REVERSIBLE INHIBITION OF CELLULAR AGGREGATION BY CYTOCHALASIN B AND COLCEMID

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Aggregation of normal mouse fibroblast-like cells, placed on the surface of a Millipore filter, was investigated quantitatively. The addition of 10 μg/ml of cytochalasin B (a substance blocking active cell movements) or 0.1 μg/ml of colcemid (a substance disturbing polarization processes in cells and, consequently, interfering with directional cell movements) completely inhibits such aggregation. The inhibition is reversible – after removal of the agents the cells again aggregate. It is concluded that aggregation of cells in this experimental system requires active cell movement and cellular polarization.

KEY WORDS: aggregation of cells; cytochalasin B; colcemid; motion of fibroblast-like cells.

Normal fibroblast-like cells, if placed on the surface of certain substrates, will grow there not in a single layer, as on glass, but will collect into compact, discrete aggregates [1, 2, 4, 5, 9]. Since ability to aggregate in this experimental system is disturbed in the case of cells of most transformed strains that have been studied [2, 5], the study of the mechanisms of aggregation of normal cells could be a useful aid to the understanding of differences between normal and tumor cells. It is important in principle to establish whether aggregation of cells on a substrate is a passive process (such as flocculation or coalescence of drops of a liquid) or whether its performance requires active and directional cell movements.

To study this problem the effect of cytochalasin B, which paralyzes various forms of cellular motor activity [7, 11], and of colcemid, which makes cell movements undirectional and prevents the formation of polarized structures in the cells and the stabilization of the cell surface [3], on aggregation were investigated.

EXPERIMENTAL METHOD

Secondary cultures of embryonic mouse fibroblast-like cells were used. HUFS membrane filters (Chemapol, Czechoslovakia) with a pore diameter of 0.3-0.5 μ, were used as the substrates to induce aggregation. The technique of the experiments to study aggregation of cells on the surface of such filters was described by the writers previously [2]. Cytochalasin B (England) was added to the culture medium as a 0.1% solution in dimethyl sulfoxide (DMSO); the final concentration of cytochalasin B in the medium was 10 μg/ml. Colcemid (Batumi Caffeine Factory) was used in a concentration of 0.1 μg/ml. The degree of aggregation of the cells on the filters was assessed, as in previous investigations [1, 2], with the aid of a special aggregation index. This index was one in the case of a random distribution of the cells on the substrate and more than one if the cells formed aggregates.

EXPERIMENTAL RESULTS

The various ways in which the cells were treated are shown in Table 1. The density of the cells on the filters, fixed 2 h, and 24 h after seeding, was 4×10^4 to 6×10^4 cells/cm^2. The addition of cytochalasin B or of colcemid caused no change in the density of the cells compared with the control. Both agents induced characteristic changes in cell morphology. Cells treated with cytochalasin B, whether on glass or on the

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TABLE 1. Effect of Colcemid and Cytochalasin B on Cellular Aggregation: Values of Aggregation Indices for Control and Treated Cells

Type of treatment of cells	Time after seeding					
	2 h		1 day		2 days	
	type of substrate					
	glass	millipore filter	glass	millipore filter	glass	millipore filter
Control	1,0±0,18	1,2±0,17	1,0±0,12	4,6±0,62	1,1±0,45	10,8±0,50
DMSO (1%) 2 h after seeding	—	—	1,0±0,16	3,4±0,40	— (24 h after removal of cytochalasin B)	—
Cytochalasin B (10 μg/ml) 2 h after seeding	—	—	0,9±0,04	1,0±0,07	1,2±0,07 (24 h after removal of colcemid)	3,4±0,50
Colcemid (0.1 μg/ml) 4 h before seeding	1,0±0,07	1,4±0,16	0,8±0,10	1,1±0,10	1,0±0,07 (24 h after removal of colcemid)	7,3±0,20
Colcemid (0.1 μg/ml) simultaneously with seeding	1,6±0,07	1,4±0,25	1,2±0,07	1,2±0,07	1,0±0,07	8,4±0,90
Colcemid (0.1 μg/ml) 2 h after seeding	—	—	1,1±0,30	1,8±0,25	—	—

Legend. Results of two experiments are pooled in the table.

filter, assumed an irregular star-shaped appearance with long and thin processes, projecting haphazardly in different directions. As a result of the action of colcemid on both types of substrate the cells with processes or elongated cells disappeared; the cells treated with colcemid spread less widely (especially on the filters) than the controls.

The results summarized in Table 1 show marked inhibition of cellular aggregation on the filters both by cytochalasin B and by colcemid. This inhibition of aggregation, however, was reversible. If filters with cells attached to them were washed to remove these agents and left in pure medium for a further 24 h, the picture of well-marked aggregation was observed on preparations fixed at that time.

Aggregation of cells fixed to the substrate is a phenomenon of the same class as the sorting of cells of different tissues in mixed aggregates [10] or the redistribution of cells on a substrate with uneven adhesiveness [6, 8]. In all these systems the cells have the power of choice of attachment (in the present case this was choice between attachment to the substrate and attachment to the neighboring cell) and this choice is always made in favor of the state corresponding to the strongest attachment. The facts described above are evidence that both cytochalasin B and colcemid prevent the normal procedure of this choice, and it can therefore be concluded that the choice of the state of best attachment by the cell is not a passive process but requires active movements and cellular polarization.

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