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Polarized Distribution of G_{M1}-Ganglioside in Human Duodenal Absorptive Enterocytes as Visualized with Cholera Toxin-Gold Complex

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Cholera toxin bound to particles of colloidal gold was used to investigate by electron microscopy the binding of the toxin in human duodenum. Cholera toxin binding was detected only in the apical (brush border) plasma membrane domain suggesting that the ganglioside G_{M1} is absent from the basolateral plasma membrane domain. Intracellularly, toxin binding became detectable in the *trans* side of the Golgi apparatus. Labeling of endosomes may indicate that the non toxin-occupied G_{M1}-ganglioside becomes internalized.

Various bacterial toxins have been reported to interact with gangliosides, and cholera toxin (CT) has been the most extensively studied in this respect [1-4]. The binding of CT to intact cells is rapid, saturable, and of high affinity [5-7]. There is now substantial evidence implicating the ganglioside G_{M1} as the only receptor for CT [4, 5, 8-10]. CT mediates its pathological effects, watery diarrhea in association with cholera, by binding to specific receptors on the intestinal mucosal cells [9-11] and activating adenylate cyclase [12, 13]. The resulting rise in cyclic AMP causes chloride and water secretion by the cells [14]. In addition to its binding to intestinal mucosal cells, CT interacts with a number of other cell types such as fibroblasts, adipocytes, neuroblastoma cells, lymphocytes and hepatocytes [15-18].

Cell surface binding and receptor-mediated endocytosis of CT have been investigated by electron microscopy in various cell types [18, 20-22]. In rat hepatocytes, endocytosis of CT bound to particles of colloidal gold has been shown to occur through non-coated membrane invaginations followed by delivery to multivesicular bodies, one type of endosomes [18]. Until now no study has been conducted to investigate the binding of CT to its classical target cells, the intestinal epithelial cells. Therefore, in the present study CT bound to particles of colloidal gold was used to define by electron microscopy the site of its binding on human intestinal cells. At the same time this procedure will provide information about the plasma membrane and the intracellular distribution of the ganglioside, G_{M1}.

Materials and Methods

Tissue Processing for Electron Microscopy

Human duodenal biopsies were obtained from 4-6 years-old children who underwent this procedure for diagnostic purposes. Donor specimens which appeared normal upon histological examination were used in this study. After aspiration, biopsy material was fixed for 2 h at room temperature in 2% freshly prepared formaldehyde-0.1% glutaraldehyde in 10 mM sodium phosphate buffer (pH 7.2) with 0.15 M NaCl (PBS). After several rinses with PBS, free aldehyde groups in the tissue were blocked by incubation with 50 mM NH_4Cl in PBS for 1 h. Tissue pieces were dehydrated in ethanol at progressively lowered temperature down to -35°C. Infiltration with the resin Lowicryl K4M [23] and UV light-polymerization (for 24 h) were done at -35°C followed by UV light-polymerization at room temperature for 2 days. Thin sections were mounted on Parlodion/carbon coated nickel grids.

Preparation of the CT-Gold Complex

Particles of colloidal gold with a mean diameter of 14 nm were prepared according to the procedure of Frens [23]. The CT-gold complex was prepared according to our previously published protocol [18, 25]. Cholera toxin was purchased from Schwarz/Mann, New York, USA.

Labeling of Ultrathin Sections with CT-Gold Complexes

Grids with the attached thin sections were placed on a droplet of CT-gold complex (100 μg/ml CT) for 1 h at room temperature. Afterwards, they were washed with PBS (2 × 2 min) and finally with distilled water. Contrasting staining was done with 2% aqueous uranyl acetate for 5 min and lead acetate for 45 sec.

Specificity Controls

Thin sections were first incubated with an excess amount of non-labeled CT (500 μg/ml) for 1 h and then exposed to CT-gold. In another type of control, thin sections were incubated with particles of colloidal gold coated with the inert protein bovine serum albumin.

Results and Discussion

In the present study CT bound to particles of colloidal gold was used to investigate by electron microscopy the distribution of G_{M1}-ganglioside in human duodenum. For this purpose ultrathin sections from tissues were incubated with the CT-gold complex. In this post-embedding approach, problems of uneven and limited reagent penetration

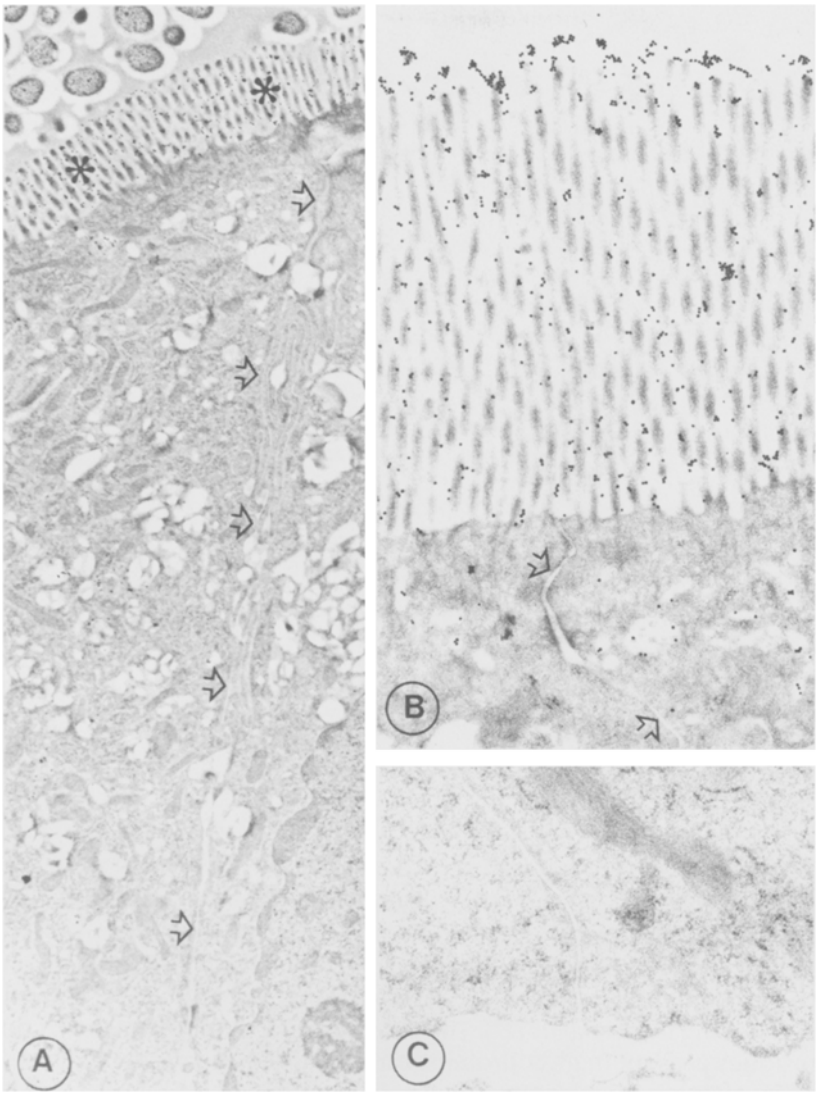

Figure 1. Ultrathin sections from Lowicryl K4M embedded human duodenum incubated with cholera toxin-gold complex. The labeling of absorptive enterocytes can be seen by the presence of the black-appearing gold particles. (A), brush border (asterisks), basolateral plasma membrane (arrows). (B), intensely labeled brush border and part of the non-labeled lateral plasma membrane (arrows). In (C), part of the non-labeled basolateral plasma membrane can be seen.
Magnifications: × 7 000 (A); × 22 000 (B); × 27 000 (C).

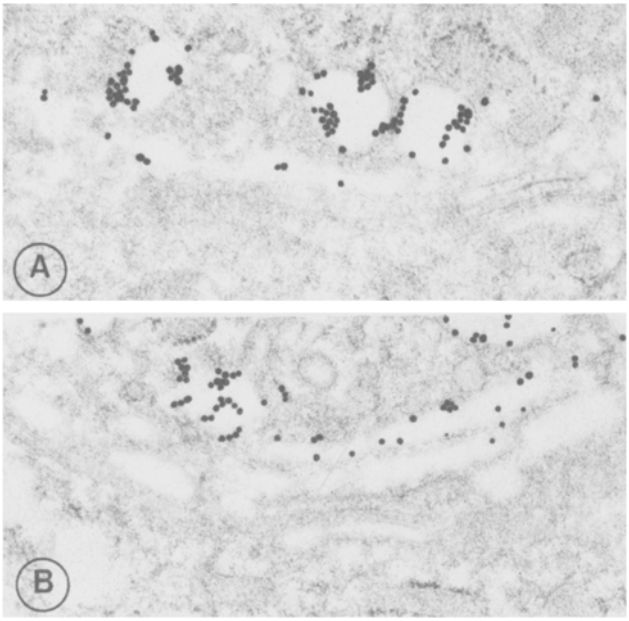

Figure 2. Gold particle label is found in absorptive enterocytes over one or two *trans* cisternae and vesicles at the *trans* side of the Golgi apparatus. *Medial* and *cis* Golgi apparatus cisternae are not labeled. Magnification: × 51 000 (A, B).

into tissue common in pre-embedding labeling procedures are circumvented. In addition, it has been demonstrated in other investigations that the tissue processing conditions of Lowicryl K4M embedding permit successful labeling of thin sections with various lectin-gold complexes [26]. Sialic acid residues could also be visualized with the lectin from the slug *Limax flavus* [27]. The present study demonstrates that not only sialic acid residues present in glycoproteins but also those found in gangliosides can be visualized by electron microscopy.

The labeling with the CT-gold complex was specific as it could be abolished by preincubation of the thin sections with non-labeled CT. Bovine serum albumin-gold complexes did not bind. Gold particle-labeling indicating the presence of CT binding was found in absorptive enterocytes where it was restricted to the apical (brush border) plasma membrane domain (Fig. 1A, asterisks). The basolateral plasma membrane (Fig. 1A-C, arrows) was completely negative. This provides another example for structural and functional cell polarity, specifically with respect to G_{M1}-ganglioside distribution. In earlier studies it was shown that certain plasma membrane proteins were present only in the apical plasma membrane whereas others were found only in the basolateral plasma membrane domain [28-31]. Such functional polarity is also reflected by the polarized budding of viruses in cultured kidney epithelial cells [32, 33]. This phenomenon was recently used by van Meers and Simons [34] to study the phospholipid composition of the

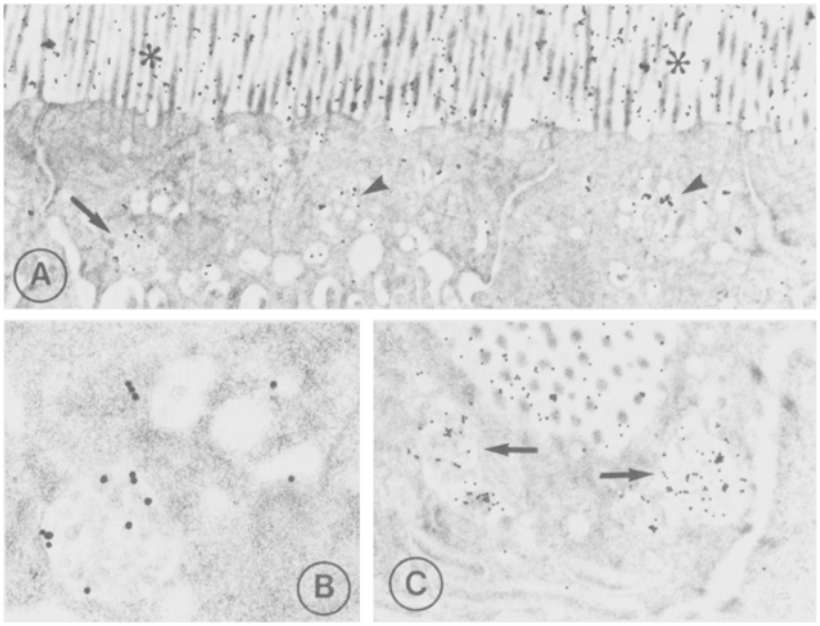

Figure 3. The apical portion of several absorptive enterocytes is shown in (A). Besides intense labeling over the brush border (asterisks), gold particles are also present over many cytoplasmic vesicles (arrowheads) and a multivesicular body (arrow) which is shown at higher magnification in (B). In (C), another apical cell region is seen with intensely labeled multivesicular bodies (arrows).
Magnifications: × 15 000 (A); × 51 000 (B); × 26 000 (C).

two membrane domains in this cell type. They showed that both plasma membrane domains are characterized by a unique phospholipid composition. Previous studies on the phospholipid composition of the plasma membrane domains of epithelial intestinal cells have demonstrated that not only the phospholipid composition but also the cholesterol content greatly differed [35-38]. The present data furnish another example of domain formation with respect to the distribution of G_{M1}-ganglioside in absorptive intestinal cells. At present it is not clear how such domains are generated, nor how they are maintained. Although tight junctions have been implicated in such processes [39], recent data [40] provided evidence that cell polarity can be established without the presence of such junctions.

Labeling with CT-gold complex was also detectable intracellularly in one or two *trans* cisternae of the Golgi apparatus (Fig. 2). In addition, cytoplasmic vesicles and multivesicular bodies were labeled (Figs. 2 and 3). The labeling in the Golgi apparatus provides evidence that sialylation of G_{M1}-ganglioside may first appear at this intracellular site as has already been shown for glycoproteins [26, 41-43].

The labeling of multivesicular bodies with CT-gold is interesting since this type of vesicle represents one form of an endosome. It is generally believed that delivery of receptors to endosomes is triggered by previous ligand binding to the cell surface receptors [44]. The present observation, however, might be taken as *bona fide* evidence that CT receptors not occupied by the toxin molecule become endocytosed in a manner as observed for toxin occupied receptors in hepatocytes [18]. Such a situation seems to be true for at least one other receptor, the transferrin receptor, which has recently been shown to be endocytosed equally rapidly in the presence or absence of transferrin [45].

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