TWO DISTINCT MECHANISMS FOR REDISTRIBUTION OF LYMPHOCYTE SURFACE MACROMOLECULES

I. Relationship to Cytoplasmic Myosin

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ABSTRACT

A detailed kinetic analysis of the distribution of cytoplasmic myosin during the capping of various lymphocytic surface molecules revealed two distinct capping mechanisms. (*a*) Some cell surface molecules, including immunoglobulin, Fc receptor, and thymus leukemia antigen, all cap spontaneously in a small fraction of lymphocytes during locomotion. Cytoplasmic myosin becomes concentrated in the cytoplasm underlying these spontaneous caps. Exposure to specific antibodies causes all three of these surface molecules to cap rapidly with a concomitant redistribution of cytoplasmic myosin to the area of the cap. These antibodies also stimulate cell locomotion. (*b*) Other lymphocyte surface molecules, including H2 and Thy.1, do not cap spontaneously. Moreover, exposure to antibodies to these molecules causes them to cap slowly without a redistribution of cytoplasmic myosin or stimulation of cell locomotion. Exposure to concanavalin A gives a response intermediate between these two extremes. We believe that the first type of capping is active and may involve a direct link between the surface molecules and the cytoplasmic contractile apparatus. The second type of capping appears to result simply from aggregation of cross-linked molecules in the plane of the membrane.

KEY WORDS capping · cell motility · lymphocyte · myosin · surface molecules

Capping of surface macromolecules is an energy-dependent process consisting of the rapid and coordinated segregation of the ligand-receptor complex to one discrete area of the cell membrane (8, 20, 24).The mechanisms of capping are of general interest because the process may reveal some features of cytoplasm-membrane interaction. The key issue is how the ligand-receptor complex on the membrane interacts, if at all, with the cytoplasm.

Any consideration of capping mechanisms must take into account the marked differences in capping of various molecules. These differences include kinetics, the use of one or two ligands, the fraction of responding cells, the response to drugs, and the relationship to cell motility (reviewed in reference 20). In the case of lymphocytes, Schreiner and Unanue (16) postulated that there are two forms of capping: (*a*) One represented by capping of surface immunoglobulin (Ig) which

appeared to involve the active participation of the cytoplasmic contractile proteins; and (*b*) another represented by capping histocompatibility molecules (H2 in the mouse) or lectin-binding sites, explained by some form of lipid flow, perhaps secondary to cell motility (6, 11).

Recent observations on the lymphocytes support the idea that capping of some membrane proteins is an active process associated with the function of contractile proteins. First, in B lymphocytes, cytoplasmic contractile proteins and surface Ig redistribute together to the same pole of the cell (4, 10, 15). Second, during active locomotion, B cells spontaneously segregate surface Ig from other membrane proteins to form caps similar to those produced by exposure to anti-Ig (14). Lastly, the effects of cytochalasins (7, 24) or agents that modulate Ca^{++} ions (13, 18, 19) on capping also suggest that surface Ig capping may involve the contractile proteins of the cell.

The experiments reported here were done for the purpose of determining, first, whether the redistribution of contractile proteins is a general phenomenon that always accompanies capping of any surface molecules; and second, whether other surface macromolecules spontaneously segregate to the motile cell and, if so, what their association with cytoplasmic myosin is. To do these experiments, we studied surface molecules of lymphocytes that differ markedly in their capping behavior.

MATERIALS AND METHODS

Cell Isolation

The lymphocytes were harvested from spleens of A/St mice (West Seneca Laboratories, Buffalo, New York) or from peritoneal exudates of mice three days after the intraperitoneal injection of 1.5 ml of 10% proteose-peptone. The spleen lymphocytes were purified by sedimentation on Ficoll-Hypaque (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) (15), whereas the peritoneal lymphocytes were separated from other cells by the method of Julius *et al.* (12). Spleen cells consisted of about equal numbers of B and T lymphocytes, whereas the peritoneal cells were ~98% T cells. Thymocytes were harvested from thymuses carefully freed of mediastinal nodes. All cell suspensions were more than 95% viable as assayed by the trypan-blue exclusion test. The medium was Hanks's balanced salt solution buffered with 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES) and containing either 0.5 mg/ml of bovine albumin or 2% fetal calf serum.

Cell Surface Ligands

The following ligands for various surface molecules were used at concentrations optimal for inducing capping. For surface Ig—a fluorescein-labeled rabbit anti-mouse Ig at 50 μg/ml (same batch as used in reference 15). For Fc receptor—an immune complex formed by fluorescein-labeled keyhole limpet hemocyanin (at 200 μg/ml) and rabbit antihemocyanin serum (at a 1 × 125 dilution). Details are given in reference 1. For the thymic leukemia antigen (TL)—a mouse anti-TL serum (B6 × A/TL-anti-ASL1) provided by Dr. E. A. Boyse (Sloan-Kettering Institute, New York) and used as a whole serum at a dilution of 1 × 100. For the Thy.1.2 antigen—an anti-Thy.1.2 (referred to as anti-θ) prepared in our laboratory by immunization of AKR mice with DBA thymocytes (batch CLS:E473) and used as whole serum at a dilution of 1 × 10. For H2—a polyvalent antibody prepared in our laboratory by repeated immunization of C57BL mice with A/St cells. It was used as serum at a 1 × 10 dilution. For Concanavalin A (Con A) binding sites—a fluorescein-conjugated Con A (from Miles Laboratories, Kankakee, Illinois) at 10 μg/ml. The fluorescein-protein ratio was 4.2.

Experimental Protocol

The experiments examined three parameters. The percentage of lymphocytes that (*a*) showed the ligands in caps, (*b*) redistributed myosin to the area of the cap, and (*c*) developed an ameboid morphology. These three parameters were examined simultaneously under two circumstances: (*a*) in cells first treated with the ligands in the cold, then warmed and fixed at various time intervals, that is, under conditions leading to induced capping; and (*b*) in cells that were cultured as described above but not treated with ligands, then fixed, and subsequently examined immunocytochemically, i.e., under conditions that would disclose the spontaneous capping of the ligand and of myosin. This experimental setup allowed us to examine individual cells at various time intervals for ligand capping, myosin redistribution, and the cell shape changes; all of these parameters were also examined in cells not stimulated by the ligand.

To study the ligand-induced capping, we proceeded as follows: (*a*) For Ig, Fc receptors, and Con-A binding sites, the cells were incubated with fluorescein-labeled ligands at 0°C for 20–30 min, then washed three times, portioned in 0.5-ml vol containing 5 × 10^6 cells, allowed to settle while still on ice for 5 min, and then warmed to 37°, or 20°C in some cases. The cells were fixed by addition of an equal volume of 2% paraformaldehyde (*b*) For H2 and θ, the cells were incubated in the cold, first with their respective antibodies, washed, and then with the fluorescein-labeled anti-mouse Ig before warming at 37°C, and proceeding as described above. This was done because these surface antigens cap only when the two ligands are used (7, 22, 24). (*c*) For TL, the cells were incubated with unlabeled anti-TL serum, washed, warmed, and fixed, then stained with a fluorescein anti-Ig to detect the ligand-receptor complex.

To investigate for spontaneous capping, a second portion of cells was treated as described above, *i.e.*,

incubated at 37°, or 20°C, for various times but without addition of the ligands and was then, after fixation, incubated with them.

Both sets of cells were suspended in fetal calf serum and smeared on cover slips, which were then dried, fixed in acetone, and stained with rhodamine antimyosin. The antimyosin was used at a final concentration of 30–50 μg/ml (15) (rabbit no. 8 of reference 9). These concentrations of antimyosin were optimal for disclosing a difference in concentrations within areas of the cell (15). In several experiments, it was varied to be sure that we were in the optimal range. It was obvious from our previous study that the segregation of myosin to one area of the cell was not absolute but relative and that we had to employ concentrations of fluorescent antimyosin which allowed one to distinguish differences in fluorescent intensity. Concerning the choice of formaldehyde-acetone as fixative, we had previously tested a variety of fixation protocols for preserving myosin distribution in cultured cells and found that the formaldehyde-acetone method used here gave results identical to those of other methods, including acetone alone, formaldehyde-ethanol, or formaldehyde-freeze thawing (9).

Cells were examined for fluorescein or rhodamine fluorescence in a Leitz Orthoplan microscope with appropriate excitation and barrier filters. ~200–300 cells were counted for each point. Each ligand was examined in at least three experiments.

RESULTS

Effect of Cell Surface Ligands on the Distribution of Cytoplasmic Myosin

Our aim in these experiments was to determine whether ligands which stimulated receptor redistribution also induced the segregation of myosin into caps.

At 37°C, surface Ig of spleen cells capped rapidly in the presence of anti-Ig. All cells had antimyosin staining of the cytoplasm under the cap, and, in many, there was segregation of myosin to the region underlying the Ig cap, as we have previously reported in detail (Fig. 1, right panel; and reference 15). Note in Fig. 1 that the segregation of antimyosin to the area of the cap reversed rapidly, a point not described in the previous study. This reversibility of antimyosin segregation occurred at the time when the cell was interiorizing the complexes from the area of the cap. As before (15), in some cells with endocytic vesicles both antimyosin and Ig could be discerned. At 20°C, Ig capping was much slower, and endocytosis was less manifest (Fig. 1, left panel). In this case, the majority of Ig caps were associated with segregation of antimyosin to the cap area at all time points. (The experiment shown in Fig. 1 also shows that only a small fraction of the B cells spontaneously redistributed myosin in the absence of ligand.)

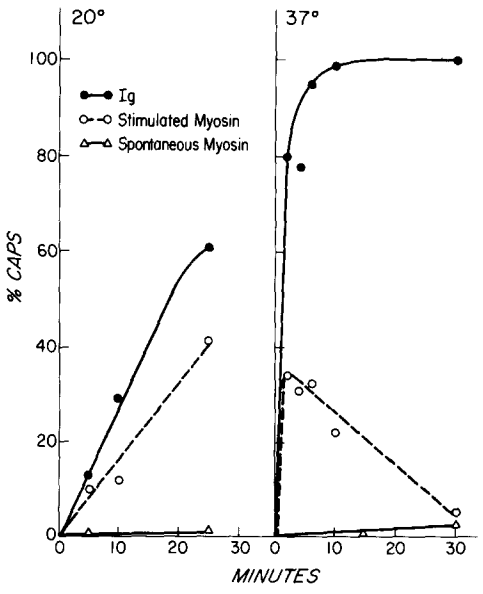

FIGURE 1 Anti-immunoglobulin capping kinetics. Splenic lymphocytes incubated with anti-Ig in the cold, washed, allowed to settle, then incubated at 20°C (left panel) or 37°C (right panel) for the indicated times, and finally fixed with paraformaldehyde to terminate the reaction. After fixation, cells were stained with antimyosin. The fraction of Ig-positive cells with Ig caps (*Ig*) and the myosin segregated to the area of the cap (*Stimulated Myosin*) were determined. Virtually all myosin segregation, when present, was localized to the cap. In a second group, cells were incubated in the absence of anti-Ig. After fixation, cells were stained with anti-Ig to identify Ig-positive cells, and the fraction of B cells with spontaneous myosin segregation (*Spontaneous Myosin*) was determined.

We also examined the capping of the Fc receptor in spleen cells and the TL antigen in thymocytes (Fig. 2). Both ligands stimulated rapid capping in nearly all cells. As with Ig, all capped cells had antimyosin staining under the caps, and, in many, the antimyosin was concentrated in the cytoplasm beneath the cap. Few cells spontaneously redistributed myosin in the absence of ligand.

Capping of Con A is more complex than anti-Ig capping, since a large dose of the ligand readily inhibits the phenomenon (7, 26). We selected a dose that would cap without the addition of colchicine (7, 26). With 10 μg/ml, about one-third of cells capped Con A; all cells had antimyosin staining under the cap, and a small number

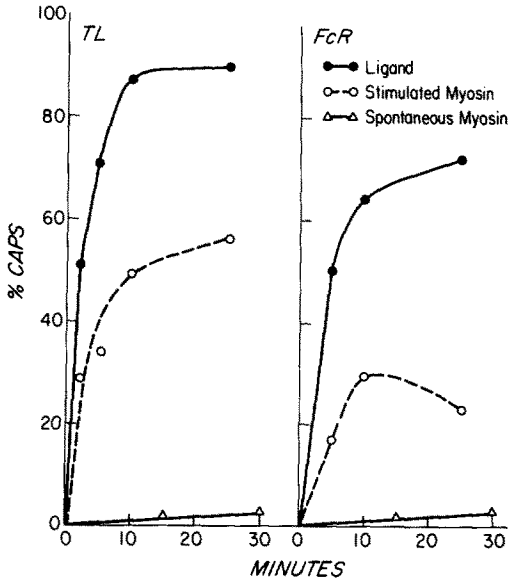

FIGURE 2 Anti-TL and Fc receptor capping kinetics. Capping of TL in thymocytes and the Fc receptor in splenic lymphocytes was examined using the same basic protocol described in Fig. 1. The ligand in the left panel was anti-TL; 80–90% thymocytes were stained. The ligand in the right panel was immune complexes; ~35% splenic lymphocytes were stained, these corresponding to B lymphocytes.

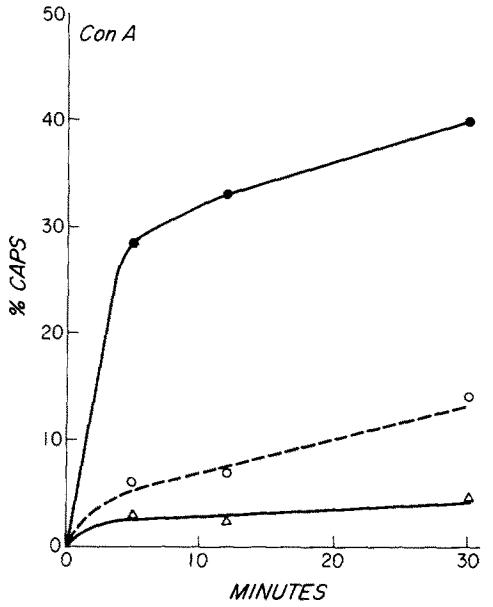

FIGURE 3 Con A capping kinetics. Con-A capping in splenic lymphocytes was examined using the same protocol described in Figs. 1 and 2. Cells were incubated at 37°C in the presence or absence of 10 μg/ml Con A. Similar results were also found with nylon-wool-purified peritoneal exudate T cells. See explanation of legend in Fig. 2.

segregated myosin to the region of the cap (Fig. 3).

Treatment of peripheral T cells or thymocytes with anti-θ or anti-H2 caused a few to slowly cap these ligands but produced no changes in myosin distribution (Fig. 4). ~5–10% of the cells treated with anti-θ and anti-H2 had ameboid shapes; in these the antimyosin was concentrated to the area of the cap. The same number of cells not treated with the ligands showed ameboid shapes with segregated myosin.

Fig. 5 shows representative photographs of the redistribution of ligands and of myosin. A, B, and C clearly demonstrate that the caps are associated with a segregation of myosin to the same area. As previously shown with surface Ig (15), we found occasional cells in which myosin was found in a bipolar distribution. Note in Fig. 5*D* that myosin is located under the caps of immune complexes as well as in the area opposite it. The two sites of myosin concentration may represent the posterior, cap associated uropod and the anterior, ruffled membrane first observed by electron microscope

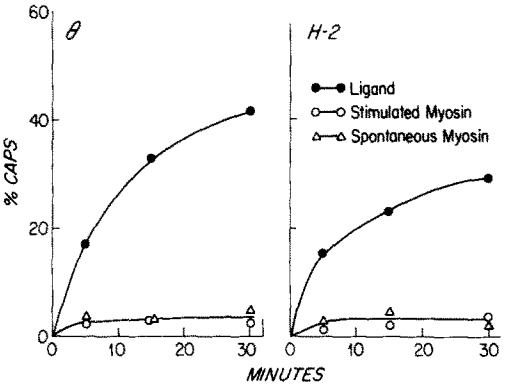

FIGURE 4 Anti-θ and anti-H2 capping kinetics. θ and H2 capping was examined using the same protocol described before. In the left panel, thymocytes were treated sequentially with anti-θ and anti-Ig as described in Materials and Methods and then warmed to 37°C for various times. Similar results were obtained with nylon-wool-purified peritoneal exudate cells. In the right panel, peritoneal exudate T cells were stained sequentially with anti-H2 and anti-Ig. These alloantibodies stained essentially all cells examined. See explanation of legend in Fig. 1.

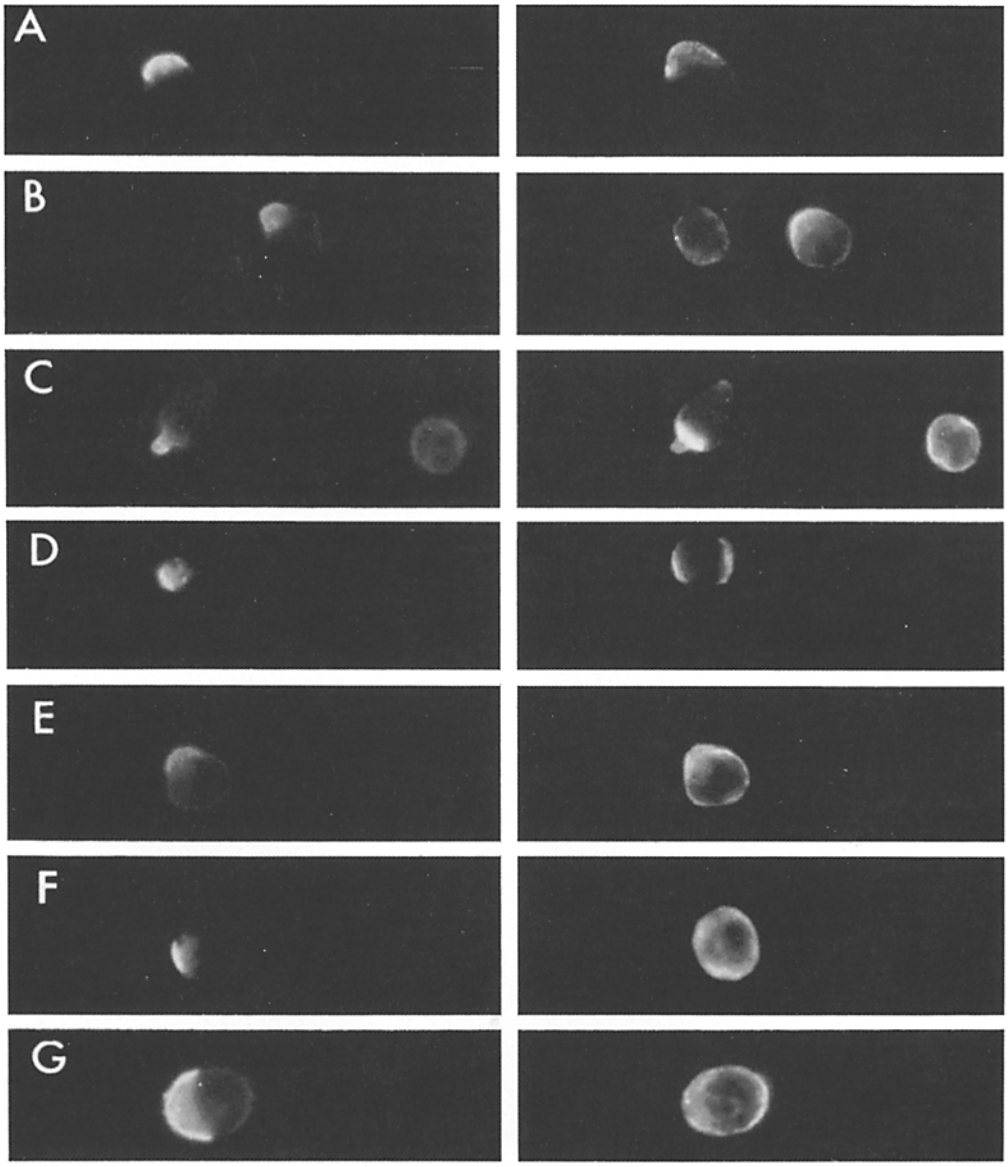

FIGURE 5 Fluorescence microscopy comparing antiligand and antimyosin staining patterns. Cells were exposed to ligands, incubated to promote capping, then fixed and stained with antimyosin. Each pair of micrographs shows the same cells stained with fluorescein-labeled ligands (left panel) and with rhodamine-labeled antimyosin (right panel). In *A*, a splenic lymphocyte with an Ig cap displays myosin segregated to the area of the cap. At this early stage of capping, there are no marked changes in cell shape, yet myosin is already segregated with Ig. In *B* and *C*, thymocytes with TL caps also display coincident, polar myosin. In *C*, note the exaggerated uropod and accentuated myosin segregation seen in certain TL caps. In *D*, a splenic lymphocyte with an Fc receptor cap is shown with bipolar distribution of myosin. A similar bipolar pattern occasionally appeared in cells capping other surface molecules and may represent myosin in the ruffled membrane as well as in the posterior uropod. However, in capping of the Fc receptor and other surface molecules, the segregation of myosin was almost exclusively unipolar. A spleen cell with a Con A cap and partially segregated myosin is shown in *E*. In *F* and *G*, a θ cap (thymocyte) and an H2 cap (peritoneal exudate T cell) exemplify the typical lack of myosin redistribution associated with these surface molecules.

examination of lymphocytes with Ig caps.[1] Figs. 5*F* and 5*G* show examples of θ and H2 caps with characteristically diffuse patterns of myosin. Fig. 6 shows several T lymphocytes with H2 caps and no segregation of myosin.

Effects of Ligands in Producing Changes in Cell Shape

Lymphocytes that are in locomotion have a characteristic ameboid morphology. Initially round, the moving cell forms a constriction that appears to displace the nucleus into an anterior part. The constrictive area, the uropod, gives the cell a hand-mirror or pear-shaped appearance that is easy to discern (reviewed in reference 20). In the absence of cell surface ligands, up to 20% of lymphocytes developed ameboid or pear-shaped morphology (Fig. 7, also see Figs. 1–6). In these moving cells, antimyosin staining frequently was segregated to the constricted uropod region. In fact, spontaneous segregation of myosin to one pole of the cell was found only in cells having ameboid morphology.

The experiments shown in Fig. 8 are the same as those depicted in Figs. 1–4 but reporting the percentage of cells that developed ameboid shape after the interaction with ligands. There is a correlation between the ability of a ligand to induce rapid capping with myosin redistribution in the cytoplasm and to stimulate a locomotory change in cell shape. Those ligands that stimulated fast capping and segregation of myosin staining – anti-Ig, anti-TL, immune complexes, and, to a lesser extent, Con A – increased the frequency of ameboid cells compared to unstimulated cultures. Those ligands that resulted in slow capping without myosin redistribution, anti-θ and H2, did not promote the formation of ameboid morphology.

In a rapid cellular event such as capping, it is difficult to establish whether the ligand-induced myosin segregation reflects a unique association to cytoplasmic myosin with the cell surface receptor or simply a coincidence of two distinct events, capping and motility. We have previously reported that, with surface Ig capping, in fact, myosin segregation and Ig capping preceded the development of ameboid shape (15). It was obvious to us that the same was true with other ligands; in fact, many cells at given times segregated myosin to the cap with no obvious changes in cell shape. With Ig, TL, and Fc receptors, a substantial fraction of the cells, from 20 to 45%, redistributed myosin without any change in cell shape.

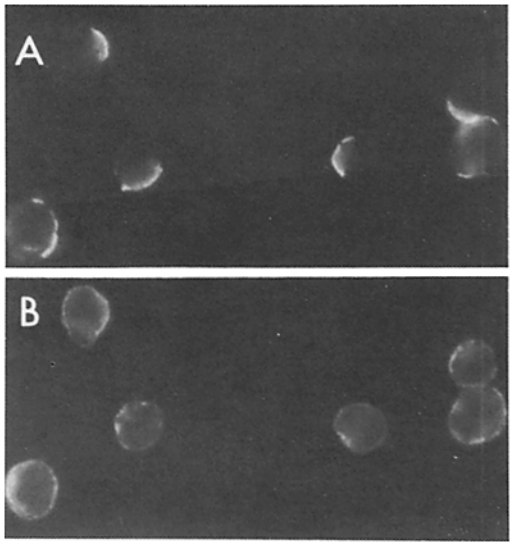

FIGURE 6 Lack of myosin redistribution in lymphocytes with H2 caps. This field shows several T lymphocytes incubated with anti-H2 (plus anti-Ig) exhibiting caps (*A*). Note the lack of redistribution of myosin (*B*).

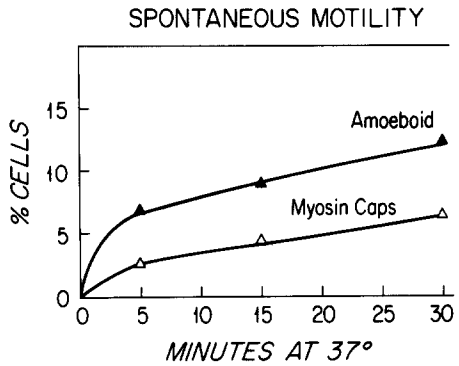

FIGURE 7 Myosin redistribution in motile lymphocytes. Splenic lymphocytes were settled in the cold, incubated at 37°C for the indicated times in the absence of ligands, then fixed with paraformaldehyde and stained with antimyosin. The fraction of cells with ameboid morphology (*Amoeboid*) and with the segregated myosin (*Myosin Caps*) were scored. Segregated myosin occurred exclusively in ameboid cells, where it localized in the uropod. Similar association of ameboid morphology and myosin segregation during spontaneous motility was also seen in thymocytes and peritoneal exudate cells.

[1] KARNOVSKY, M. J., and E. R. UNANUE. Scanning electron microscopy study of immunoglobin capping of lymphocytes. Manuscript submitted for publication.

Relationship of Spontaneous Myosin Segregation to Spontaneous Capping

To study spontaneous capping in the various experiments described above, cells were incubated at 37°C, fixed with paraformaldehyde, and then stained with fluorescein-labeled ligands, followed by rhodamine antimyosin. Figs. 1–4 include the results with each of the ligands—note "spontaneous capping." Table I is a detailed description of one representative experiment.

As before, we found that a certain percentage of B lymphocytes capped Ig spontaneously. All the small fractions of B lymphocytes with a spontaneous Ig cap had ameboid morphology; and, of these, 60% had myosin segregated to the area of the uropod underlying the cap. The same results were obtained for the Fc receptors.

In contrast, TL spontaneously redistributed in >10% of thymocytes. The majority of spontaneous TL caps was found in round cells in which myosin was distributed diffusely throughout the cytoplasm. However, ameboid cells with myosin concentrated to the uropod had a high frequency of the TL antigen spontaneously redistributed to the same area (Table I). The appearance of cells with spontaneous caps is shown in Fig. 9.

Under the same conditions, Con A, H2, and θ did not cap spontaneously (Table I). In fact, in ameboid cells the intensity of these three ligands to the area of the uropod was diminished, although not absent. This finding was distinct for about one-third of ameboid cells and is in agreement with the findings of de Petris and Raff (8).

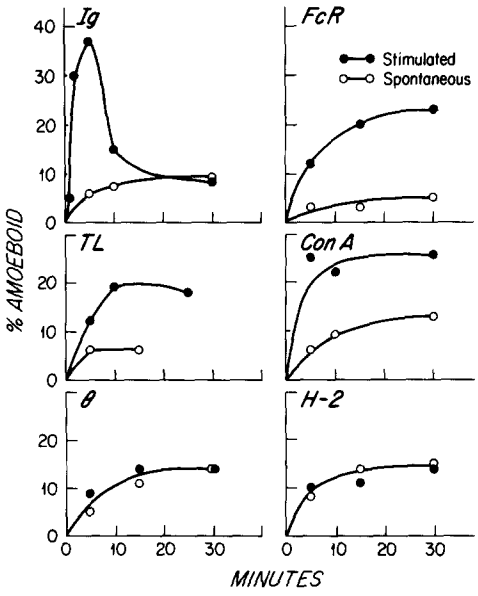

FIGURE 8 Changes in cell shapes induced by ligands. The occurrence of motile cells in the presence (*Stimulated*) or absence (*Spontaneous*) of different ligands was determined in lymphocytes incubated at 37°C. These data were obtained from the identical experiments shown in Figs. 1–4.

DISCUSSION

These studies delineate two distinct types of capping of lymphocyte surface molecules. One type occurs with surface Ig, the Fc receptor, and the TL antigen. All these surface molecules cap rapidly upon interaction with a single ligand. Their capping is associated with a coincident segregation of cytoplasmic myosin to the area of the cap and with the production of changes in cell shape resulting most likely in the stimulation of cell motility. Furthermore, in actively moving lymphocytes, these three molecules spontaneously segregate in a few cells to the area of the cell where myosin is also segregated. A second type of capping occurs with H2 and θ alloantigens. These cap slowly, and only if two ligands are used. Their caps are not associated with segregation of myosin. In spontaneously moving lymphocytes, these molecules do not redistribute to the sites where myosin concentrates.

Our results differ from those reported recently by Bourguignon and Singer (4, 5), who found segregation of myosin to the area of H2 or θ caps in the lymphocyte. In analyzing their results, we find it difficult to reconcile this discrepancy. Their studies were made on frozen sections of cells. Perhaps this method is more sensitive to detection of minor differences in the distribution of contractile proteins. On the other hand, their studies did not include a kinetic analysis of capping and information on the extent of background redistribution of myosin. These could be critical if the cells used in the experiments were highly active in translatory motion. Indeed, we have shown that lymphocytes in locomotion had spontaneous segregation of myosin to one pole of the cell. This point, however, does not explain the results with patching. In their hands, H2 patches coincided with myosin aggregates, whereas in ours, this was not the case (see accompanying paper). Our method, however, was sensitive enough to detect myosin patches with anti-Ig plus azide.

Our results suggest that, for the redistribution

TABLE I

Spontaneous Redistribution of Surface Molecules

	Cells with caps			Ameboid cells	
	% Total cells	% With ameboid shapes	% With segregated myosin	% Total cells	% With caps
Ig	2.7	100	57.1	4.7	57.1
TL	10.4	27.8	33.3	4.8	62.5
Fc Receptors	1.5	100	100	3.9	38.5
Con A	0	0	0	15.5	0
θ	0	0	0	12.5	0
H2	0	0	0	16.1	0

A representative experiment showing the spontaneous redistribution of surface molecules and myosin. Cells were incubated for 30 min at 37°C in the absence of ligands and fixed with paraformaldehyde. Fixed cells were then stained with ligands and antimyosin. Ig, Fc receptors, and Con A were examined on spleen cells; TL and θ on thymocytes; and H2 on peritoneal exudate T cells.

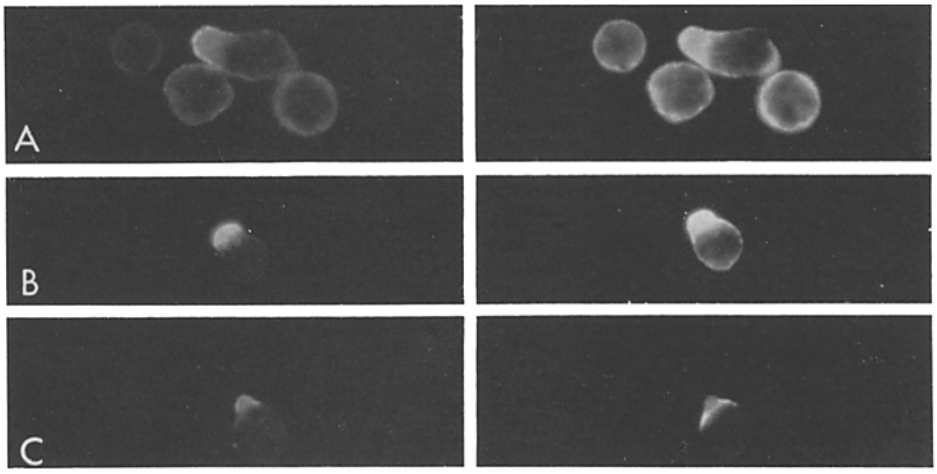

FIGURE 9 Fluorescence micrographs of cells showing spontaneous capping. Splenic lymphocytes (*A* and *B*) or thymocytes (*C*) were settled in the cold, incubated at 37°C for 30 min in the absence of ligands, and fixed with paraformaldehyde. Fixed cells were then stained with ligands and antimyosin to determine the location of surface molecules and myosin in spontaneously active lymphocytes (see Materials and Methods). Each pair of micrographs shows the same cells with fluorescein-labeled ligands (left panel) and rhodamine antimyosin (right panel). Surface Ig (*A*) and the Fc receptor (*B*) cap spontaneously in ameboid cells, and the caps were nearly always associated with segregated myosin. Spontaneous caps were not seen in round cells. In thymocytes, TL spontaneously capped (*C*); and as shown, the caps were often associated with segregated myosin.

of various molecules, there are at least two different mechanisms (a) microfilament dependent, and (*b*) microfilament independent. We hypothesize that the first type of capping is a contractile event which involves a direct reversible link between the surface molecules and underlying microfilaments (7, 20, 21). The microfilament-surface molecule association forms, perhaps, by a yet unidentified transmembrane structure either during external stimulation by specific ligands or by internal activation of the contractile apparatus in the moving cell. A contraction in the lymphocyte cortex parallel to the plane of the membrane could cause the simultaneous redistribution of the linked surface molecules and accumulation of myosin to one region of the cortex. Successive activation of microfilament-surface molecule units throughout the cell cortex eventually organizes the sprawling network of microfilaments into a discrete area where both myosin and the surface molecules

become concentrated as a single cap. Deformation of the microfilament network could also induce a concomitant change in cell morphology, resulting in the orderly sequence of ameboid motion. How the network of contractile elements is organized in the cell cortex to bring about such a rapid coordinated aggregation remains to be established.

We believe that the second type of capping occurs by an entirely different mechanism—the progressive aggregation of mobile surface molecules by cross-linking reagents. Most surface molecules possess a large degree of lateral mobility; this property permits random collisions to occur between groups of membrane proteins, the frequency of which depends primarily on the concentration of molecules in the membrane and their rate of diffusion. When a group of surface molecules is bound with multiple layers of antibody, these collisions result in aggregate formation; successive collisions produce complexes of increasing size. However, the frequency of collisions eventually declines because extensive aggregation reduces the number of complexes in the membrane, while the accompanying rise in mass of these complexes limits their rate of diffusion. Thus, the complexes may reach a size where they lie relatively motionless in the plasma membrane. The final accumulation of scattered aggregates into a single cap depends on processes which further concentrate the sluggish complexes. This might occur through contractile events such as cell motility, which segregates large aggregates to boundary areas by inducing rapid deformation of membrane regions, thereby excluding the sluggish aggregates (the result would be a countercurrent flow of lipids and cross-linked aggregates, as suggested by de Petris and Raff [8]); alternatively, segregation of large aggregates could occur by directed flow of lipids and large molecular aggregates, as suggested by Bretscher (6) and Harris (11). Thus, the second type of capping, in our view, is primarily the aggregation of cross-linked molecules in the plane of the membrane regulated and/or accelerated by contractile activity; it does not stimulate or require a direct association between molecules and cytoplasmic microfilaments.

The two forms of capping in lymphocytes suggest the presence of distinct classes of surface macromolecules, one capable of reversibility linked with contractile apparatus, and the other apparently unencumbered by similar cytoplasmic interactions. While the latter class of surface molecules may interact with ligands without altering cytoplasmic activity, the former class of surface molecules, when bound by ligands, induces a highly integrated sequence of contractile events; and, conversely, internal activation of the contractile apparatus promotes reorganization of the surface molecules in the plasma membrane. The unique capability of this class of molecules to interact directly with the contractile apparatus suggests that the function of these surface and cytoplasmic structures is related. One such function may be the chemotactic response of cells to ligands. For one example, surface Ig serves as the antigen receptor in B lymphocytes and may direct these cells to sites of immunological activity. Exactly what molecular mechanism controls the interaction of these surface molecules with the contractile apparatus is unclear, although previous studies (13, 18, 19) demonstrate striking sensitivity of this interaction to levels of cytoplasmic calcium. In fact, we demonstrate in the accompanying paper that agents which perturb calcium metabolism simultaneously disrupt the segregation of myosin and surface molecules that occurs in this type of capping. Significantly, the same agents had little effect on the second type of capping exhibited by H2 and θ.

In regard to the appearance of changes in cell shape, we should mention that previously we had established, using Ig, that such changes accompanied motility as judged by direct microscope studies or by use of migration chambers. Previously, too, we had failed to see effects of anti-H2 in stimulating motility (17). Studies are in progress in which migration chambers are used with these different ligands to further examine this point. However, the evidence supports, so far, that the changes in cell shape in the lymphocyte are an expression of locomotion.

We noted above that, in spontaneously motile cells, the surface molecules that cap rapidly were concentrated in the uropod, whereas in some cells the surface molecules that cap slowly (such as θ and H2) were partially excluded from the uropod. The complementary redistribution of these two classes of surface molecules may be related in a simple way: when the former class of surface molecules accumulates in the uropod, the concentration of membrane protein may be considerably increased; "exclusion" of other surface molecules may represent the equilibration of proteins in the capped region with regions where the protein concentration is lower.

Finally, some features of Con-A capping also deserve note. Previously, the segregation of Con-A binding sites with contractile proteins was described by electron microscopy (2, 3) and by immunofluorescence in fibroblasts (25), HeLa cells (4), and lymphoblastoid cells (23). Upon further examination, however, the same ultrastructural features associated with capping occurred with similar frequency in the absence of Con A, suggesting that Con-A redistribution did not induce the rearrangement of microfilaments (3). Con A binds to many different types of surface molecules; and, as a tetravalent ligand, it produces large aggregates of membrane proteins. For these reasons, the nature of capping induced by Con A may not be unique, but instead, eclectic—the summation of diverse properties exhibited by the heterogeneous, cross-linked molecules.

The basic experiments upon which the study was based were carried out by Dr. George F. Schreiner, who is now going through the labors of a medical internship. We thank him for his advice.

This work was supported by National Institutes of Health grants AI 10091, GM 19654, GM 23531, and by United States Public Health Service Training Grant GM 02220.

Received for publication 30 March 1978, and in revised form 9 June 1978.

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