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”Pseudo-cap” formation in Ehrlich ascites tumor cells induced by cytochalasin B.

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Abstract

Cytochalasin B (CB) treatment induces or accelerates the capping phenomenon in some cells. In Ehrlich ascites tumor cells (EATC) CB treatment apparently induced the capping of Con A binding sites as observed under a fluorescent microscope. However, electron microscopic examinations revealed that the CB treatment did not induce a rearrangement of Con A binding sites, but rather it only induced a change in cell shape. On the contrary, CB treatment inhibited the capping phenomenon induced by treatment with Con A. Electron microscopic observations may give exact information on the distribution of lectin binding sites.

KEYWORDS: Ehrlich ascites tumor cell, concanvalin A, cytochalasin B, cap formation

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—— BRIEF NOTE ——

"PSEUDO-CAP" FORMATION IN EHRLICH ASCITES TUMOR CELLS INDUCED BY CYTOCHALASIN B

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Abstract. Cytochalasin B (CB) treatment induces or accelerates the capping phenomenon in some cells. In Ehrlich ascites tumor cells (EATC) CB treatment apparently induced the capping of Con A binding sites as observed under a fluorescent microscope. However, electron microscopic examinations revealed that the CB treatment did not induce a rearrangement of Con A binding sites, but rather it only induced a change in cell shape. On the contrary, CB treatment inhibited the capping phenomenon induced by treatment with Con A. Electron microscopic observations may give exact information on the distribution of lectin binding sites.

Key words : Ehrlich ascites tumor cell, concanavalin A, cytochalasin B, cap formation.

Concanavalin A (Con A) rearranges the distribution of lectin binding sites on the cell surface of various kinds of cells and induces cap formation (1-3). It is generally believed that the phenomenon is closely related to the function of microfilaments (MF) (1, 4), as the capping is supressed by the treatment of cells with cytochalasin B (CB) (5, 6), which arrests the function of MF. It is also known that CB induces or accelerates the capping phenomenon in some cells, as can be seen by fluorescent microscopy (7-9). In such cases, CB treatment induces a change in cell shape and the formation of a uropode, at which capping may be observed. Such an effect of CB on the cell surface was confirmed by us in Ehrlich ascites tumor cells (EATC). However, electron microscopy revealed that the CB-induced capping was not accompanied by the rearrangement of Con A binding sites. In this paper the cap formation of EATC induced by CB is demonstrated in relation to the distribution of Con A binding sites.

EATC were harvested from the peritoneal cavity of Swiss mice, 10 days after inoculation with 2×10^6 cells. After the cells were washed 3 times by repeated centrifugation at 4℃ with phosphate buffered saline (PBS), they were

suspended in PBS, at 2×10^6 cells/ml.

EATC incubated with FITC-Con A formed a cap as seen under the fluorescent microscope in about 50% of the cells (Fig. 1a). As observed under the electron microscope the cap was composed of a number of accumulated microvilli. Con A binding sites were dense around the roots of the microvilli (Fig. 1b). EATC incubated with CB, fixed and exposed to FITC-Con A also showed

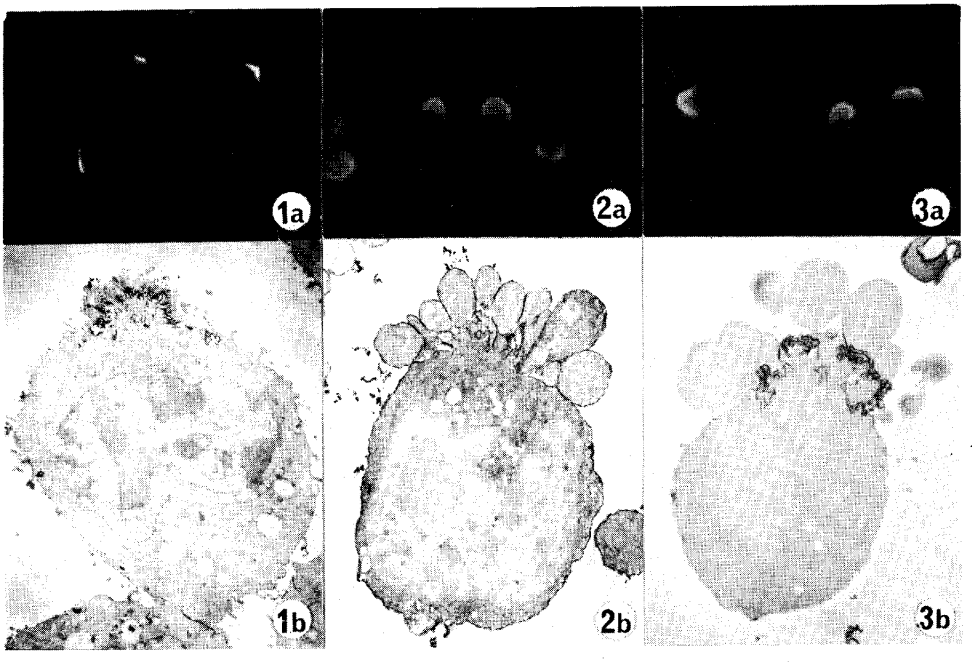

Fig. 1. EATC were preincubated for 20 min at 37℃ without any reagents, and then incubated with Con A (50 μg/ml) for 15 min. For fluorescent microscopy, FITC-Con A (20 μg/ml) was used instead of Con A. For electron microscopy, cells were fixed with 2% glutaraldehyde and stained by the horse radish peroxidase diamino-benzidine method (10) to visualize Con A binding sites.

Fig. 2. EATC were incubated with CB (10 μg/ml) for 20 min and fixed with 2% glutaraldehyde for 15 min and then treated with Con A (50 μg/ml). For fluorescent microscopy, movement of Con A binding sites was stopped with NaN_3 (5 mM) after the treatment with CB, and then the cells were treated with FITC-Con A. NaN_3 inhibits the movement of Con A binding sites (11).

Fig. 3. EATC were preincubated with CB (10 μg/ml) for 20 min and further incubated with Con A or FITC-Con A. Other conditions are as in Fig. 1.

a; Capping observed under the fluorescent microscope. (× 1,000)

b; Distribution of Con A binding sites observed under the electron microscopy. Dense deposits on the surface are Con A binding sites visualised by the horse radish peroxidase diaminobenzidine method. (× 4,500)

capping in about 70% of the cells. Caps were bigger than those formed by incubating with Con A (Fig. 2a). Electron microscopically, caps were made of

uropodes which consisted of blebs and microvilli (Fig. 2b). Con A binding sites were distributed diffusely over the surface of these cells (Fig. 2b). Therefore the capping observed under the fluorescent microscope was formed without the induction of a rearrangement of Con A binding sites. EATC incubated with CB followed by reincubation with Con A also showed capping in about 70% of cells (Fig. 3a). As observed under the electron microscope, the morphology of the cells was the same as the CB-treated cells (Fig. 3b), but in about 40% of the cells the uropode was closely associated with Con A binding sites (Fig. 3b), though in the others, Con A binding sites were evenly distributed. The phenomenon indicates that CB treatment partially inhibits the rearrangement of Con A binding sites with apparent acceleration of capping.

As mentioned above, CB treatment induces or accelerates cap formation in some cell types (7-9). The morphological changes of these cells were very similar to those of our observations, except that electron microscopic observations were not performed on the former.

In general, distribution of membrane components is observed with the fluorescent microscope, but present observations show that the intensity of fluorescence is not always indicative of the amount of FITC per unit of membrane surface. Electron microscopic observations may give more exact information.

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