Proc. Natl. Acad. Sci. USA
Vol. 78, No. 2, pp. 1072–1076, February 1981
Cell Biology

Inhibition of the mobility of mouse lymphocyte surface immunoglobulins by locally bound concanavalin A

(cytoskeleton/anchorage modulation/concanavalin A-platelets/mouse B lymphocytes/photobleaching recovery)

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Communicated by Robert L. Baldwin, November 10, 1980

ABSTRACT **Fluorescence photobleaching recovery was used to study directly and quantitatively the inhibition of the lateral mobility of surface immunoglobulins (sIg) on mouse lymphocytes by localized binding of concanavalin A (Con A) coupled to platelets. Up to a threshold occupancy of about 10% of the upper cell surface by Con A-platelets, the diffusion coefficient and mobile fraction of sIg remained as in untreated cells (5.3×10^{-10} cm^2/sec and 0.65, respectively). At higher surface occupancy, these values decreased to 8×10^{-11} cm^2/sec and 0.11. The magnitude of the effect was independent of the percentage occupancy above the threshold and of the distance from the bound Con A-platelets, indicating a cooperative and propagated phenomenon. Treatment with colchicine or cytochalasin B separately induced only partial reversal of the Con A-induced modulation. Treatment with both drugs together was synergistic and fully reversed the mobility inhibition. The modulation was unaffected by NaN_3 and 2-deoxyglucose, suggesting no dependence on metabolic energy. Con A-platelets did not affect the mobility of a lipid probe. Models for the Con A-induced modulation and the relationship between the effects of Con A on sIg mobility and patch formation are discussed.**

Recent experimental work from several laboratories has suggested that forces in addition to the viscosity of the plasma membrane lipid bilayer retard the lateral mobility of cell-surface proteins (1–4). Measurements of the lateral mobility of a number of membrane proteins on several different cell types have shown that a fraction of the molecules is immobile on the experimental time scale and that the diffusion coefficients of the mobile molecules are $\frac{1}{10}$ to $\frac{1}{100}$th those of lipid-like molecules (1, 2, 5, 6). This difference is too large to explain in terms of the expected difference between the frictional coefficients of lipids and proteins (7). The interactions responsible for retarding membrane protein mobility are unknown. Considerable evidence exists suggesting interactions between surface proteins and the cytoskeleton (8–12). Attempts to demonstrate the effects of drug-induced disruption of the cytoskeleton on membrane protein mobility have, however, been negative or equivocal (2, 5, 6). Only in the specialized example of erythrocytes are there demonstrable effects on membrane protein mobility of interactions with the spectrin-actin cortex underlying the membrane (12, 13) and a structural interpretation of these interactions (14).

The greater structural variability and complexity of mammalian nucleated cells make the analysis of mobility constraints more difficult. One approach would be to investigate a system in which these constraints could be varied by some treatment and then to seek structural and biochemical changes in the cell that could be responsible for the changes in mobility. A system that holds some promise for this kind of study is provided by the inhibitory effect of concanavalin A (Con A) on the formation of patches by various cross-linked membrane proteins in several cell types (8, 15–17). This effect, which has been termed "anchorage modulation" (8), can also be induced by Con A bound to a localized region of the cell surface demonstrating that the inhibitory effect can propagate throughout the entire cell (18–20). It was proposed that anchorage modulation arises from interactions between the membrane proteins and a "surface-modulating assembly" involving the cytoskeleton (8, 20, 21).

In earlier work it was assumed that inhibition of patch and cap formation by Con A reflected inhibition of mobility. In this communication we report direct and quantitative measurements of the effect of locally bound Con A on the lateral diffusion of surface immunoglobulins (sIg) on mouse lymphocytes. The most striking result is a demonstration of the central role played by cytochalasin B (CB) and colchicine-sensitive microfilaments and microtubules in the process. By comparing the effects of Con A on patch formation and on mobility, we can also draw conclusions about the relationship of the two phenomena.

MATERIALS AND METHODS

Reagents. Monovalent tetramethylrhodamine-labeled Fab' fragments (Me_4R-Fab') of rabbit IgG directed against mouse IgG were prepared from Me_4R-Fab'$_2$ fragments (Cappel Laboratories, Cochranville, PA) by reduction with 2-mercaptoethanol, followed by alkylation with iodoacetamide (22) and chromatography on Sephadex G-100. Polyacrylamide/NaDodSO_4 slab gel electrophoresis under nonreducing conditions detected no contamination by Me_4R-Fab'$_2$ fragments. Specificity was tested by labeling in the presence of excess unlabeled rabbit anti-mouse IgG antibodies, which prevented 90% of the labeling with Me_4R-Fab'.

Fresh human blood platelets (American Red Cross) were fixed and labeled (19) with Con A [platelets (1.5×10^8 per ml) were incubated with Con A (200 μg/ml) in phosphate-buffered saline for 30 min at 22°C]. Paraformaldehyde replaced glutaraldehyde as the fixative to reduce autofluorescence.

N-4-nitrobenzo-2-oxa-1,3-diazole derivative of phosphatidylethanolamine (NBD-PE) was obtained from Avanti Biochemicals; Con A (grade III), colchichine, and CB were from Sigma. Goat anti-Con A antibodies (Miles-Yeda, Rehovot, Israel) were labeled with Me_4R isothiocyanate (23).

Mouse Splenic Lymphocytes. Spleen cell suspensions from 12- to 16-week old BALB/c mice (Jackson Laboratory) were prepared in Dulbecco's modified Eagle's medium containing

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Abbreviations: Con A, concanavalin A; sIg, surface immunoglobulins; FPR, fluorescence photobleaching recovery; Me_4R, tetramethylrhodamine; Me_4R-Fab', Me_4R-labeled Fab' fragments of rabbit IgG directed against mouse IgG; CB, cytochalasin B; NBD-PE, *N*-4-nitrobenzo-2-oxa-1,3-diazole derivative of phosphatidylethanolamine; D, diffusion coefficient.

20 mM Hepes (pH 7.3) (GIBCO) with 0.2% bovine serum albumin (24). Suspensions were prepared and stored at 22°C to avoid cold-induced microtubule disruption. Cell viability was 90% 10 hr after preparation as judged by trypan blue exclusion.

Cell Labeling. After Con A binding to the platelets, the Con A-platelets were washed three times with the same medium. By using ^{125}I-labeled Con A, we have verified that a negligible amount of Con A is released from the platelets during 1 hr (19).

Con A-platelets (4×10^7) were then incubated in 0.5 ml of the same medium with 3×10^6 spleen lymphocytes at 22°C for 20 min, followed by 10 min incubation at 22°C with 100 μg/ml Me_4R-Fab' in the same medium or with 0.02 mg of NBD-PE per ml of Dulbecco's modified Eagle's medium/Hepes containing 1% ethanol. The suspension was washed twice by centrifugation with Dulbecco's modified Eagle's medium/Hepes (without phenol red), and 3 μl of the suspension was placed between a microscope slide and a coverslip. The cells settled onto the slide within minutes. Approximately 40% of the cells showed staining with Me_4R-Fab', a further indication of the labeling specificity. By measuring the fluorescence intensity from 2.5-μm^2 spots on cells labeled with Con A-platelets and stained by Me_4R-labeled goat anti-Con A antibodies, we have verified that platelet-bound Con A does not dissociate and rebind to the cell surface (20).

Treatment with Drugs. Lymphocytes (10^7 cells per ml) were incubated 45 min at 37°C in Dulbecco's modified Eagle's medium/Hepes/albumin with either colchicine (1 or 100 μM), CB (10 μg/ml), colchicine (1 μM) with CB (10 μg/ml), or NaN_3 (10 mM). The incubation mixture contained 1% of ethanol or demethyl sulfoxide for colchicine and CB, respectively. The presence of 1% of the drug solvent did not affect sIg mobility. After this preincubation, the cells were labeled with Con A-platelets or Me_4R-Fab', or both. The drugs were present in the medium during and after the labeling. As judged by trypan blue exclusion, no increase in cell death resulted from these drug treatments in the presence or absence of Con A-platelets.

Fluorescence Photobleaching Recovery (FPR). Diffusion coefficients and mobile fractions of fluorescently labeled membrane components were measured by FPR (25–27). The FPR apparatus described (25) has been modified for continuous monitoring of fluorescence by using an arrangement of beam splitters and a shutter similar to that of Koppel (28).

The attenuated laser beam (λ = 529.5 nm for rhodamine, 476.5 nm for NBD-PE, at 0.3–0.6 μW) from an argon ion laser was focused on the cell membrane through a Zeiss Universal fluorescence microscope with an oil immersion lens (×100) to a spot of 0.9-μm radius. Fluorophores in this region were irreversibly bleached by a brief intense pulse (3–6 mW for 4–10 msec) of the laser beam. By using the 10^4-fold attenuated beam to monitor the rate of fluorescence recovery in the bleached spot due to the entry of unbleached fluorophores, the diffusion constants and mobile fractions of the fluorescent probe were determined (26). Incomplete fluorescence recovery was interpreted as indicating probe molecules which were immobile on the experimental time scale. FPR measurements were performed at 22°C on spots at various distances from the Con A-platelets. The fraction of the upper cell surface covered by platelets was estimated as described earlier (20). The relative error in these estimates is ±20% of the measured values (e.g., 14 ± 3% coverage). Measurements started 50 min after the initiation of the incubation with the Con A-platelets and were continued for 1 hr, with the sample of cells on the microscope slide being replaced every 10–15 min.

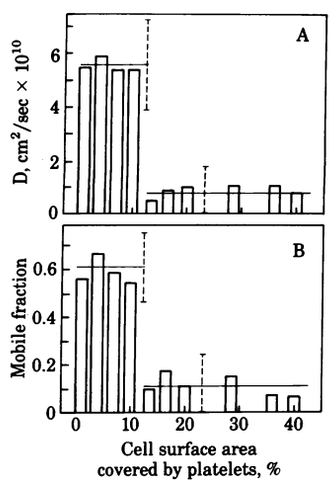

FIG. 1. Effect of Con A-platelets on the D (*A*) and mobile fraction (*B*) of sIg on B lymphocytes. The abscissa shows the percentage of the upper cell surface area covered with Con A-platelets. The relative error in the estimates of coverage is ±20% of the estimated value (e.g., 14 ± 3%). Each bar represents measurements performed on 10–20 cells. ——, Averages of measurements on cells with low (<10 ± 2%) and high (>14 ± 3%) coverage by Con A-platelets; – – –, error bars indicating SD.

RESULTS

Effect of Con A-Platelets on the Diffusion of sIg and a Lipid Probe. In the absence of treatment with Con A-platelets, Me_4R-Fab'-labeled sIg gave a mean ± SD diffusion coefficient (D) of 5.3 ± 1.4 × 10^{-10} cm^2/sec, and a mean ± SD mobile fraction of 0.65 ± 0.16 (Table 1) in reasonable agreement with earlier reports (6).

Coverage of over 14 ± 3% of the upper cell surface by Con A-platelets reduced the D and mobile fraction to 0.8 ± 1.0 × 10^{-10} cm^2/sec and 0.11 ± 0.13, respectively. Below 10 ± 2% coverage, no inhibition of mobility was observed (Fig. 1, Table 1). The modulation of sIg mobility by Con A-platelets in the majority of the B lymphocytes is even larger, because 53% of the B lymphocytes with surface occupancy over 14 ± 3% showed completely immobilized sIg (Fig. 2). This total immobilization indicates that dissociation of Me_4R-Fab' from the cell surface followed by rebinding does not contribute to the measured lateral mobility. To further rule out this possibility, cells were prefixed with 2% (wt/vol) paraformaldehyde (excess reagent was quenched with 20 mg/ml glycine) and then labeled with Me_4R-Fab'. These cells did not show fluorescence recovery on the time scale of the FPR experiments. The modulation of sIg mobility by Con A-platelets did not depend on the distance from the platelets (data not shown), indicating the propagated nature of the Con A-platelets effect.

In order to rule out the possibility that the platelets themselves induce sIg immobilization, the effect of platelets not la-

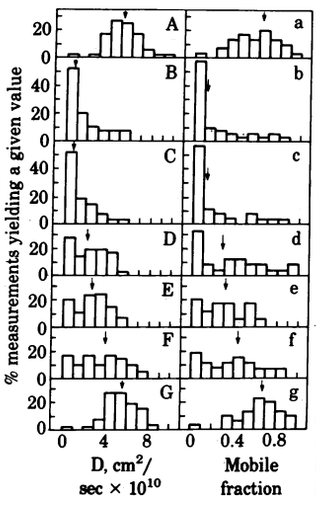

FIG. 2. Effect of drugs on the distribution of sIg D values and mobile fraction on lymphocytes treated with Con A-platelets. Capital letters, D; small letters, mobile fraction. Arrows indicate the mean values. Except in *A*, where the cells were not treated with Con A-platelets, the values shown are on cells with upper surface occupancy over 14 ± 3% by Con A-platelets. Prior to the treatment with Con A-platelets, the cells were incubated 45 min at 37°C with medium only (*B*), 10 mM NaN_3 (*C*), 10 μg of CB per ml (*D*), 1 μM colchicine (*E*), 100 μM colchicine (*F*), and 1 μM colchicine together with 10 μg of CB per ml (*G*). Thirty to 50 cells were measured in each category. The difference between *E* and *F* may be due to further microtubule breakdown (29).

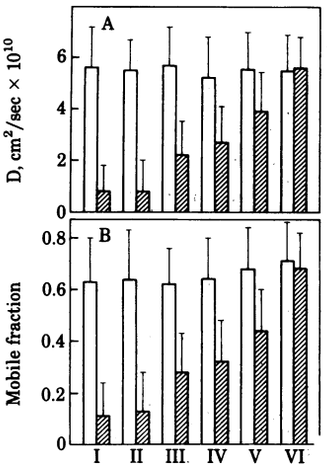

FIG. 3. Drug effects on D (*A*) and mobile fraction (*B*) of sIg on B lymphocytes occupied with Con A-platelets above and below the modulation threshold. Empty bars, upper surface coverage with Con A-platelets below 10 ± 2%; cross-hatched bars, coverage above 14 ± 3%. Cells were preincubated 45 min with medium only (I), 10 mM NaN_3 (II), 10 μg of CB per ml (III), 1 μM colchicine (IV), 100 μM colchicine (V), and 1 μM colchicine together with 10 μg of CB per ml (VI). Each bar represents measurements on 30–50 cells.

beled with Con A was tested. Very few unlabeled platelets adhered to the cells. The D and mobile fraction of Me_4R-Fab'-labeled sIg on cells with a high coverage of unlabeled platelets were not altered (Table 1).

The modulation of sIg mobility by Con A-platelets could result from a decrease in membrane fluidity or from a change in membrane topography (e.g., an increased amount of microvilli). In both cases, the lateral mobility of a lipid probe should be diminished as well. These possibilities were tested by using the lipid probe NBD-PE. Even at 50% occupancy of the upper cell surface by Con A-platelets, no effect on either the D or mobile fraction of NBD-PE was observed (Table 1).

Effect of Cytoskeleton Disruption and Metabolic Inhibition. To test the role of the cytoskeleton and of metabolic energy in the modulation phenomenon, we employed drugs that disrupt microtubules (colchicine), microfilaments (CB), and the metabolic energy inhibitor NaN_3. The results are depicted as histograms in Fig. 2. The mean values of the modulation in the presence and absence of these drugs are shown in Fig. 3 and Table 1.

The threshold at 8–17% Con A-platelets occupancy was retained in the presence of the drugs (Fig. 3). Colchicine induced partial reversal of the modulation of both the D and the mobile fraction. The effect of the drug was larger at 100 μM than at 1 μM, but the reversal of the modulation was incomplete even at the higher drug dose, which is well over the concentration required to disrupt the microtubules. CB also caused partial reversal of the modulation. Interestingly, the combination of 1 μM colchicine with CB (10 μg/ml) was synergistic and induced full reversal of the modulation, whereas 1 μM colchicine or CB (10 μg/ml) alone induced only 40% and 30% reversal, respectively (Fig. 3). 10 mM NaN_3 had no effect on the modulation of sIg mobility (Figs. 2 and 3), and similar results were obtained when 10 mM 2-deoxyglucose were present together with the azide. This indicates that the modulation does not depend on metabolic energy and that the CB effect is *not* due to its inhibition of glucose transport. None of the drug combinations affected the mean values and distribution of the lateral mobility and mobile fraction of Me_4R-Fab'-labeled sIg on cells occupied with Con A-platelets below the threshold (Figs. 2 and 3) or on cells which were not treated with Con A-platelets. This is in accord with the report (6) that none of these drugs influenced the lateral mobility of sIg or Thy-1 antigens on mouse lymphocytes.

DISCUSSION

The interactions and structures that in general constrain the lateral mobility of plasma membrane proteins remain a mystery.

Table 1. Effect of local Con A binding on the lateral mobility of sIg and NBD-PE on lymphocytes

Marker	Treatment	D, $cm^2/sec \times 10^{10}$	Mobile fraction
sIg	None	5.3 ± 1.4	0.65 ± 0.16
sIg	Platelets (high, no Con A)	5.2 ± 1.1	0.70 ± 0.13
sIg	Con A-platelets (low)	5.6 ± 1.6	0.63 ± 0.17
sIg	Con A-platelets (high)	0.8 ± 1.0	0.11 ± 0.13
sIg	Con A-platelets (high) + 10 mM NaN_3	0.8 + 1.2	0.13 ± 0.15
sIg	Con A-platelets (high) + CB at 10 μg/ml	2.2 ± 1.3	0.28 ± 0.15
sIg	Con A-platelets (high) + 1 μM colchicine	2.7 ± 1.4	0.32 ± 0.17
sIg	Con A-platelets (high) + 100 μM colchicine	3.9 ± 1.5	0.44 ± 0.16
sIg	Con A-platelets (high) + 1 μM colchicine + CB at 10 μg/ml	5.6 ± 1.2	0.68 ± 0.14
NBD-PE	None	120 ± 30	0.89 ± 0.10
NBD-PE	Con A-platelets (low)	115 ± 32	0.88 ± 0.11
NBD-PE	Con A-platelets (high)	125 ± 37	0.90 ± 0.11

sIg were labeled with Me_4R-Fab'. "high" and "low" Con A-platelets stand for >14% ± 3% and <10% ± 2% coverage, respectively, of the upper cell surface area by the platelets. Thirty to 50 cells were measured in each category. With both sIg and NBD-PE, similar results were obtained whether the labeling was done before or after the binding of Con A-platelets to the cells. Data are shown as mean ± SD.

However, this study does demonstrate a role for microfilaments and microtubules in a specialized example of the modulation of mobility. Although the physiological role of this modulation is unknown, characterization of the structures and mechanisms involved could provide important information about dynamic interactions between cell surface components and the cytoskeleton.

Modulation of Lateral Mobility and of Patch and Cap Formation. Anchorage modulation by Con A has been most extensively characterized on lymphocytes in terms of the inhibition of antibody-induced patching and capping of sIg (8, 15–19). A basic assumption in the interpretation of these experiments was that inhibition of patching and, therefore, of subsequent capping results from an inhibition of the mobility of individual sIg molecules (8, 18–20). This assumption may be tested by comparing the inhibition of patching and capping with the modulation of mobility of sIg molecules labeled with monovalent Fab' fragments.

The main features of the anchorage modulation are quite similar when characterized by the two different kinds of observations. Localized binding of Con A inhibits patching and capping of sIg (18, 19) and also reduces the lateral mobility and mobile fraction of Me_4R-Fab'-labeled sIg (Fig. 1). A threshold seems to exist for inhibition of patching as it does for inhibition of mobility, although the phenomenon is less clear-cut for patching due to the nonquantitative nature of the measurement (19). The inhibition both of patch formation and of mobility is partially reversed by microtubule-disrupting drugs, but the reversal is incomplete even at high drug doses (8, 16) (Figs. 2 and 3; Table 1). Neither effect requires metabolic energy as demonstrated by the failure of NaN_3 to reverse the inhibition of sIg patching (30) or mobility (Figs. 2 and 3; Table 1). The analogy appears to fail, however, in the response to treatment by CB: no effect on the inhibition of patching (8, 17) and partial reversal of the inhibition of mobility (Figs. 2 and 3; Table 1). However, this discrepancy may be due to the difficulty of detecting a small

effect on patching and capping; CB reverses the inhibition of sIg mobility by only 30% and CB itself inhibits cap formation (31). Our results indicate parallel characteristics of the Con A-induced inhibition of patching and capping and of the lateral mobility of sIg. This is consistent with the assumption that patching inhibition results from inhibition of the mobility of individual receptors. Moreover, this parallelism and lack of dependence on metabolic energy are in accord with the notion that patching (unlike capping) is a diffusion-limited process.

Involvement of the Cytoskeleton in the Con A-Induced Modulation. Our results provide strong evidence that *both* microfilaments and microtubules are involved in the modulation of mobility by Con A. Treatment with either colchicine or CB individually reverses the inhibition of sIg mobility only partially. Together, however, the two drugs completely reverse the inhibition (Figs. 2 and 3; Table 1). It is important to recognize that colchicine and CB reverse only the incremental inhibition of mobility caused by Con A-platelets. In the presence of these drugs the mobility and mobile fraction of sIg return to the values observed in the absence of Con A. However, this mobility, like that of most membrane proteins yet examined, is still less than that expected for diffusion limited only by the viscosity of the plasma membrane (1–3, 5–7), and an immobile fraction is still present. This suggests that sIg mobility is constrained by factors beyond that of bilayer viscosity that are insensitive to treatment by colchicine and CB either in the presence or absence of Con A. Therefore, we have direct evidence for the involvement of the cytoskeleton only in the propagated, long-range inhibition induced by Con A but not in the restraints on mobility in unperturbed cells. If surface–cytoskeleton interactions are involved in the latter, they must be insensitive to the drug treatments used.

Restraints on the mobilities of membrane proteins are reflected not only in the relatively low diffusion rates of mobile molecules but also in the presence of immobile molecules. Immobile fractions of a number of specific membrane proteins have been observed in several cell types (2, 5, 6, 32, 33), including sIg on unperturbed lymphocytes (6). A striking result of the current work is that Con A binding induces a parallel decrease in both the fraction and the D of mobile sIg molecules (Fig. 1). The reversal of the decrease in the mobile fraction by colchicine and CB demonstrates that cytoskeletal interactions are involved in shifting sIg molecules from the mobile to the immobile fraction and in retarding the diffusion of molecules that remain mobile.

However, the classification of sIg molecules as "mobile" and "immobile" may be quantitative rather than qualitative. Immobile molecules are operationally defined as having $D \leq 5 \times 10^{-12}$ cm^2/sec. It is probable that both mobile and immobile molecules have distributions of D, those of the latter being below the detection limit. It is therefore possible that Con A binding induces a simple shift in the overall distribution of D, thereby increasing the fraction of molecules below the detection limit (Fig. 2). Moreover, the response to Con A-platelets is heterogeneous over the cell population; on some cells sIg appears to be entirely immobile. A further noteworthy observation from Figs. 2 and 3 is the continuity in the variation of the sIg D values. Therefore, even though the threshold onset and global character of the modulation suggest a highly cooperative process, it is not sufficient to consider the phenomenon in terms only of two end states (modulated or not, above and below the threshold). It also will be important to understand the intermediate conditions produced by CB and colchicine acting separately. The action of these drugs indicates that the initiation of the modulation, which seems to occur unimpaired in their presence, is separable from its magnitude, which is differentially affected by the kind and amount of anticytoskeletal agent present.

These results agree with previous findings on the mobility of unselected antigens on mouse 3T3 cells (20), which was found to decrease above a threshold coverage by Con A-platelets. This reduction was partially reversed by colchicine. A decrease in the mobile fractions was not detected, however. This difference may be quantitative rather than qualitative, because no 3T3 cells exhibiting complete immobilization of their surface antigens were observed, suggesting the inhibition does not bring the mobility below the detection limit. Differences between the Con A-induced modulation in lymphocytes and in other cells will be discussed elsewhere.

Models for Con A-Induced Modulation. Characteristics of the phenomenon that should be considered include: (*i*) propagation of the inhibition of mobility over the whole cell independent of the distance from the Con A-platelets; (*ii*) requirement for crosslinking Con A receptors (8, 18, 30); (*iii*) apparent stabilization by Con A of preexisting microtubule organization (29); (*iv*) involvement of both microtubules and microfilaments subject to complete reversal by the combined action of CB and colchicine; (*v*) lack of effect on lipid fluidity and membrane topography indicated by measurements on NBD-PE (Table 1); (*vi*) independence of metabolic energy indicated by failure of NaN_3 to affect modulation (Figs. 2 and 3; Table 1).

These observations lead to the models in Fig. 4. The basic premises are that inhibition of mobility depends on an interaction between sIg and the cytoskeleton and that crosslinking a critical number of Con A receptors (perhaps of a single or a

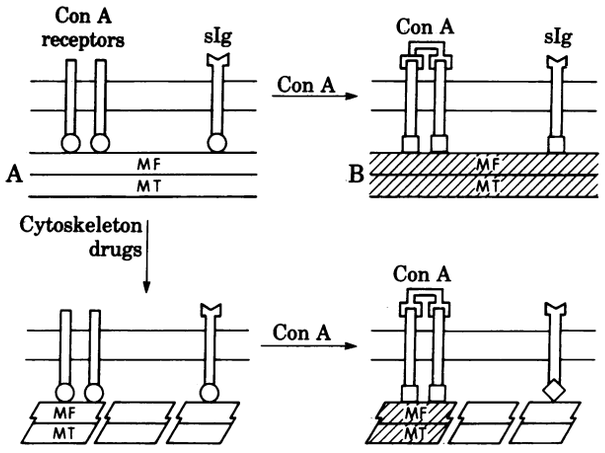

FIG. 4. Schematic representation of Con A-induced modulation of membrane protein mobility. Components are not drawn to scale. Con A receptors penetrate the membrane and interact directly or indirectly with the cytoskeletal complex composed of microfilaments (MF), microtubules (MT), and possibly additional components. Indirect interaction may occur through a linkage molecule, represented as a circle or a square to denote different states before and after Con A binding. The ◇ state (*Lower Right*) is equivalent to the ○ state in mechanisms 2 and 3, which assume that the Con A effect propagates through the cytoskeleton, or to the □ state (in mechanism 1). The different mechanisms for the propagation of the Con A modulation are described in the text. Extensive crosslinking by Con A (shown with only 2 sites, for simplicity) induces a change in the interactions of membrane proteins with the cytoskeleton, either by stabilizing a certain state of the cytoskeleton (*B*, designated by diagonal lines) or by generating (or enhancing) linkage of membrane proteins with the cytoskeleton. This results in a restriction of membrane protein mobility. The propagation of this effect involves *both* microtubules and microfilaments. The restriction of membrane protein mobility in the absence of Con A, which is not affected by drugs that disrupt the cytoskeleton, may be either due to local interactions that do not require an intact cytoskeleton, or due to factors other than cytoskeletal interactions.

few specific species) changes either the state of the cytoskeleton or sIg–cytoskeleton interaction. Biochemical evidence in favor of a link between actin and sIg has been obtained (11).

The mechanism by which a critical extent of Con A binding initiates or enhances the immediate interactions that inhibit sIg mobility is not yet clear, nor is the exact nature of these interactions. The inhibition of mobility could be propagated over the cell by the activation of a diffusible enzyme that acts on the cytoskeleton or on the sIg–cytoskeleton linkage, by a change in conformation or aggregation state propagated through the cytoskeleton or by a change in the ionic content of the cytoplasm. [Nevertheless, Con A does not act simply as a Ca^{2+} ionophore, because the latter prevents only capping but not patching of sIg (34).]

We consider three possibilities for the interactions generating the mobility restriction:

(*i*) Enhancement of specific interactions between membrane proteins and the cytoskeleton without changing the structure and stability of the latter.

(*ii*) Stabilization of the cytoskeleton in a state (Fig. 4, state B) with higher affinity for sIg either directly or indirectly through a linker molecule. This stabilization need not involve a change in the aggregation state of the cytoskeleton, but could result from a conformational change of one or more of its constituents.

(*iii*) Stabilization of the cytoskeletal matrix subjacent the membrane so that it acts more strongly as a nonspecific barrier to the diffusion of sIg and other membrane proteins. A portion of membrane proteins is supposed to project into a cytoskeletal matrix, which sterically blocks lateral movement. The links of this matrix are supposed to break and reform continually, thereby allowing lateral diffusion of the entrapped protein (refs. 35 and 36; D. E. Koppel, personal communication).

The accumulation of actin, myosin, and tubulin beneath patches (9, 11, 37) seems more consistent with mechanisms 1 and 2 than with 3.

The extent to which any of these mechanisms may operate to retard membrane protein mobility in the absence of Con A is unknown. Nevertheless, such restrictions must be insensitive to CB and colchicine because of the insensitivity of the baseline mobility to these drugs.

The models presented here agree in most respects with the model proposed by Edelman *et al.* (8, 17–20) but differ in some features. The supposition that Con A binding triggers microtubule assembly (8) has been omitted due to the failure to detect changes in microtubule distribution following Con A binding to lymphocytes (29). The proposition that CB-sensitive microfilaments serve only to link microtubules and membrane proteins (8, 20) was omitted because the experiments with colchicine and CB do not distinguish between the roles of microtubules and microfilaments in the modulation. In addition, the current model emphasizes the distinction between the restraints on sIg mobility induced by Con A and those present in unperturbed cells.

We thank Dr. Nils O. Petersen for his help in constructing the FPR instrument and Dr. H. M. McConnell for a helpful remark. This research was supported by National Institutes of Health Grant GM 21661 and by a Chaim Weizmann Postdoctoral Fellowship to Y.I.H. The Washington University Center for Basic Cancer Research (funded by Department of Health and Human Services Grant 5P30 CA 16217) provided tissue culture media.

1. Schlessinger, J. & Elson, E. L. (1980) in *Biophysical Methods, Methods of Experimental Physics*, eds. Ehrenstein, G. & Lecar, H. (Academic, New York), in press.
2. Schlessinger, J., Axelrod, D., Koppel, D. E., Webb, W. W. & Elson, E. L. (1977) *Science* **195**, 307–309.
3. Cherry, R. J. (1979) *Biochim. Biophys. Acta* **559**, 289–327.
4. Smith, B. A., Clark, W. R. & McConnell, H. M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5641–5644.
5. Schlessinger, J., Koppel, D. E., Axelrod, D., Jacobson, K., Webb, W. W. & Elson, E. L. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2409–2413.
6. Dragsten, P., Henkart, P., Blumenthal, R., Weinstein, J. & Schlessinger, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5163–5167.
7. Saffman, P. G. & Delbruck, M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3111–3113.
8. Edelman, G. M. (1976) *Science* **192**, 218–226.
9. Bourguignon, L. Y. W. & Singer, S. J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5031–5035.
10. Ash, J. F., Louvard, D. & Singer, S. J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5584–5588.
11. Flanagan, J. & Koch, G. L. E. (1978) *Nature (London)* **273**, 278–281.
12. Sheetz, M. P., Schindler, M. & Koppel, D. E. (1980) *Nature (London)* **285**, 510–512.
13. Golan, D. E. & Veatch, W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2537–2541.
14. Fowler, V. & Bennett, V. (1978) *J. Supramol. Struct.* **8**, 215–221.
15. Yahara, I. & Edelman, G. M. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 608–612.
16. Yahara, I. & Edelman, G. M. (1973) *Nature (London)* **246**, 152–155.
17. Edelman, G. M., Yahara, I. & Wang, J. L. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 1442–1446.
18. Rutishauser, U., Yahara, I. & Edelman, G. M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1149–1153.
19. Yahara, I. & Edelman, G. M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1579–1583.
20. Schlessinger, J., Elson, E. L., Webb, W. W., Yahara, I., Rutishauser, U. & Edelman, G. M. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1110–1114.
21. Yahara, I. & Edelman, G. M. (1975) *Exp. Cell. Res.* **91**, 125–142.
22. Braun, J., Shaafi, R. I. & Unanue, E. R. (1979) *J. Cell Biol.* **82**, 755–766.
23. Goldman, M. (1968) in *Fluorescent Antibody Methods* (Academic, New York), pp. 108–109.
24. Ryan, J. L., Arbeit, R. D., Dickler, H. B. & Henkart, P. A. (1975) *J. Exp. Med.* **142**, 814–826.
25. Koppel, D. E., Axelrod, D., Schlessinger, J., Elson, E. L. & Webb, W. W. (1976) *Biophys. J.* **16**, 1315–1329.
26. Axelrod, D., Koppel, D. E., Schlessinger, J., Elson, E. L. & Webb, W. W. (1976) *Biophys. J.* **16**, 1055–1069.
27. Elson, E. L., Schlessinger, J., Koppel, D. E., Axelrod, D. & Webb, W. W. (1976) in *Membranes and Neoplasia: New Approaches and Strategies*, ed. Marchesi, V. T. (Liss, New York), pp. 137–147.
28. Koppel, D. E. (1979) *Biophys. J.* **28**, 281–292.
29. Yahara, I. & Kakimoto-Sameshima, F. (1978) *Cell* **15**, 251–259.
30. Yahara, I. & Edelman, G. M. (1973) *Exp. Cell Res.* **81**, 43–155.
31. de Petris, S. (1975) *J. Cell Biol.* **65**, 123–146.
32. Schlessinger, J., Webb, W. W., Elson, E. L. & Metzger, H. (1976) *Nature (London)* **264**, 550–552.
33. Jacobson, K., Derzko, Z., Wu, E.-S., Hou, Y. & Poste, G. (1976) *J. Supramol. Struct.* **5**, 565–576.
34. Braun, J., Fujiwara, K., Pollard, T. D. & Unanue, E. R. (1978) *J. Cell Biol.* **79**, 419–426.
35. Schindler, M., Osborn, M. J. & Koppel, D. (1980) *Nature (London)* **283**, 346–350.
36. Lux, S. E. (1979) *Nature (London)* **281**, 426–429.
37. Albertini, D. F. & Anderson, E. (1977) *J. Cell Biol.* **73**, 111–127.