LIGAND-INDUCED MOVEMENT OF LYMPHOCYTE MEMBRANE MACROMOLECULES

V. Capping, Cell Movement, and Microtubular Function in Normal and Lectin-Treated Lymphocytes*

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Interaction of ligands with the antigen receptor on B lymphocytes leads to a rapid and dramatic series of surface and cytoplasmic events that have been mostly studied using as a probe anti-immunoglobulin (Ig) antibodies (anti-Ig) in murine lymphocytes. Four separate but overlapping events can be identified as occurring during capping: (*a*) an initial formation of aggregates of variable sizes on the cell surface, which is temperature but not energy dependent (1, 2); (*b*) a rapid distribution of the complexes into a single aggregate or cap (3, 4); (*c*) the stimulation of translational motility of the cell (5); and (*d*) the internalization of the aggregates by pinocytosis with subsequent partial degradation of the complexes (3, 6). These latter three events are both temperature and energy dependent. There is general agreement that capping results from the interaction of a critical amount of cross-linking, bivalent anti-Ig with surface Ig and that it involves an energy-dependent step not directly associated with the translation of the cell. We have recently discussed the possibility that movement of complexes on the cell surface may be brought about by some form of cell surface "activity," perhaps in the form of "waves" or "membrane flow" (5). This activity directed towards one zone of the cell surface moves the aggregates of complexes initially distributed at random into one lattice forming the cap. This putative "surface activity" can be separated from the actual process of movement of the cell on a solid surface, since by several experimental maneuvers capping can be induced in the absence of actual translation (5). However, it should be clearly pointed out that an association also exists between the surface activity involved in translation and capping. It was shown both in the neutrophil polymorphonuclear leukocyte (PMN)[1] (7) and in the lymphocytes that movement serves to direct the

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; FITC, fluorescein isothiocyanate; HBSS, Hanks' balanced salt solution; PMN, neutrophil polymorphonuclear leukocyte.

cap to the trailing part of the cell. In the lymphocytes, the caps in stationary cells occupy a large area of the cell surface, while those in cells in translation are tight and occupy a small area at the posterior part of the cell (5).

Several recent publications implicate microtubular proteins as exerting some form of control of events occurring at the cell surface, including capping. Berlin and co-workers (8–10) based their conclusions on experiments in which the relationship between two surface sites was altered by colchicine treatment of the cells. In a normal state, the two sites were discriminated; but after colchicine treatment, the sites were randomized. One of us (Karnovsky), together with Berlin and associates, observed that the mobility of Con A-binding sites in SV40-transformed fibroblasts was enhanced by colchicine treatment (11). Edelman and co-workers (12–15) inhibited capping of Ig on lymphocytes by concanavalin A (Con A) treatment at physiological temperatures. The inhibition by Con A of Ig capping was counteracted, and capping was obtained, by colchicine treatment or by initial reactions with the ligands at low temperatures with subsequent warming of the labeled cells (12–15). Similar results have been seen in the PMN where high concentrations of Con A inhibited capping (7). However, in the PMN it was noted that the colchicine effect was not observed on cells in which cell movement was inhibited. Hence, the question was raised as to whether the colchicine effect of reversing Con A inhibition of capping was mediated through cell movement itself, particularly in view of the observation that cell movement in itself would apparently favor capping of both monovalent and multivalent ligands, apparently through a sweeping of the label to the trailing edge of the cell (7).

In view of the above observations, it was of interest to re-examine similar phenomena as they occur in lymphocytes. We now report on a series of experiments on capping of Ig-anti-Ig complexes on lymphocytes in which the effects of drugs that bind to microtubular protein have been analyzed. The murine lymphocyte is an ideal cell for studying behavior of surface molecules: the end point (capping) is very distinct and can be easily scored; and the relationship between a stationary cell or one in motion can be reasonably well established by scoring for the presence of motile or ameboid forms (5). One problem with capping in lymphocytes is its rapidity, but this problem can, in part, be obviated by reducing temperature of the test system from 37°C to 21°C.

Previous experiments had indicated that colchicine-treated lymphocytes capped complexes of Ig-anti-Ig without any apparent difficulty (3, 16). We now report that colchicine has a synergistic negative effect with cytochalasin B on capping and that the effect of colchicine on the Con A inhibition of capping can be explained by increased motility of the cell. All these experiments could be interpreted as supporting the hypothesis that microtubules have some effect in regulating the surface activity generated by complexing of receptors with their ligand, but call attention to cell motility as an important factor to take into consideration, at least in some systems, whenever interpreting the role of intracellular organelles in surface behavior.

Materials and Methods

Cells. Lymphocytes were harvested from spleens of A/St mice (West Seneca Laboratories, Buffalo, N. Y.) by conventional procedures. Cells were harvested using Hanks' balanced salt solution

(HBSS) containing 10 mM HEPES, and 1% fetal calf serum. After harvesting, the cells were centrifuged on a Ficoll-Hypaque gradient in order to eliminate dead cells, erythrocytes, and debris.

Reagents

CULTURE MEDIA. HBSS with 10 mM HEPES and 1% fetal calf serum were used in most experiments.

FLUORESCENT ANTI-IG ANTIBODY. It was an IgG from polyvalent rabbit antimouse Ig with a fluorescein (FITC)-protein molar ratio of 4. 20–100 μg were used per test.

FERRITIN-LABELED ANTI-IG ANTIBODY. The same IgG as described above was conjugated to ferritin. It was the same preparation used by Unanue et al. (4).

COLCHICINE. Colchicine (Sigma Chemical Co., St. Louis, Mo.) solutions of 10^{-4}–10^{-6} M were prepared immediately before use.

VINBLASTINE. Vinblastine (Sigma Chemical Co.) was used at a concentration of 10^{-4} M.

CYTOCHALASIN B. Cytochalasin B, (ICI Research Laboratories, Cheshire, England) a 1 or 10 μg/ml solution was prepared in dimethylsulfoxide.

CON A. Con A (Miles Laboratories, Inc., Miles Research Div., Kankakee, Ill.), was kept sterile at room temperature and used at concentrations of 1–100 μg/ml.

Experimental Procedures. The basic design was to determine the effect of the various drugs on the capping of fluorescent anti-Ig-Ig complexes. The assays were usually carried out on 2-cm diameter flat-bottomed culture dishes (Multi Dish Disposo-Tray, Linbro Chemical Co., New Haven, Conn.). 0.5 ml of a cell suspension (10^7/ml) was incubated with anti-Ig either at 21°C or at 37°C. At appropriate times, usually 30 min at 21°C or 10 min at 37°C, the reaction was stopped by the addition of an equal volume of 2% paraformaldehyde. The cells were left 15 min in the fixative, washed once, resuspended in media, and examined under the fluorescent microscope. Cells were scored for those showing fluorescence distributed in caps or throughout the whole cell surface; in the latter case, attention was paid to the pattern of fluorescence, whether delicately speckled, forming a finely interrupted ring at the periphery, or coarsely aggregated into patches. 200–300 cells were scored in each experiment.

One set of experiments evaluated the effects of colchicine or cytochalasin B, or both, on capping. In these experiment, the cell suspension was first incubated with the drug for 30 min to 2 h; then anti-Ig was added, and the reaction was allowed to proceed. The concentration of colchicine was 10^{-4} M in most experiments. Cytochalasin B was used at 1–10 $\mu g \times$ ml.

Most of the experiments using Con A followed the original protocol of Yahara and Edelman (12). The cells were incubated with Con A for a period of 30 min before addition of anti-Ig. If necessary, colchicine, vinblastine, or cytochalasin B were added together with Con A.

Results

Effects of Colchicine and Cytochalasin B. We studied the effects of colchicine or cytochalasin B, or both, on capping of Ig-anti-Ig complexes. Colchicine did not interfere with capping of Ig, either at 21°C or at 37°C, in accordance with past results (5, 16). In the present experiments, the tempo of capping was evaluated by counting caps at different times from 10–30 min at 21°C in the presence or absence of the drug. In normal cells, the rate of capping was markedly reduced at 21°C as compared with 37°C, and hence it was possible that effects of any drug could be observed better under conditions of reduced temperature. The results of Table I clearly indicate no effect of colchicine on the rate of capping. The time of incubation of the lymphocytes in 10^{-4} M colchicine was varied from 30 min to 2 h and was found not to affect the capping of anti-Ig complexes (results not shown).

The effects of colchicine were also tested on cells in suspension or settled on a dish. Previous studies indicated that lymphocytes settled on a dish showed translational motility upon interaction with a bivalent anti-Ig. This motility was

reduced in cells in suspension, at least as judged by the presence or absence of ameboid forms (5). It was possible that a role of microtubules in capping could be evident in cells in suspension, that is, in conditions where the cells were not involved in translation.

Spleen cells were harvested and incubated at 4°C with anti-Ig, washed, and resuspended on a dish at 10^7/ml or maintained in suspension (at 10^6/ml) for 30 min at room temperature. Under each condition, cells were suspended in media with or without 10^{-4} M colchicine. Colchicine had no effect on capping in cells settled on a dish or in suspension, despite the fact that translational motility was known to be reduced in the last condition (5).

Incubation of lymphocytes at 37°C with cytochalasin B did not interfere with capping except for the changes already described in previous papers (5). These

TABLE I
Effect of Colchicine on Capping at 21°C

Time of incubation	Cells incubated with:	
	Regular media	10^{-4} M colchicine
min	%	%
5	19.5	16.3
15	36.8	41.2
30	80.6	88.2

Cells were incubated with 10^{-4} M colchicine at room temperature for 30 min before the addition of FITC-labeled anti-Ig.

are the appearance of caps occupying a large area of the cell surface, with a tendency for the complexes to separate into loose, individual aggregates. However, at 21°C, the number of caps in the presence of cytochalasin B was reduced usually about 30%; the cells not showing caps had large masses of complexes in various areas of the membrane.

There was a marked reduction in capping by the exposure of lymphocytes to both colchicine and cytochalasin B but only when the experiments were done at 21°C. At 37°C, these effects were minimal. Fig. 1 and Table II depicts representative experiments.

Effects of Con A. Following the original protocol of Yahara and Edelman (12), we found an inhibition of Con A on the capping of Ig-anti-Ig (Table III). Patching of the complexes, as assayed by fluorescent and electron microscopy (using ferritin-labeled anti-Ig in thin sections or freeze etching), was also greatly inhibited. The inhibition of capping was best seen in lymphocytes incubated at 21°C with Con A; at 37°C, the inhibitory effects were present, albeit to a lesser extent (Table III, exp. 2). In agreement with the observation of Edelman and co-workers, we found that drugs that bind to microtubular proteins, like colchicine or vinblastine, reversed the inhibition (Table III, exps. 2, 3, and 4). It became very apparent, however, that the caps seen in Con A-treated cells (treated or not treated with colchicine) were distinctly different from the caps

seen in normal lymphocytes. The caps in Con A-treated cells occupied a larger area of the surface, the fluorescence was finely stippled, and, moreover, the caps were generally observed in lymphocytes having ameboid or motile forms. By electron microscopy, the complexes in the capping area were evenly distributed and showed little aggregation or patching.

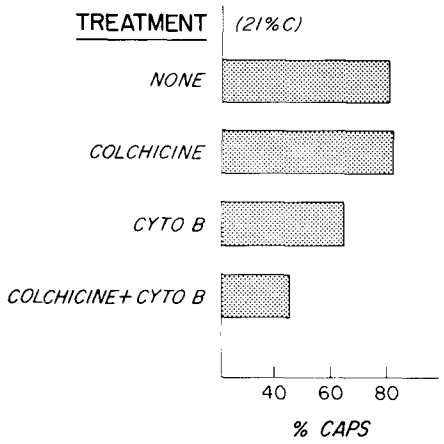

FIG. 1. Lymphocytes were incubated with the drugs for 30 min before addition of anti-Ig. Colchicine was used at 10^{-4} M. Cytochalasin B (cyto B) was used at 1 μg/ml.

TABLE II
Effects of Colchicine and Cytochalasin B on Capping

Treatment	Caps at:	
	21°C	37°C
	%	%
Regular media	81.1	93.5
Colchicine (10^{-4} M)	82.0	93.0
Cytochalasin B (1 μg/ml.)	64.6	78.6
Colchicine (10^{-4} M) plus cytochalasin B (1 μg/ml.)	46.5	92.1

The lymphocytes were incubated with the drugs for 30 min at 21°C or 37°C, anti-Ig was then added, and capping allowed to proceed for 30 min at 21°C or for 20 min at 37°C.

In the experiment of Fig. 2, we scored the caps on the basis of the area of the lymphocyte that they occupied; that is to say, whether they covered one-fourth, one-half, or three-fourths of the cell surface. Previously, we showed that stationary lymphocytes tended to have larger caps (i.e., those occupying one-half to three-fourths of their surface), while motile lymphocytes had small caps occupying a small area (one-fourth caps). In this experiment, we allowed the lymphocytes to settle on a dish, carried out the reaction, i.e. added Con A, then anti-Ig, and then stopped the reaction with fixative, and then scored the caps.

TABLE III
Inhibition of Capping by Con A

	Dose of Con A	Other treatment	Temperature	Caps
	μg/ml			%
I	No Con A	None	21°C	97.1
	1	None	21°C	94.5
	5	None	21°C	36.5
	10	None	21°C	25.6
	50	None	21°C	22.0
	100	None	21°C	14.3
II	No Con A	None	21°C	87.7
	100	None	21°C	19.5
	100	Colchicine 10^{-4} M*	21°C	65.9
	100	Vinblastine 10^{-4} M*	21°C	65.3
	No Con A	None	37°C	93.0
	100	None	37°C	62.8
III	No Con A	None	21°C	87.7
	100	None	21°C	19.5
	100	Colchicine 10^{-4} M*	21°C	65.9
IV	None	None	21°C	74.0
	100	None	21°C	36.0
	100	Colchicine 10^{-4} M*	21°C	58.0
	100	Colchicine 10^{-4} M‡	21°C	62.0

The lymphocytes were incubated with Con A 30 min before the addition of anti-Ig.
* Drug was added together with Con A
‡ Lymphocytes were incubated with colchicine 30 min before incubation with Con A.

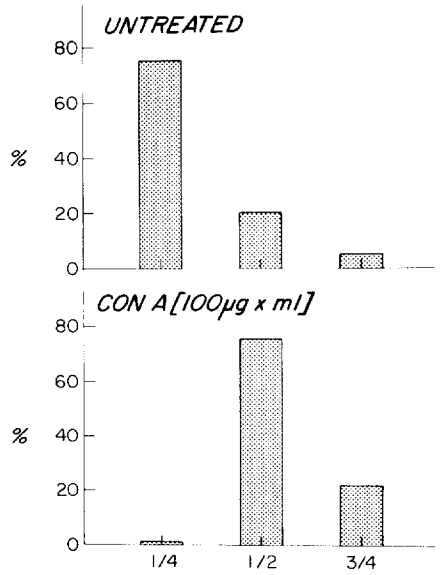

FIG. 2. This figure shows the number of lymphocytes having caps of different sizes. Most untreated lymphocytes have small tight caps occupying one-fourth of the cell surface. The caps seen in Con A-treated lymphocytes occupy a much larger area of the surface.

Note that while most normal lymphocytes had tight (one-fourth) caps, the great number of caps in Con A-treated lymphocytes occupied half or more of the cell surface.

It was of interest to note that Con A-treated lymphocytes containing caps of Ig-anti-Ig exhibited very abnormal shapes, being irregularly long and stretched. Fig. 3 is a scanning electron micrograph of one such cell. In the experiment of Table IV, we incubated cells with Con A, with or without colchicine, for 30 min, then added FITC anti-Ig for 30 min, and fixed. The cells were examined for presence of Ig-anti-Ig in caps or over the entire surface and/or for the presence of normal or ameboid shapes. One can note that Con A-treated cells had a reduced

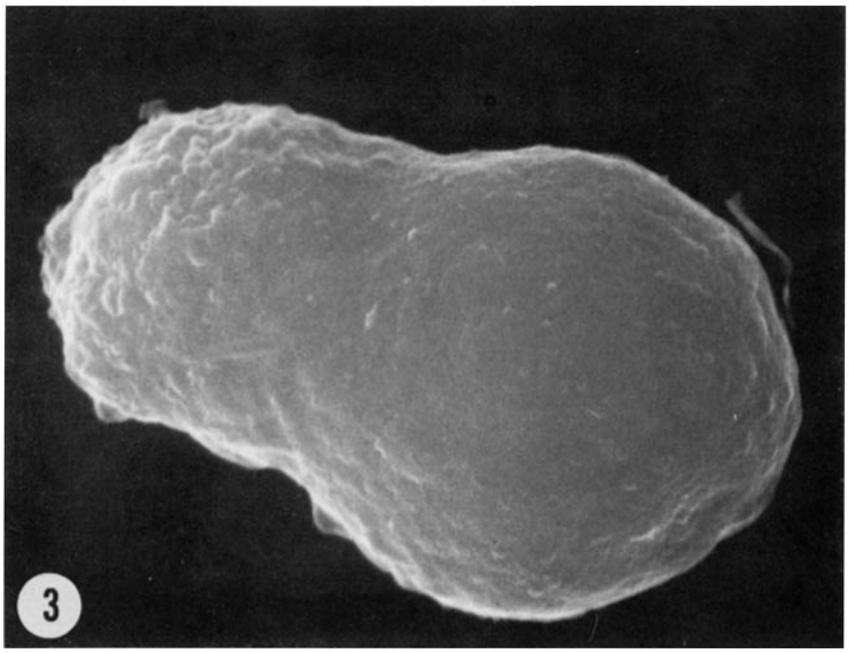

FIG. 3. Scanning electron micrograph of lymphocytes treated with Con A and anti-Ig under the conditions described in the text. Note the markedly elongated hand-mirror form of the cell. One pole is smooth; the other (presumably the tail) is roughened. × 14,000.

TABLE IV

Motile and Stationary Lymphocytes in the Con A Effect

Anti-Ig plus:		Capped lymphocytes				Noncapped lymphocytes		
Con A	Colchicine	%	Round	Ameboid	Ratio (ameboid: round)	%	Round	Ameboid
No	No	60	23	37	1.6	40	39	1
No	10^{-4} M	63	21	42	2.0	37	37	0
100 μg	No	20	1	19	19.0	80	76	4
100 μg	10^{-4} M	47	5	42	8.4	53	51	2

Stationary and ameboid lymphocytes were judged on the basis of morphology (5).

number of caps. Indeed, most caps in Con A lymphocytes were in ameboid cells. Colchicine increased the number of caps in Con A-treated lymphocytes increasing the number of cells with ameboid features (Fig. 4).

We investigated the relationship between cell motility and the Con A inhibition of capping by examining microscopically live lymphocytes settled on culture dishes. This was done by examining the dishes on an inverted microscope either at room temperature or at 37°C with the aid of an air-curtain incubator.

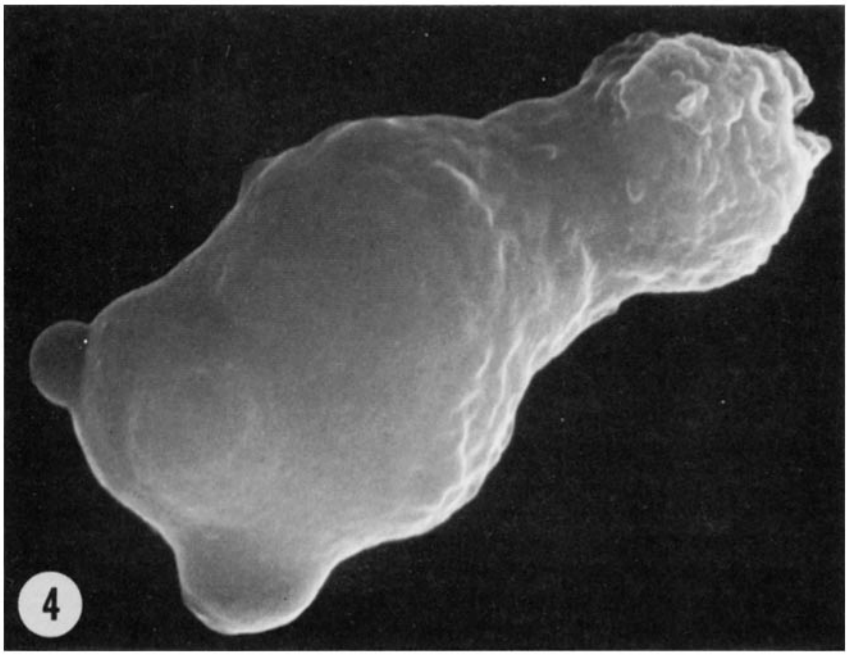

FIG. 4. Scanning electron micrograph of lymphocytes treated with Con A, colchicine, and anti-Ig. Note the markedly elongated form. One pole is smooth and shows blebbing of the surface membrane; the other (presumably the tail) is roughened. × 10,000.

Most normal lymphocytes were round and did not move. Addition to them of anti-Ig produced an increase in random translation in about one-fourth of the cells. This translational process, previously described (5), is slow and random. Addition of Con A produced a striking series of changes. Some cells became elongated and very stretched, very slowly displacing themselves. (Some of these elongated forms were rather striking; note the scanning electron micrograph in Figs. 3 and 4.) After about 5 min, these ameboid lymphocytes no longer moved, although retaining their abnormal shapes. These ameboid shapes persisted for at least 2 h of culture, suggesting that the lymphocytes were "frozen" in their peculiar morphology. (In contrast, normal lymphocytes treated with anti-Ig displaced themselves for about 20 min and then became round.) The addition of colchicine plus Con A brought about one significant change from Con A alone, and that was that the lymphocytes having the ameboid shapes

continued their slow migration and change of shape for up to 30 min of culture at which time they stopped but retained their abnormal morphology. Cytochalasin B (1 μg/ml) added at the same time with Con A, or Con A and colchicine, inhibited all cell motility and development of ameboid shapes in the lymphocytes.

In contrast to its effects on normal lymphocytes, cytochalasin B markedly affected capping in Con A-treated cells. First, cytochalasin B enhanced the inhibition of capping produced by Con A; secondly, it counteracted the effect of colchicine on the Con A inhibition of capping. These experiments are depicted in Table V and Fig. 5.

We were interested in determining whether the inhibition of Con A on capping

TABLE V
Effects of Colchicine and Cytochalasin B on the Inhibition of Capping by Con A

Con A	Colchicine	Cytochalasin B	Caps
μg/ml		μg/ml	%
No	No	No	56.5
100	No	No	25.2
100	10^{-4} M	No	53.9
100	No	1.0	2.0
100	10^{-4} M	1.0	1.0
No	No	1.0	41.7
No	10^{-4} M	1.0	24.8

Lymphocytes were incubated with the different drugs for 30 min at 21°C before addition of anti-Ig.

could be influenced if the lymphocytes were on a solid substrate or in suspension. Lymphocytes were placed in a small culture flask at room temperature and maintained in rotation for the whole time of the experiment. Another portion of cells was placed on a culture dish. Both sets were exposed to the drugs for 1 h at room temperature, then FITC anti-Ig was added, and the reaction stopped after 30 min. The results of one representative experiment are depicted in Fig. 6. Capping was inhibited in lymphocytes placed on a culture dish and treated with Con A; the effect was reversed by colchicine treatment; cytochalasin B counteracted the effects of colchicine and enhanced the inhibition produced by Con A. Lymphocytes in suspension and treated with Con A exhibited as many caps as normal cells; these lymphocytes with caps were also markedly ameboid, and the caps occupied a large area of the surface. We surmised that in suspension the lymphocytes treated with Con A could still produce the changes in shape which, as discussed before, were not always associated with movement. The capping of these cells, however, was totally inhibited by addition of cytochalasin B which also inhibited the changes in cell shape.

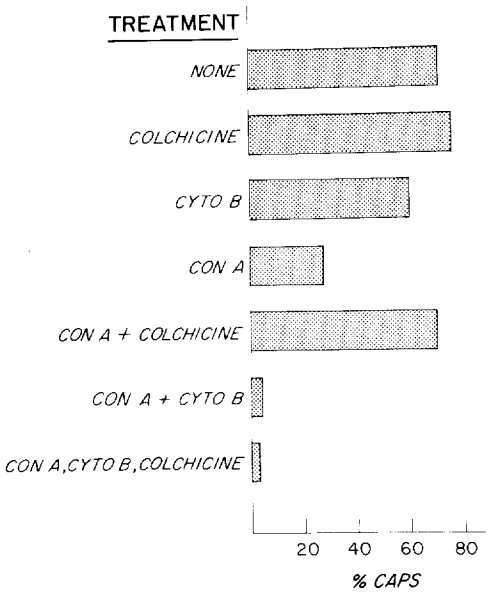

FIG. 5. Lymphocytes were incubated with the drugs for 30 min before addition of Con A. Colchicine was used at 10^{-4} M, cytochalasin B (cyto B) at 1 μg/ml, and Con A at 100 μg/ml.

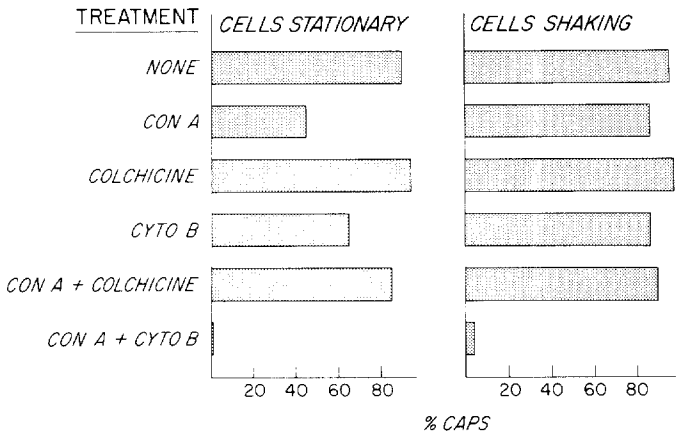

FIG. 6. The description of the experiment is in the text. The concentrations of the drug as in the experiment of Fig. 5. Cells were shaken on a rotating platform at about 150 cycles/min.

Discussion

Several results emerge from these experiments, and tentative conclusions can be drawn; however, it is fully realized that the great problem in interpreting the results of these experiments and others like them is that they depend on the actions of various chemicals, whose specificity of action is not altogether clear, and which may also have several sites of interaction with the cell. Cytochalasin B

is a drug that clearly inhibits movement, but its site of action is controversial; we have accepted the notion that in a broad sense it acts on contractile proteins and microfilaments, although other effects, such as inhibition of glucose transport, have been indicated (17). Colchicine and similar drugs definitely have specificity binding to microtubular protein, but other effects such as inhibition of nucleotide transport (18) have been reported. Apart from these problems, we face the general observation that microtubules and microfilaments are not seen in abundance in B lymphocytes. Whether or not colchicine-sensitive and cytochalasin B-sensitive structures in the B cells are organized as in other cells needs to be determined, as does their pattern of response to these drugs.

The movement of surface complexes into a cap involves an energy-dependent process and is not the result of simple diffusion. It is our hypothesis that a cross-linking ligand initiates a series of reactions that result in a rapid flow of membrane producing the cap, and in the stimulation of the translation of the cell if it happens to sit on a solid substrate. Basically, capping involves a membrane perturbation that needs some directional control for orienting the complexes towards a focal point. Indeed, as one analyzes the different experiments, they strongly suggest that the energy-dependent membrane activity that results in capping is directly or indirectly influenced or regulated by two interrelated but distinct internal events, one related to cell translation involving microfilaments, i.e., to a cytochalasin B-sensitive system; the other associated with the activation of the microtubular system. This statement is based on the fact that the chain of events can be stopped at certain steps by appropriate drugs. What is the role of microtubules in these processes? This point is not clear, although the overall evidence, albeit indirect, suggests that they do play a role perhaps by orienting the membrane activity. There is no doubt that colchicine-treated lymphocytes capped Ig and moved, perhaps even more than normal cells. This clearly implies that microtubules are not essential in the translational process of the lymphocyte, although the possibility of involvement of some long-lived colchicine-resistant structure has not been ruled out. Also the kind of movement in the normal and colchicine-treated lymphocyte has not been studied in depth, and qualitative differences have not been explored. The effects of microtubules became noticeable in conditions where the actual translation of the cell was stopped, such as by cytochalasin B. (There is an apparent discrepancy between this experiment and that in which colchicine-treated lymphocytes in suspension still capped despite the absence of ameboid forms indicative of cell translocation. We conclude that in the latter instance the mechanisms subserving translocation were operative despite the lack of movement since the cells were not attached to a substrate.) Our conclusion is that in a normal lymphocyte the process of translation orients the cap towards its trailing portion, microtubules most likely, although not necessarily, playing a role. If, however, translation event is stopped at some critical step in its triggering, such as by cytochalasin B, then the microtubular system becomes more essential, serving in some way to direct the flow of complexes. The important point that we are stressing here is the need for some internal regulating process in orienting the flow of complexes. The experiments of Berlin and co-workers (8–11) in the neutrophil and macrophage do show more clearly the regulation of membrane activity by microtubules than

do the present experiments with lymphocytes. Likewise, the experiments of Ryan et al. (7) in the neutrophil very clearly demonstrate the role of translation in the orientation of the capped complexes.

The interrelationship between microtubules and surface Ig has been stressed by Yahara and Edelman (12–15) in a series of experiments confirmed herein. The basic observation is that Con A stops both membrane aggregation of complexes and capping and that colchicine counteracts these effects. Edelman's work calls attention to two states of microtubules with the possibility of their activation of fixation when Con A binds to the membrane. We interpret these results in a different way based on the present experiments. Our indications are that colchicine is allowing the Con A-treated lymphocyte to increase its translational process as a result of which there is a backflow of the complexes to the most posterior part of the cell. It appears that Con A at the doses employed among other things restricts the translation of the cell, although these do develop very abnormal shapes. This restriction seems to be alleviated by certain maneuvers, such as treating the cell with colchicine, increasing the temperature of the reactions (Table III), or placing the cells in suspension (Fig. 6). The point to stress is that capping in the Con A lymphocyte, treated or not with colchicine, appears to be dependent on movement or at least on a cytochalasin B-sensitive system, which is not the case in the normal cells. Still, the basic effect of Con A on the membrane seems to us to be its "stabilization", leading to the blocking of patching or aggregation. This effect was not counteracted by colchicine (our cytological observations; experiment of Fig. 2). The nature of the Con A effect on membrane remains a fascinating point to explore.

Summary

Capping of surface Ig by anti-Ig antibodies involves a membrane perturbation requiring an energy-dependent step. Lymphocytes treated with anti-Ig are stimulated to move. Previously, we had shown that movement was not essential for capping, although it influenced the localization of the cap. We have investigated the role of cell movement and of microtubular proteins in this phenomenon. Treatment of B lymphocytes with colchicine does not affect capping of Ig nor does it affect the increase in translational movement produced by anti-Ig antibodies. Treatment of lymphocytes with cytochalasin B stops translational movement and may affect capping to some degree under appropriate circumstances. Lymphocytes treated with both drugs are impaired in capping. We surmise that there may be two cytoplasmic events regulating directly or indirectly capping: one associated with the process of translational movement, the other associated with the activity of microtubules. Lymphocytes treated with concanavalin A do not cap Ig. Colchicine reverses this inhibition. Certain experimental procedures antagonize the colchicine effect, the most striking of which is the use of cytochalasin B. Colchicine appears to increase movement of the Con A-treated lymphocyte, and this increased movement appears responsible for the accumulation of complexes to the posterior part of the

cell. Con A inhibits patching of Ig by anti-Ig, and this is not reversed by colchicine.

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Note Added in Proof. After this manuscript was submitted for publication, a paper by Dr. S. de Petris appeared in *Nature (Lond.)*. (**250:**54, 1974), containing experiments very similar to ours. de Petris presented data showing that both colchicine and cytochalasin B inhibited capping of Ig-anti-Ig and that cytochalasin B had a marked inhibitory effect on capping of FITC-Con A.

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