EFFECT OF CYTOCHALASINS ON THE SURFACE TOPOGRAPHY OF TUMOR CELLS IN SUSPENSION

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Scanning electron microscopy (SEM) has demonstrated the presence of various microscopic outgrowths on the surface of many types of cells: vesicles, microvilli, folds, etc. These formations may have important functions: They are the "storehouses" of the cell surface, utilized during changes in shape of the cell. They also take part in absorption of substances from the medium, and so on, by the cell [1, 3]. Mechanisms of formation of these microscopic outgrowths are not clear: It can be tentatively suggested that their character is determined by the state of the cytoskeleton and, in particular, the state of the cortical layer of actin microfilaments. To test this hypothesis cytochalasins (CCH), which reversibly disorganize the microfilament system [5, 12], may be used. Several effects of CCH be on these microscopic outgrowths have been described in the literature: the formation of focal concentrations of microvilli or microfolds [4], the appearance of large vesicles [6, 9], reduction of microvilli [8, 11], and the appearance of coarsely folded regions of the surface [8]. No systematic quantitative analysis of the action of CCH has yet been undertaken on the various microscopic outgrowths on the surface of cells of one type.

In the investigation described below the effect of CCH was studied on the character of microscopic surface outgrowths of neoplastic fibroblasts and of ascites tumor cells in culture; the cells were suspended in liquid medium, for the number of microscopic outgrowths on such cells is particularly large. Besides CCH B it was also decided to use CCH D, which gives no side effects and has the most selective action on the microfilament system.

EXPERIMENTAL METHOD

Neoplastic mouse fibroblasts of the L line and its LS subline, adapted to conditions of culture in a suspended state (obtained by V. I. Gel'fand), and also cells of an Ehrlich's mouse ascites tumor were used. A suspension of L cells was obtained by treating a monolayer culture with a mixture of trypsin and versene; LS cells were separated by centrifugation; ascites tumor cells were obtained on the 7th-8th day of growth of the tumor, separated by centrifugation from ascites fluid, and washed twice with medium 199. The cells were resuspended in medium 199 with 10% bovine serum (the LS cells in medium 199 with 20% bovine serum) and incubated with CCH B (2 μg/ml) or CCH D (0.2 μg/ml) for 30 min-3 h at 37°C in siliconized petri dishes. At the end of incubation the cells were fixed in suspension and prepared for SEM by the method described previously [2]. Besides qualitative evaluation, SEM also included quantitative analysis of the relative numbers of different types of surface microrelief of the suspended cells [10]. The following types of microrelief were considered: microvillous — the microrelief consisted entirely of microvilli (Fig. 1a); vesicular — the microrelief consisted entirely of vesicles (Fig. 1c); mixed, vesiculo-microvillous (Fig. 1b). Cells with localized areas of coarsely folded surface against a general background of one particular microrelief also were counted (Fig. 1f).

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TABLE 1. Effect of CCH B on Surface Topography of Suspended Cells

Incubation time	Treatment of cells	Percentage of cells with different types of microrelief			Percent of cells with areas of coarsely folded surface
		microvillous	mixed	vesicular	
		Cells of line L			
30 min	Control	43,3±9,0	46,7±9,1	10,0±5,5	—
	CCH B	—	12,5±8,1	87,5±8,1	6,7±4,2
1 h	Control	66,7±8,6	26,7±8,1	6,7±4,6	—
	CCH B	—	31,8±9,9	68,2±9,9	23,4±7,7
3 h	Control	63,2±11,0	36,8±11,0	—	—
	CCH B	—	25,0±9,7	75,0±9,7	33,3±9,0
		Cells of subline LS			
1 h	Control	39,2±10,0	56,5±10,0	4,3±4,0	—
	CCH B	—	100,0	—	—
3 h	Control	46,0±7,6	48,8±7,6	4,6±3,2	—
	CCH B	5,1±3,6	23,1±6,7	71,8±7,2	58,7±7,8
		Ehrlich's ascites tumor cells			
30 min	Control	83,0±6,8	14,0±6,3	3,0±3,1	—
	CCH B	34,5±8,6	65,5±8,6	—	—
3 h	Control	82,6±7,8	13,0±7,0	4,3±4,0	—
	CCH B	—	—	100,0	78,0±13,7

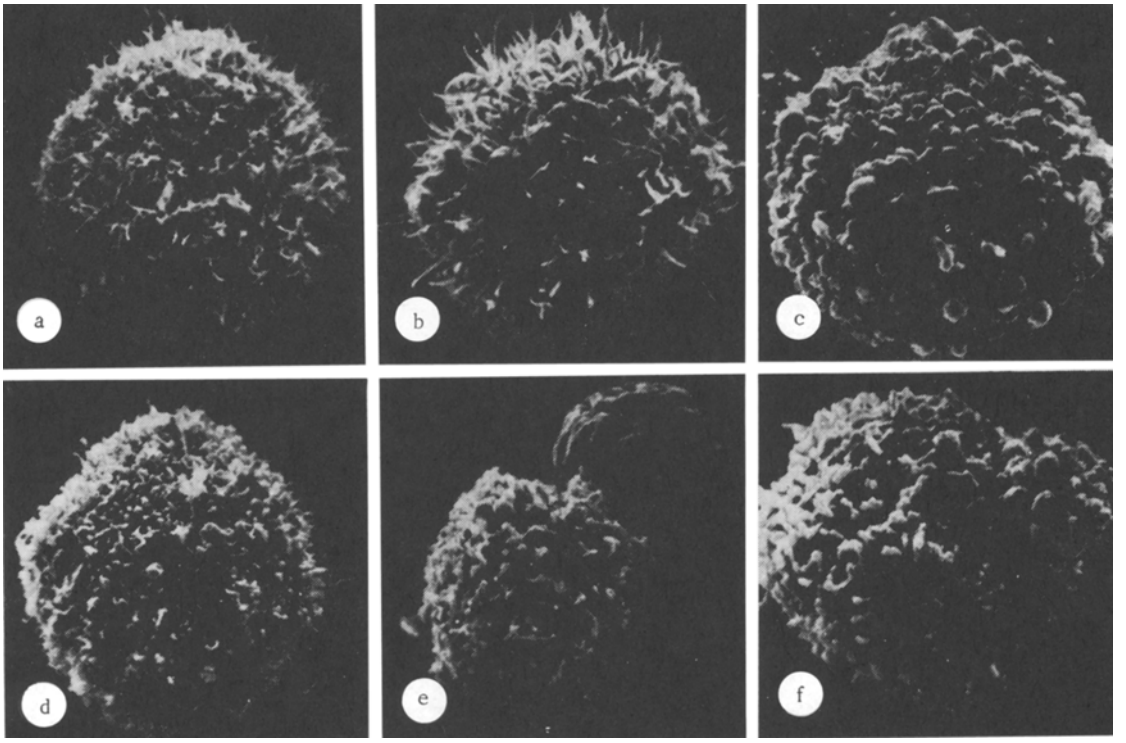

Fig. 1. Effect of CCH B on character of microvilli on surface of neoplastic fibroblasts and ascites tumor cells in culture. a) L cell: microvillous relief; b) L cell: mixed vasiculo-microvillous relief; c) L cell: vesicular relief; d) L cell + CCH B: shortened microvilli with reduced density of distribution; e) LS cell + CCH B: shortened microvilli, very large single vesicles; f) Ehrlich's ascites tumor cell + CCH B: polar distribution of vesicles, area of coarsely folded surface. Magnification 3700.

EXPERIMENTAL RESULTS

Most frequently two types of microrelief of L and LS cells were observed: microvillous and mixed; among L cells most were of the microvillous type. The overwhelming majority of ascites cells had a microvillous relief (Table 1). As a result of incubation (30 min to 1 h) of the cells with CCH B the following changes were observed in cell surface topography.

Even though the relative percentage of cells with a microvillous relief was considerably reduced, there was a corresponding increase in percentage of cells with vesicular (L cells) or mixed (LS cells, Ehrlich's ascites tumor cells) type of microrelief (Table 1).

The microvilli were shortened (from the normal length of 0.9-1.7 μ to 0.2-0.6 μ) and the density of their distribution on the cell surface was reduced (Fig. 1d). Localized areas with a coarsely folded relief appeared (Table 1). Some very large (5-13 μ in diameter) single vesicles appeared against the background of a mixed microrelief on LS cells, less frequently on L cells (Fig. 1e). Changes in the character of distribution of the vesicles also were observed on Ehrlich's ascites tumor cells: They were connected in one particular area of the cell surface (polar arrangement of vesicles), and the rest of the surface appeared to be coarsely folded (Fig. 1f).

After incubation for 3 h with CCH B, the overwhelming majority of the cells consisted of those with a vesicular microrelief (Table 1). The irregular character of distribution of vesicles described above now was observed not only on Ehrlich's ascites tumor cells, but also on L cells. A polar arrangement of vesicles was the commonest type of ascites cells. CCH D had the same effect on the surface topography of L and LS cells as CCH B.

The results show that the mechanism of formation of two different types of microscopic outgrowths (microvilli and vesicles) may differ. To form microvilli a normal state of the actin cytoskeleton is required, and this process is inhibited even by small doses of CCH. Conversely, vesicle formation is undisturbed after partial disorganization of the cortical layer of the actin microfilaments. During the formation of microvilli polymerization of the "rod" of the microfilaments may perhaps take place first, followed by evagination of the plasma membrane, whereas polymerization of microfilaments of this kind is unnecessary for vesicle formation. For example, vesicles may be formed as a result of disturbance of the continuity of the microfilament network of the cortical layer: Under the influence of CCH, "windows" are formed in it, through which the cytoplasm bulges to form vesicles [7]. The mechanism of formation of the polar concentrations of vesicles which we observed is not yet clear. The microfilament system may somehow control the uniformity of distribution of microscopic outgrowths on the cell surface.

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