Brief Definitive Report

REDISTRIBUTION OF MYOSIN ACCOMPANYING CAPPING OF SURFACE Ig*

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There is widespread interest in the possibility that contractile proteins in the cytoplasm may control the movement of molecules on the outer surface of cells. In one of the best studied systems, that of the B lymphocyte, observations from several laboratories have implicated contractile microfilaments in the rapid redistribution of surface immunoglobulin (Ig) into polar caps after complexing with antigen or with anti-Ig antibodies (anti-Ig) (reviewed in reference 1). The capping of Ig requires energy (2, 3); it is inhibited by low temperature and reduced by cytochalasins (4), treatments which interfere with the activity of cytoplasmic contractile systems. Ca^{2+} has an important role in the formation and maintenance of Ig caps (5–7). Finally, filaments similar in size to actin are found in the cytoplasm under the Ig caps (8).

In addition to this work dealing with Ig capping, there is considerable evidence that nonmuscle cells, in general, contain the contractile proteins actin and myosin (9). Actin filaments are bound to several types of isolated plasma membrane, and myosin may be associated as well (reviewed in reference 10).

In this study we have used fluorescence microscopy to follow the redistribution of surface Ig and cytoplasmic myosin during the process of B-lymphocyte capping. We report that the surface molecule and the cytoplasmic contractile protein become concentrated together in the same region of the cell when the cap forms. This morphological finding supports the hypothesis that cap formation is a contractile process.

Materials and Methods

Spleen lymphocytes were harvested from A/St mice (West Seneca Laboratories, Buffalo, N. Y.) and purified by Ficoll-Hypaque centrifugation. The medium was Hanks' balanced salt solution (Grand Island Biological Co., Grand Island, N. Y.) with 2% fetal calf serum (Associated Biomedic Systems, Inc., Buffalo, N. Y.) and 10 mM HEPES buffer (Microbiological Associates, Bethesda, Md.); pH was adjusted to 7.4. Capping reactions with fluorescein-labeled anti-Ig, at a concentration of 100 μg/ml, were carried out at 20°C, unless otherwise noted (5, 6). At the noted times, the reaction was terminated by addition of an equal volume of 2% paraformaldehyde in phosphate-buffered saline. The fixed cells were washed three times in saline, once in fetal calf serum, smeared, and dried on cover slips, which were then placed in ice-chilled acetone for 15 min and air dried. The cover slips were then incubated with rhodamine-labeled anti-myosin for 30 min at 37°C, washed three times in saline, and examined on a Leitz Orthoplan microscope with Ploem epillumination (E. Leitz, Inc., Rockleigh, N. J.). Appropriate excitation and barrier filters were

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employed. The changes in myosin distribution described below can be obscured using a high concentration of the anti-myosin, which stains the entire cytoplasm strongly. We used a diluted concentration of rhodamine-anti-myosin Ig of 60 μg/ml to maximize the contrast between regions of high and low concentration. The preparation and specificity of the fluorescein-anti-Ig and rhodamine-anti-myosin antibodies, both made in rabbits, have been detailed (3, 11). The anti-myosin was obtained from rabbit no. 8 in reference 11. It was prepared against purified human platelet myosin rod. Its specificity for myosin and its cross-reactivity with mouse myosin were determined in a series of studies: (*a*) absorption of rhodamine-anti-myosin with human platelet myosin completely blocked the labeling of murine, acetone-treated lymphocytes as well as human lymphocytes; (*b*) unlabeled anti-myosin antibody completely blocked cytoplasmic staining by rhodamine-anti-myosin; (*c*) rhodamine-anti-myosin extensively absorbed against glutaraldehyde-precipitated rabbit IgG had no effect on the intensity of cytoplasmic staining of lymphocytes treated with rabbit anti-Ig; (*d*) anti-myosin did not label anti-Ig-treated lymphocytes if the cells were viable or had been fixed only by paraformaldehyde, i.e., an acetone fixation was required; and (*e*) adding limiting quantities of fluorescein-anti-Ig and large quantities of rhodamine-anti-myosin simultaneously to viable cells did not affect the binding of anti-Ig. B lymphoblasts were generated in 24-h cultures with 20 μg/ml of *Escherichia coli* lipopolysaccharide.

Results and Discussion

The binding of anti-Ig to surface Ig initiates a sequence of events in the B lymphocyte which can be divided into four stages. Initially, the anti-Ig is uniformly distributed on the cell membrane. In the first stage, the complexes form microclusters disseminated over the cell surface. In the second stage, these microclusters flow rapidly in an energy-requiring step to one pole of the round cells, forming the cap. In the third stage, the cytoplasm under the cap appears to contract, forming a protuberance or uropod. Cytoplasm simultaneously flows forward into a pseudopod-like structure in a direction opposite that of the cap; the cell becomes ameboid in appearance and initiates translatory movement. In a final stage, the surface complexes are endocytosed during this motile period. New antigen receptors appear on the cell surface hours later (1).

When lymphocytes were stimulated to cap surface Ig by fluorescein-anti-Ig for varying times, fixed, and then stained with rhodamine-anti-myosin, we found a striking distribution of anti-myosin staining concomitant with the capping of the surface Ig. This mutual reorganization follows a regular course and is presented in the sequence shown in Fig. 1 and analyzed quantitatively in Table I. Throughout all stages of capping, 70–80% of capping lymphocytes show clear polarization of myosin, always toward the pole of the cell occupied by the cap or toward which the surface Ig is moving (Table I).

During the first 1–3 min after fluorescein-anti-Ig binds to the cell surface, the rhodamine-anti-myosin uniformly stains the rim of cytoplasm surrounding the nucleus (Fig. 1 A). This pattern of anti-myosin staining changes during the earliest stages of Ig cap formation in one of two ways. Usually, the anti-myosin staining is concentrated at the cytoplasmic periphery under the patches of surface Ig and is fainter in regions of cytoplasm covered by membrane free of surface Ig (Fig. 1 B). In a minority of cases, there is an additional area of myosin labeling in a small transient cytoplasmic spur found at the pole opposite the cap (Fig. 1 C). A small spur of cytoplasm is usually observed opposite the cap at this early stage (5) and appears to correspond to membrane ruffles (Morris J. Karnovsky and Emil R. Unanue, unpublished observation).

As time progresses, the anti-myosin-staining intensity is maintained or in-

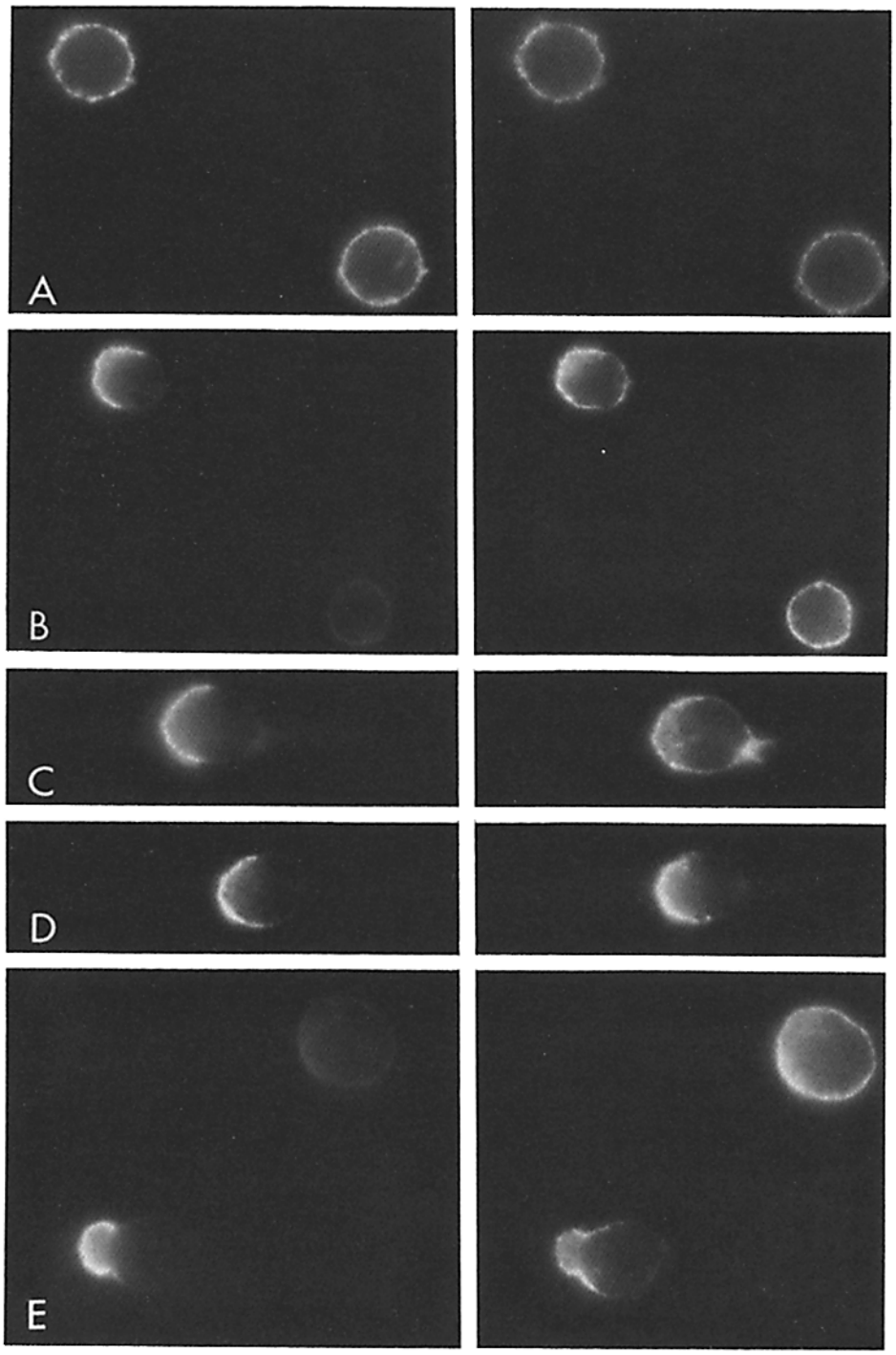
A
B
C
D
E

TABLE I

Anti-Myosin Distribution in Lymphocyte Subpopulations During Ig Cap Formation

Time	Ig+ cells with caps	Ig-positive cells		Ig-negative cells
		Capped cells with polar anti-myosin*	Noncapped cells with polar anti-myosin	Cells with polar anti-myosin
min	%	%	%	%
Exp. 1				
0	0	–‡	0	2
5	12	76	0	6
10	31	78	0	6
25	57	74	4	6
Exp. 2				
0	0	–‡	0	0
5	18	78	2	8
10	31	70	0	12
25	64	72	4	12

Splenic lymphocytes exposed to fluorescein-anti-Ig were assayed for the percent displaying asymmetrical myosin staining after 0, 5, 10, and 25 min of the capping reaction, carried out at 20°C. Three categories of lymphocytes were assayed: (*a*) Ig+ cells in the process of capping; (*b*) Ig+ cells with diffuse surface staining; and (*c*) Ig– lymphocytes.

* Polar myosin is always localized in the cap region.

‡ There are no Ig caps at 0 minutes.

creases under the forming cap. Labeling of myosin in the rest of the cytoplasm is decreased (Fig. 1 D).

There are several interesting aspects of the third stage of Ig capping when the lymphocyte assumes the ameboid shape and starts moving. Although polar myosin staining is always localized in the Ig cap region, the staining usually extends beyond the boundaries defined by the tightly aggregated cap and encompasses the base of the uropod (Fig. 1 E). While anti-myosin stains the uropod, as expected for an area containing much of the cell's cytoplasm, it is most intense in the cortical region of the cytoplasm, in close apposition to the membrane area bearing surface Ig. This is even more apparent in the B lymphoblasts, which follows an identical pattern of Ig and myosin redistribution during surface Ig capping (Fig. 2 A).

In the last stage, the complexes are interiorized, becoming incorporated into endocytic vesicles. We have, in several instances, observed distinct granularity in the myosin underlying such caps (Figs. 2 B and 2 C). This possible association between myosin and endocytic vesicles is currently under study.

It should be noted that a small percentage of Ig– lymphocytes (i.e., T cells) demonstrates spontaneous myosin shifts (Table I). This is always observed in the

FIG. 1. Each pair of micrographs shows the same cells with fluorescein-anti-Ig fluorescence on the left and rhodamine-anti-myosin fluorescence on the right. (A), patterns of anti-Ig and anti-myosin before redistribution; (B), the cell at the top shows the early formation of an anti-Ig cap (left panel) and an accentuation of the anti-myosin staining to the region of the cap (right panel), the cell stained with myosin at the bottom right is an Ig– cell (5 min at 20°C); (C), in this cell there is a typical cap of Ig (left panel) with a concentration of anti-myosin to the cap area and in a small zone opposite the cap (right panel) (5 min at 20°C). (D), the cell shows heavy concentration of anti-myosin (right panel) to the region of the Ig cap (10 min at 20°C); (E), the lymphocyte at the bottom left shows the Ig cap and the formation of the uropod, the myosin is concentrated in the cap and slightly beyond it (25 min at 20°C). Initial magnification, 790.

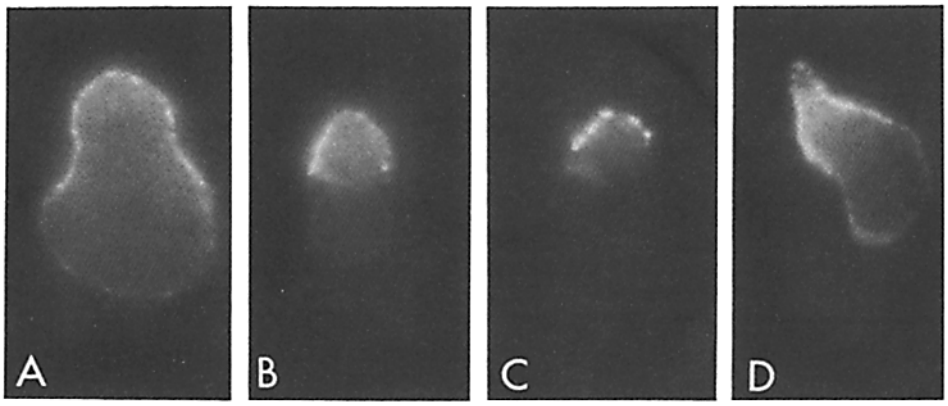

FIG. 2. (A), B lymphoblasts show the myosin concentrated to the cortical area of the cell associated with the Ig cap (not shown); (B and C), late in the sequence (usually at 20–30 min) the fluorescent Ig complexes acquire a punctate appearance (B), the myosin appears in discrete spots (C); (D), an Ig− T cell with a motile configuration showing myosin concentrated to one area of the cytoplasm. × 790.

ameboid Ig− cells with the intense staining localized to the uropod (Fig. 2 D). The T cells differ from resting B cells in that they spontaneously exhibit translatory motion (1). If the asymmetrical myosin distribution found in spontaneously motile T lymphocytes is essential to lymphocyte motility, perhaps one purpose of Ig capping is to induce such a myosin shift in the normally nonmotile B cell and thus stimulate movement.

To conclude, this study has disclosed a very close relationship between surface redistribution of Ig and the cytoplasmic accumulation of myosin. We hypothesize that this membrane-cytoplasmic association may reasonably account for the rapid reorganization of membrane topography stimulated by ligand binding to antigen receptors and perhaps also for the subsequent stages of stimulated cell motility and endocytosis of the receptors. The apparent role of cytoplasmic actomyosin in simultaneously altering the external order of the membrane and the internal order of the cytoplasm over both time and space necessitates that this form of membrane-cytoplasmic interaction no longer be thought of in static terms. There is increasing evidence that, at least in the lymphocyte, it is a means by which the cell can exert modulatory control over its membrane and thus, by extension, over its interactions with its environment.

Summary

The capping of surface Ig on B cells occurs with a striking redistribution of cytoplasmic myosin. Our results suggest a close association between surface Ig and myosin which could be the basis for Ig redistribution and stimulated motility.

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