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Morphology, Motility, and Surface Behavior of Lymphocytes Bound to Nylon Fibers

(cell shape/cell mobility/cap formation/concanavalin A)

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ABSTRACT **Mouse B lymphocytes that were specifically bound to dinitrophenylated bovine serum albumin on nylon fibers exhibited continuous morphological changes, whereas bound T lymphocytes remained more or less spherical. Cinematomicrographic studies showed that the shape changes were associated with local and global movements, although the attached cells did not translocate along the fiber. Cap formation induced by anti-immunoglobulin was always found to be opposite to the point of attachment. The movements and the shape changes were prevented by cytochalasin B and colchicine. Treatment with these agents did not prevent cap formation but led to randomization of the position of the caps with respect to the fiber. Exposure to concanavalin A or attachment of cells to concanavalin A fibers prevented both movement and patch and cap formation, suggesting that cellular structures regulating the mobility of various receptors are altered by binding to concanavalin A fibers. These observations also indicate that interactions of local areas of the lymphocyte surface with certain ligands and substrates can strongly affect the movement and morphology of the entire cell.**

The relationship of cell movement to cell surface events is poorly understood (1). In studying this problem, a number of factors can be distinguished: local movement of cell surface receptors (diffusion and patch formation), global movements of these receptors (capping), local morphological changes (microvillus formation, blebbing, and ruffling), global morphological changes altering cell shape, and translocation of the whole cell.

It is experimentally difficult to analyze these events at both the microscopic and molecular level in a single cell type. To reduce this difficulty, we have applied the method of fiber fractionation (2) to make lymphocytes sessile by attaching them to derivatized nylon fibers via their Ig or lectin receptors. The bound lymphocytes do not translocate on the fiber; for this reason, they may be identified and readily observed *in situ* and polarity effects may be studied with relative ease.

In the present experiments, we have studied the morphological changes undergone by fiber-bound lymphocytes, the identification of cellular elements affecting their movement and shape, and the connection between these factors and those controlling the local movement of cell surface receptors. Our experimental results are consistent with the proposal that surface receptor mobility is modulated by colchicine-sensitive cytoplasmic structures that can respond to cross-linkage of lectin receptors (3). They also suggest that at least some of these structures may be important in altering and maintaining cell shape.

Abbreviations: MCC, morphologically changed cells; Dnp-BSA, dinitrophenylated bovine serum albumin; Con A, concanavalin A; MEM, minimal essential medium, Eagle's, with Earle's balanced salts, without bicarbonate; PBS, phosphate-buffered saline, pH 7.4; fl-anti-Ig, fluorescein-labeled rabbit Ig directed against mouse Ig.

MATERIALS AND METHODS

Fractionation of Cells by Derivatized Nylon Fibers. Two- to 4-month-old Balb/c mice (Jackson Lab., Bar Harbor, Me.) were immunized twice intraperitoneally with 100 μg of Dnp-hemocyanin adsorbed on bentonite. Spleen cell suspensions with a viability of 75–85% were prepared from immunized mice 5–6 days after the second immunization.

General procedures for preparation of derivatized nylon fibers have been described previously (2). Derivatization of the fibers was carried out at 21° for 30 min using 0.25 mg/ml of protein and 1.25 mg/ml of the carbodiimide reagent (2).

Cells (10^8) in 4.0 ml of Eagle's minimal essential medium (MEM)–DNase (4) were incubated with derivatized fibers at 4° for 1 hr with gentle shaking as described previously (2). Unbound cells were removed by immersion of the fibers in a series of bowls containing cold phosphate-buffered saline, pH 7.4, (PBS) and finally cold MEM. The binding of cells to dinitrophenylated bovine serum albumin (Dnp-BSA) fibers was over 90% inhibitable by 100 μg/ml of soluble Dnp-BSA or 300 μg/ml of anti-Ig. Binding to concanavalin A (Con A) fibers was over 90% inhibitable by α-methyl-D-mannoside. The initial viability of the fiber-bound cells determined by trypan-blue dye exclusion was over 95% and did not decrease by more than 10% during any experiment. As previously observed (4), the viability of bound cells was always greater than that of unfractionated cells.

Observation of Morphological Changes and Cap Formation. In most experiments, fiber-bound cells were incubated in MEM at 21° or 37° for 1 hr and the changes in cell shapes were scored immediately after incubation. A water-immersion lens was used to examine the cells with a Zeiss Universal Microscope at 400× magnification. 0.05 M NaN_3, 10^{-6} M valinomycin, 20 μg/ml of cytochalasin B, 10^{-4} M vinblastine sulfate, 10^{-4} M colchicine, 100 μg/ml of Con A (5), or 100 μg/ml of Dnp_{10}-BSA were used as inhibitors.

Fiber-bound cells were incubated with 100 μg/ml of fluorescein-labeled rabbit Ig directed against mouse Ig (fl-anti-Ig) at 21° for 15 min and washed with PBS at 21°. Observations were carried out with a Zeiss Universal Microscope equipped with a mercury lamp, vertical illuminator and a water-immersion lens. Photographs were taken with Kodak

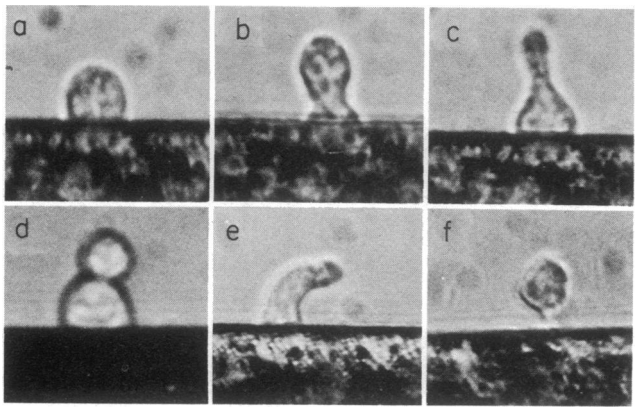

FIG. 1. Various shapes of lymphocytes bound to Dnp-BSA fibers. (*a*) round cells, (*b*) to (*f*) morphologically changed cells.

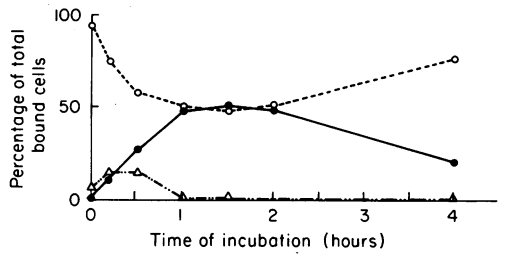

FIG. 2. Time course of the morphological changes of lymphocytes bound to Dnp-BSA fibers. Cells bound to the fibers were incubated at 21° up to 4 hr. (●) Morphologically changed cells of various shapes. (△) Bulb-like cells. (○) Round cells.

FIG. 3. Continuous changes of cell shapes (from *left* to *right*) as observed by cinematography. Silhouettes were obtained by tracing cell shapes on positive prints of every 50 frames (6.7-min intervals).

Tri-X film with an exposure time of 3 min. The nylon fibers displayed a green autofluorescence but this did not interfere with the observations. Because of rapid cell movements, pictures of morphologically changed cells (MCC) were occasionally defocused.

Time-lapse cinematomicrography was used for continuous monitoring of cell shapes. An Arriflex motion picture camera was used with a Universal Zeiss Microscope and water-immersion lens. A time lapse of 2 sec between 2-sec exposures with Plus-X film gave a final speed-up of 96 times at 24 frames/sec. Fig. 3 was obtained by tracing positive prints (×20) of cinematographic films.

RESULTS

Morphological Changes of Cells Bound to Dnp-BSA Fibers. Mouse splenic lymphocytes initially bound to Dnp-BSA fibers at 4° were observed to be more or less spherical in shape but at 21° or 37° they underwent pronounced changes in their morphology. A round cell and various types of MCC are shown in Fig. 1. Up to 50% of the bound cells changed in shape in the first hour of incubation; this number remained constant between 1 and 2 hr and then declined to about 25% at 4 hr (Fig. 2). Bulb-like cells (Fig. 1*b*) were observed only for the first 15–30 min of incubation (Fig. 2) and, therefore, may represent early stages of morphological change. All cells were firmly attached to the fibers, did not detach even after incubation for 4 hr, and in no case was translocation of a cell along the fiber observed. A small number of cells nonspecifically bound to Dnp-BSA fibers were obtained by shaking the fibers with cells in the presence of soluble Dnp-BSA. These cells also exhibited morphological changes during incubation.

TABLE 1. *Effects of drugs on morphological changes of lymphocytes bound to fibers*

Inhibitor	Concentration (μg/ml)	% Morphologically changed cells
None	—	34–58
NaN_3	3000	19–29
Valinomycin	1	0–10
4°*	—	0–10
Cytochalasin B	10	0
Vinblastine	90	7–23
Colchicine	40	15–28
Dnp-BSA	100	41–55
Con A	100	4–25
Con A fibers†	—	3–10

Cells bound to Dnp-BSA fibers were incubated in MEM containing the indicated inhibitors, at concentrations described in this table, at 37° for 30 min except where noted. The percentages shown reflect the range obtained in three to five experiments.

* Cells bound to the fiber were incubated at 4° for 30 min.

† Con A fibers used instead of Dnp-BSA fibers.

In order to distinguish B cells from T cells, the bound cells were treated at 21° for 15 min with fl-anti-Ig after incubation at 37° for 1 hr (4, 6). This procedure did not affect the morphology of the cells. The percentages of cells stained with fl-anti-Ig ranged from 61 to 86% for the Dnp-BSA fiber and from 52 to 62% for the Con A fiber. The MCC were stained with fl-anti-Ig and developed caps similar to those observed in free B cells (5, 7); MCC that did not stain with fl-anti-Ig were very rare.

Cinematomicrographic observations that started just after the temperature was raised to 21° gave direct information about the dynamic state of the cells bound to the fibers. About half the cells bound to Dnp-BSA fibers began changing in shape within 30 min and they continued to change for 2 hr. Analysis of the films showed that the shape changes took place continuously (Fig. 3).

Effects of Suppression of Cellular Metabolism. To determine whether the induction of morphological changes was dependent on cellular metabolism, we tested the effects of low temperature and of NaN_3 and valinomycin added to the medium. As shown in Table 1, 0.05 M NaN_3 was partially inhibitory, 10^{-6} M valinomycin was completely inhibitory and incubation of bound cells at 4° failed to induce morphological changes. The effects of NaN_3 and low temperature were reversed when the fiber-bound cells were transferred to fresh medium without drugs or to 21°. Although valinomycin did not diminish the cell viability, its effects remained even after washing the cells in medium that contained no drugs.

TABLE 2. *Reversibility of effects of drugs on morphological changes and the effects of drugs on pre-formed MCC*

Inhibitor and conditions		% Morphologically changed cells	
1st incubation	2nd incubation	1st incubation	2nd incubation
—*	—	55	50
NaN_3	—	20	40
Valinomycin	—	8	7
4°	37°	7	47
Vinblastine	—	23	45
Cytochalasin B	—	0	38
Con A	—	25	37
Con A	50 mM αMM†	25	49
—‡	NaN_3	34–55	15
—	Valinomycin		13
—	4°		4
—	Vinblastine		3
—	Cytochalasin B		0
—	Con A		22

* Cells bound to Dnp-BSA fibers were incubated in MEM containing inhibitors for 30 min, washed, and incubated for an additional 30 min in MEM containing no inhibitor. Concentrations of inhibitors are the same as those in Table 1.

† α-Methyl-D-mannoside.

‡ Cells bound to Dnp-BSA fibers were incubated in MEM for 30 min and incubated for 30 min in MEM containing inhibitors.

All of these results suggest that establishment of the morphological changes depends upon cellular metabolism.

We also tested whether maintenance of the morphological changes required cellular metabolism. Bound cells were incubated in the standard medium at 37° for 1 hr and then transferred either to media containing drugs or to media at 4°. The results (Table 2) revealed that addition of NaN_3 or valinomycin to the medium or a shift-down of the temperature resulted in decreases in the number of MCC.

Effects of Cytochalasin B, Colchicine, and Vinblastine. In the presence of 20 μg/ml of cytochalasin B none of the fiber-bound cells showed changes in shape under cinematographic observation. Vinblastine (10^{-4} M) was observed to reduce both the number of cells changing in shape and the rate of change of individual cells. The effect of cytochalasin B (Table 1) was not due to the presence of dimethylsulfoxide in which the drug was dissolved. Although both colchicine and vinblastine reduced the number of MCC, their effects were partial and by 30 min to 2 hr after incubation, the number of MCC in the presence of colchicine and vinblastine remained almost constant. Addition of either one of these drugs to pre-formed MCC also caused a decrease in their number (Table 2). The effects of all drugs were reversed after removing them from the media (Table 2). These data suggest that cellular structures susceptible to cytochalasin B, colchicine, and vinblastine are involved in the morphological changes of fiber-bound cells.

Effects of Con A, Soluble Antigen, and Anti-Ig. The addition of 100 μg/ml of Con A to the medium caused a decrease in the number of MCC. This effect was not reversed by washing with fresh medium but was reversed by addition of 0.05 M α-methyl-D-mannoside. In addition to preventing the formation of MCC, Con A also appeared to induce their transformation to round cells (Table 2). Con A exerted its effects even when attached to only one region of the cell, for morphological changes of cells bound to Con A fibers occurred much less frequently than on Dnp-BSA fibers (Fig. 1). As viewed by cinematography, cells bound to Con A fibers changed their shapes more slowly than those bound to Dnp-BSA fibers and the period during which the changes of shapes took place continuously was shortened. The inhibition of shape changes by Con A fibers could be reversed by adding 0.05 M α-methyl-D-mannoside to the medium even though this treatment did not remove the cells from the fiber. In contrast to these observations, anti-Ig affected the morphological changes only slightly and 50 μg/ml of Dnp-BSA and 20 μg/ml of ε-Dnp lysine had no effect on the MCC bound to Dnp-BSA fibers.

Cap Formation by Fiber-Bound Cells. All of the MCC stained with fl-anti-Ig showed polar distributions of fluorescence in so-called caps (7) (Table 3). Caps on MCC labeled with fl-anti-Ig were always found to be away from the fiber (distal caps) (Fig. 4*a–c*). In contrast, round cells showed various types of caps, and the direction of cap formation was not fixed (Fig. 4*d–f*).

TABLE 3. *Morphology and staining patterns of cells bound to Dnp-BSA fibers and Con A fibers*

Inhibitor*			% Cells with indicated shape and staining pattern†					
Fiber	Incubation‡	Staining‡	MCC (cap)	MCC (no cap)	MCC (unst.)	R (cap)	R (no cap)	R (unst.)
Dnp-BSA	—	—	51	1	0	7	21	20
	NaN_3	NaN_3	10	8	1	1	63	17
	Valinomycin	Valinomycin	0	0	0	0	74	26
	—	Valinomycin	1	30	0	0	55	14
	4°	Valinomycin	0	0	0	0	77	23
	Vinblastine	Vinblastine	7	0	0	43	17	33
	Cytochalasin B	Cytochalasin B	0	0	0	20	45	35
Con A	Con A	Con A	2	2	0	3	58	35
	—	—	0	0	3	5	52	40
	Vinblastine	Vinblastine	0	0	0	23	29	48

* The concentrations of inhibitors were as in Table 1.

† Bound cells were classified in terms of shape and staining patterns with fl-anti-Ig as follows: MCC, morphologically changed cell; R, round cell; cap, cap with fl-anti-Ig; no cap, cells stained with fl-anti-Ig showing patterns other than caps; unst., cells unstained by fl-anti-Ig.

‡ Incubation at 37° for 1 hr except where noted. Staining at 21° for 10 min.

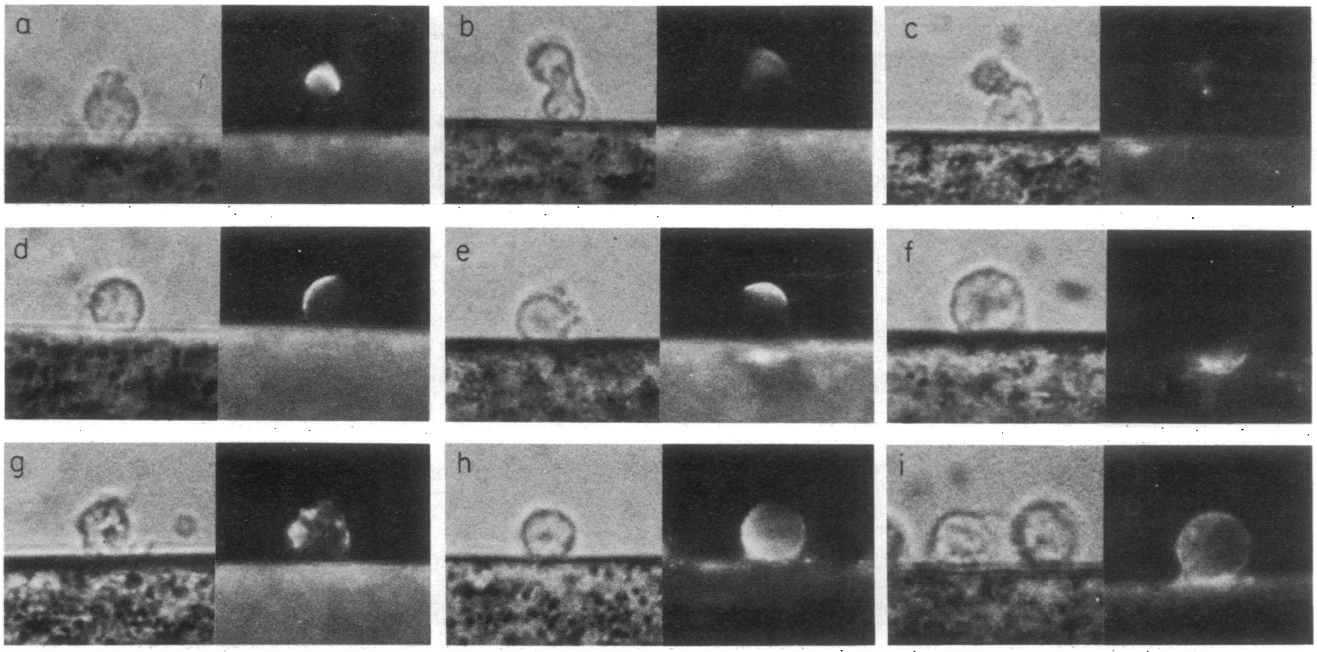

FIG. 4. Various types of fiber-bound cells labeled with fl-anti-Ig. Dnp-BSA fibers were used for (*a*) to (*h*) and Con A fibers were used for (*i*). (*a*) to (*c*)—morphologically changed cells showing distal caps. (*d*) to (*f*)—round cells showing caps with various orientations. (*g*) A round cell showing patchy distribution of fl-anti-Ig. (*h*) A round cell showing diffuse staining. (*i*) Two cells bound to a Con A fiber, one (*right*) showing a diffuse pattern and the other (*left*) showing no staining with fl-anti-Ig.

Treatment of MCC with fl-anti-Ig at 21° for 15 min in the presence of NaN_3 to inhibit cap formation (5, 7) resulted in MCC and round cells that were uniformly stained or contained patches. Prolonged incubation of MCC with NaN_3 in the presence of fl-anti-Ig resulted in increases in the number of round cells uniformly labeled with fl-anti-Ig. Typical patches were also observed in MCC and round cells that were incubated with fl-anti-Ig in the presence of 10^{-6} M valinomycin. Both patch and cap formation were inhibited by exposure to low temperature and by Con A treatment. Cytochalasin B (20 μg/ml) partially inhibited cap formation but did not decrease the viability of the bound cells. Neither colchicine nor vinblastine inhibited cap formation.

Although 60% of the cells bound to Con A fibers were stained with fl-anti-Ig, only a very small number of cells showed cap formation. In agreement with previous observations on free lymphocytes in the presence of Con A (5), when vinblastine was added to the medium at a concentration of 10^{-6} M, the proportion of cap-forming cells increased to as much as 40% of the stained cells (Table 3). These experiments suggest that polar contact of one region of the cell with Con A is sufficient to inhibit patch and cap formation and that the inhibition is reversed by colchicine and *Vinca* alkaloids.

DISCUSSION

The specific selection of lymphocyte populations by nylon fibers derivatized with various antigens has been successfully employed for cell fractionation and characterization. Fiber-fractionated cell populations bind the same antigen used for the fractionation and contain cells capable of an immune response (8). The present experiments suggest that the method may be used to study the movement and surface behavior of single cells as well as populations. It is particularly useful for exploration of the polar effects of various ligands and for molecular analysis of cell surface events.

The cinematographic experiments on cells bound to fibers strongly suggest that the morphological changes are due to movements of the lymphocytes attached to fibers. It has been observed that cap formation always occurs toward the posterior region of free lymphocytes (9, 10). The present finding that all MCC showed distal caps suggests that cells bound to Dnp-BSA fibers act as if they are moving toward the fibers. This seems to be directed by attachment to the fiber itself rather than by the antigen (Dnp-BSA) coupled to fibers, inasmuch as cells nonspecifically bound to fibers also behaved in the same way. The direction of movement of fiber-bound cells may be determined by the characteristics of the surface to which the cells are bound, for it has been reported that caps were always formed at the sites of attachment of cells to nylon fibers derivatized directly with Dnp by a different method (11).

In the present experiments, the direction of cap formation appeared to be determined only when the bound cells exhibited morphological changes and movement. Round cells, which were identified as nonmoving cells by cinematomicrography, showed cap formation in various directions. These observations suggest that the lymphocytes attached to the fibers in a random way at 4° and then oriented themselves with respect to the fibers. It was evident that cells were not able to move freely along the surface of the fiber. Fixation by Ig receptors apparently prevents translocation, possibly by the formation of a specialized structure in or on the cell. The preferential formation of a distal uropod-like structure may also restrict the possibility of translocation along the fiber.

As shown in Table 3, most of the MCC possessed Ig molecules on their surface, suggesting that they are B cells (4, 6) and implying that, at least on the fibers, B cells are more mobile than T cells. This can be explained by one of the following possibilities: (*a*) B cells are generally more mobile than T cells; (*b*) movement of B cells but not of T cells is induced

by binding to fibers; and (*c*) T cells are intrinsically mobile but their mobilities are restricted by binding to fibers. A comparison of the movements of bound and free cells must be made to determine which of these possibilities is correct. The morphological and motility differences between T and B cells observed on Dnp-BSA fibers might be correlated with their capacity to form microvilli (12, 13).

The most important question raised by the present studies concerns the nature of the system responsible for the morphological changes as well as the structures modulating the behavior of that system. It is not surprising that metabolic inhibitors such as NaN_3 inhibited the morphological changes, because cell movement depends upon metabolic events. Thus, the decrease in the number of MCC at long times of incubation is probably attributable to depletion of metabolites. More specific changes are implied by the effects of cytochalasin B, which appears to cause alterations in some microfilamentous structures (14). The strict inhibition of morphological changes by this drug suggests that microfilaments are important in lymphocyte movement. It is noteworthy that cap formation on bound cells was inhibited more effectively by cytochalasin B than that on free cells (7). Binding of the cells to the fibers may have modified microfilaments that may be involved in cap formation, making them more susceptible to cytochalasin B. The marked differences in the susceptibility of cell motility and cap formation to cytochalasin B may be explained by the presence of sheath-type microfilaments that are resistant to the action of this drug (14).

Structures in addition to microfilaments are also involved in the shape changes, as indicated by the action of colchicine and vinblastine. The prevention of shape changes by these agents suggests the possibility that microtubules are necessary for movement and for maintenance of morphology. Thus, both microtubules and microfilaments appear to be required to convert a round cell to an MCC. Our results agree with reports that colchicine affects the mode of the cell movement (15, 16) but not cap formation (7). The global mobility of lymphocytes does not seem to be directly associated with the capacity to form caps, for caps were observed on both round cells and MCC.

We have previously observed (5, 17, 18) that Con A inhibits patch and cap formation by free lymphocytes and that this effect is related to cross linkage of glycoprotein receptors for the lectin. In the present studies, Con A was found to inhibit both shape changes and patch and cap formation by bound cells. Most strikingly, the Con A effect was polar; binding of cells to Con A fibers was sufficient to suppress both cell movement and cap formation, and, although the effect was partial, it appeared to be specific. This suggests that local interaction with Con A receptors strongly modifies in a cooperative fashion the behavior of cellular structures that are involved both in cell movement and the movement of cellular receptors. We have proposed that the structures modulating receptor movement are submembranous colchicine-binding proteins or possibly microtubules themselves (3). It is noteworthy that colchicine and *Vinca* alkaloids prevent cell movement and reverse the inhibitory effect of Con A fibers on bound cells as well as that of Con A on bound and free cells (17). This raises the intriguing possibility that some of the structures modulating the motion of cell-surface receptors are also involved in cell movement and in the alteration and stabilization of cell shape.

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