ELECTRON-CYTOCHEMICAL INVESTIGATION OF CHOLERA TOXIN ABSORPTION BY EPITHELIUM OF PEYER'S PATCHES IN GUINEA PIGS

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Lympho-epithelium interactions in Peyer's patches play an important role in the development of immune mechanisms acting at the level of the intestinal wall and lumen. It has been shown, for example, that the M cells of the epithelium of the apices of the Peyer's patches carry out active pinocytosis of intact protein macromolecules (antigens) from the lumen of the intestine and transfer them to the subjacent lymphoid cells [1, 2]. Lymphocytes activated by antigen migrate into the mesenteric lymph nodes and along the thoracic duct into the blood stream. After a certain time, having migrated into the tunica propria of the intestinal mucosa, the lymphocytes differentiate into plasma cells, which synthesize specific immunoglobulins of class A. In conjunction with the secretory component, these immunoglobulins emerge on the surface of the mucous membrane and into the lumen of the intestine, where they inactivate the antigen [3].

Injection of cholera exotoxin into the lumen of the small intestine is known to lead to the development of local antitoxic immunity in experimental animals, due to the appearance of plasma cells synthesizing specific antitoxic class A immunoglobulins, in the tunica propria of the mucous membrane of the small intestine [4, 5]. This fact suggests that the cholera toxin or its subunits penetrate through the epithelium of the small intestine. However, the results of experiments carried out to study this problem have not proved convincing. Some workers, using labeled cholera toxin, found at the light-optical level that toxin penetrates through the epithelial layer of the intestine [6], whereas other workers, using a highly sensitive electron-immunocytochemical method of detection of toxin in the tissue, showed that cholera toxin does not penetrate into the intestinal epithelium, but interacts only with the apical plasmalemma of the epitheliocytes [7].

Meanwhile, despite existing data on the direct role of the Peyer's patches in the development of antitoxic immunity [8], the possibility of selective absorption of cholera toxin by the epithelium of the Peyer's patches has not been investigated.

Accordingly, the aim of the investigation described below was to study the possibility of absorption of cholera toxin by the epithelium of Peyer's patches.

EXPERIMENTAL METHOD

Experiments were carried out on 15 male guinea pigs weighing 100-120 g. Before the experiment the animals were deprived of food for 24 h but allowed water ad lib. Highly purified cholera toxin (Choleragen, from the "Mikrob" Research Institute, Saratov), labeled with horseradish peroxidase by means of glutaraldehyde by a two-stage method [9], was used. Under hexobarbital anesthesia laparotomy was performed and a segment of the proximal part of the jejunum, containing a Peyer's patch, 1.5 cm long was ligated. Into the lumen of the ligated loop, 50-100 μg of labeled cholera toxin in a volume of 100-150 μl of 0.9% NaCl solution was injected. In the control experiments labeled cholera toxin was injected into the lumen of a ligated loop not containing a Peyer's patch. To study the distribution of endogenous peroxidase activity, 0.9% NaCl solution was injected into the lumen of a ligated loop. The ligature was removed from the jejunum 60 min after injection of the labeled toxin. The contents of the loop were washed out with 2-2.5 ml of 2.5% glutaraldehyde solu-

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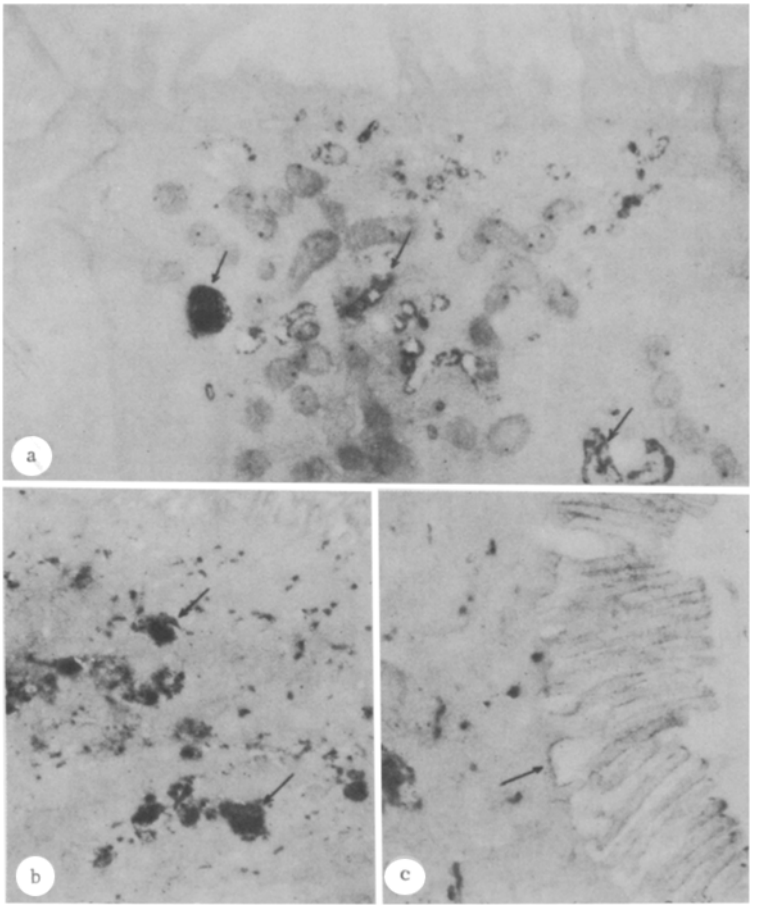

Fig. 1. Absorption of cholera toxin labeled with horseradish peroxidase by epithelium of Peyer's patch in guinea pig 60 min after injection into intestinal lumen. a) Deposition of reaction product in tubules and vesicles of smooth endoplasmic reticulum of M cells, 28,800×; b) presence of reaction product on apical plasmalemma of epitheliocytes of intermediate villi of Peyer's patch, 27,000×; c) deposition of reaction product in elements of smooth endoplasmic reticulum of epitheliocytes of intermediate villi of a Peyer's patch, 15,000×.

tion in 0.1 M cacodylate buffer, pH 7.4, containing 7% sucrose. The excised loop of jejunum was spread out on a paraffin wax dish, covered with a layer of fixative, and fixed for 40 min. The jejunum was then cut transversely and the pieces thus obtained were postfixed for 20 min. The pieces were washed in 0.1 M cacodylate buffer containing 7% sucrose overnight, after which frozen sections were cut to a thickness of 25 μ. Peroxidase activity was detected with the aid of 3,3'-diaminobenzidine [10]. After washing three times with distilled water, the sections were postfixed for 1 h in a 1.33% solution of osmium tetroxide in 0.1 M cacodylate buffer, pH 7.4. The material was dehydrated in acetone of increasing strength and embedded in a mixture of Epon and Araldite. Unstained ultrathin sections, gray in color, were examined in the JEM 100S electron microscope.

EXPERIMENTAL RESULTS

The investigation showed that 60 min after injection of cholera toxin into the intestinal lumen a homogeneous electron-dense reaction product was localized on the inner surface of most tubules and vesicles of the smooth endoplasmic reticulum of the M cells of the epithelium covering the apices of the Peyer's patch (Fig. 1a). Tubules (from 30 to 35 nm in diameter) and vesicles (from 245 to 490 nm in diameter) were concentrated in the cytoplasm of the supranuclear zone of the M cells and were surrounded by numerous mitochondria. Occasionally these structures were adjacent to the lateral plasmalemma.

Meanwhile the homogeneous electron-dense reaction product was discovered on the membrane of the microvilli, in invaginations of the apical plasmalemma (Fig. 1b), and in most of the tubulo-vesicular elements of the smooth endoplasmic reticulum of the supranuclear zone of the columnar cells of the epithelium covering the intermediate villi of the Peyer's patch (Fig. 1c). As a rule the diameter of the tubules was from 30 to 35 nm and of the vesicles from 260 to 520 nm.

No electron-dense reaction product was found in the material studied in the intercellular spaces of the epithelial layer of the Peyer's patch.

By contrast with the findings described above, a study of absorption of labeled cholera toxin by the epithelium of the jejunum outside the region of the Peyer's patch showed that the cytochemical reaction product was present only on the apical plasmalemma of the epitheliocytes and was absent in their cytoplasmic structures.

In control experiments endogenous peroxidase activity was detected in erythrocytes in the blood capillaries and in lysosome-like structures of the cytoplasm of the macrophages of the Peyer's patch.

The results thus indicate that labeled cholera toxin is absorbed by M cells of the Peyer's patch epithelium. This fact confirms the view already expressed in the literature, that M cells at the apices of Peyer's patch can absorb intact protein macromolecules [1, 2].

Meanwhile, the investigation showed that columnar epitheliocytes of the intermediate villi of the Peyer's patch are also capable of absorbing labeled cholera toxin. In our opinion this process is probably the result of close topographical connection between the intermediate villi and T-dependent zones of the Peyer's patch. It is also possible that absorption of labeled cholera toxin by the epithelium of the intermediate villi is a particular feature of interaction between cholera toxin and the epithelium of the Peyer's patch.

The presence of reaction product in structures of the smooth endoplasmic reticulum of the epitheliocytes and its absence in the intercellular spaces of the epithelium of Peyer's patch 60 min after injection of the toxin into the intestinal lumen suggest that the process of liberation of cholera toxin from the epitheliocytes takes place at later times. Such a possibility is indicated by observations made on other models [11]. However, the experimental technique used, with ligation of the intestine, did not enable the subsequent fate of the cholera toxin to be traced because of hemodynamic disturbances arising in the intestinal wall with the passage of time. Of course the possibility of release of very small quantities of labeled cholera toxin from epitheliocytes which had absorbed it cannot be ruled out, although probably these amounts could not be detected by the method used.

It can be tentatively suggested that the mechanism of absorption of labeled cholera toxin by the epithelium of Peyer's patches, as described above, also acts in the case of absorption of unlabeled cholera toxin. This view is based on observations showing that cholera toxin labeled with horseradish peroxidase preserves its toxic properties and its ability to bind with the specific Gm_1 receptor [12].

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