteria in the intestinal loops and in the intestine of piglets infected by mouth also were attached to the supramembranous cover of the microvilli of the epithelial cells and proliferated all over the surface of the mucous membrane but without penetrating into the epithelium or causing changes in it or inflammation.

The results showing the morphological manifestations of increased functional activity of the intestinal epithelium in the experiments with ETEC support the hypothesis of the leading role of hypersecretion or enteroabsorption in the pathological processes produced by these microorganisms. At the same time, the presence of autophagosomes in the cytoplasm of the epithelial cells and the desquamation of large numbers of these cells (although without disturbing the continuity of the epithelium) point to damage to the epithelium detectable with the electron microscope. In experiments in which mice were infected intranasally with ETEC death of individual bronchial epithelial cells was observed with the formation of small defects in the epithelial lining [2].

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