

SPONTANEOUS REDISTRIBUTION OF SURFACE
IMMUNOGLOBULIN IN THE MOTILE B LYMPHOCYTE*

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Most macromolecules are assumed to be distributed randomly on the cell surface, diffusing freely within the plane of the plasma membrane. However, upon interaction with ligands, surface proteins undergo an orderly redistribution and segregation from other macromolecules – the phenomenon of capping (1). Capping of various molecules shares certain requirements: a cross-linking ligand producing the stimulus, plus metabolic energy, membrane fluidity, and the participation, still ill defined, of cytoplasmic microfilaments and microtubules (for example, see references 1-5).

In this paper we report an example of a surface protein selectively segregated from other surface macromolecules in the absence of any stimulation by ligands. B lymphocytes, during translatory motion, accumulate immunoglobulin (Ig) to one area of their surface. In contrast, two other surface macromolecules remain diffusely distributed. This capping of surface Ig demonstrates a spontaneous change in the distribution of a surface protein during a particular functional state of the cell and may reflect a reversible association between membrane proteins and cytoplasmic structures.

Materials and Methods

Spleen lymphocytes, harvested from A/St mice, were purified by Ficoll-Hypaque centrifugation. The cells were examined immediately after harvesting or, in most instances, after several days of culture. The cells were cultured in RPMI 1640 medium with 5% fetal calf serum at a density of 1.5×10^6 /ml. Some cultures contained 20 μ g of *Escherichia coli* lipopolysaccharide (LPS) (Difco Laboratories, Detroit, Mich.) for the entire period, usually 1-2 days. In other cultures 5×10^6 spleen cells/ml were incubated with 200 μ g of trypsin (trypsin TPCK; Worthington Biochemical Corp. Freehold, N. J.) for 90 min, then washed and cultured as indicated before for 24 or 48 h. Both LPS and trypsin (6) are mitogens selective for B cells under the above culture conditions.

At the time of examination (referred to as the test period), the cells were distributed into new tubes (at $2-5 \times 10^6$ /ml), allowed to settle at room temperature or at 0°C, then incubated at 37°C for 20 to 90 min at which time an equal volume of 2% paraformaldehyde was added. After 15 min, the cells were washed and then incubated at room temperature with the various fluorescent reagents, the preparation and details of which have been previously given (5, 7). Hence, the cells were examined by fluorescent microscopy for the distribution of various surface molecules always after fixation. Surface molecules in cells fixed in 1% paraformaldehyde do not redistribute, a point consistently checked in various experiments. The test reagents were: (a) fluorescein-labeled anti-Ig, a polyvalent anti-Ig antibody made in rabbits; a fluorescein-labeled Fab fragment was used in one experiment; (b) fluorescein-labeled concanavalin A (Con A); and (c) an anti-H-2^a made in C57BL mice immunized with B10.A spleen cells; this reagent was used in a two-layer reaction using fluorescent anti-Ig for detection.

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Caps refer to fluorescence occupying a segment, one-half or less, of the cell surface. Surface fluorescence was easily distinguished from a diffuse cytoplasmic fluorescence exhibited by a rare cell in the preparation. Motile forms refers to lymphocytes showing irregular, long and oval configurations, sometimes pear shaped, easily distinguished from the round, nonmotile cell (8). Some experiments included the use of various drugs, the details of which are given in the Results section. The source of the drugs and conditions of use were previously detailed (5, 7).

Results

Lymphocytes fixed immediately after incubation in the cold were round and exhibited surface Ig all throughout their surface. After incubation at 37°C, however, some of the B cells exhibited translatory motion which could be easily ascertained by their ameboid appearance after fixation. The motile appearance was particularly striking in transformed B cells first cultured for 24 to 48 h in LPS or trypsin. About 40-90% of the B cells in such cultures showed various types of ameboid changes, in contrast to no more than 5% of B cells from nonstimulated spleens (8). In cultures of cells exposed to LPS or trypsin, the bulk of cells exhibiting motile morphology were, in fact, B cells. After 20 min of the test period, many of the cells exhibiting motile morphology (usually more than 75%) showed surface Ig in one portion of their surface in a typical cap (Figs. 1 A-C). Many of the motile forms had Ig caps placed over clearly defined uropods; other ameboid cells lacking classical uropods also displayed asymmetrically located caps. As expected, since the cells were already fixed, identical results were obtained using a labeled Fab anti-Ig antibody. Ig in round, nonmotile cells was always found distributed all over the cell surface, never in caps.

The dependence of spontaneous Ig capping on cell movement was further supported by two experiments, one examining the time sequence of the phenomenon, the other using drugs that affect cell motility. In experiment 1 of Table I, LPS-treated lymphocytes were allowed to settle on the tube at 0°C and then brought to 37°C for varying lengths of time before fixation. Within minutes of incubation at 37°C, the blast cells formed uropods and extended pseudopods, becoming ameboid in appearance. As the percent of motile cells increased steadily, the percent of Ig in caps, low at first, rose to maximum level by 20 min. This suggested that motility preceded Ig capping and that Ig was only gradually swept into a cap as the cell moved. This was suggested, too, by observing motile cells in the earlier stage of the culture with pronounced Ig caps but with additional faint Ig staining over the rest of the cell.

Cells exposed to metabolic inhibitors or drugs known to stop motility (such as cytochalasin B or chlorpromazine) showed very few motile forms and spontaneous Ig caps: It is of interest that drugs that presumably increased cyclic AMP (i.e., dibutyryl cyclic AMP plus theophylline) also stopped cell motility and spontaneous capping. This last result contrasts with their effects on anti-Ig-induced capping. In previous studies, it was shown that anti-Ig-induced capping, a phenomenon in our view not dependent on cell motility, was never affected by drugs that increased cellular cyclic AMP (7). The microtubular-disrupting drug, colchicine, had no effect on spontaneous motility or capping. Experiment 2 of Table I shows a representative result.

Although a great many of our studies were made in cells cultured in LPS, this manipulation was not necessary for the spontaneous development of Ig caps.

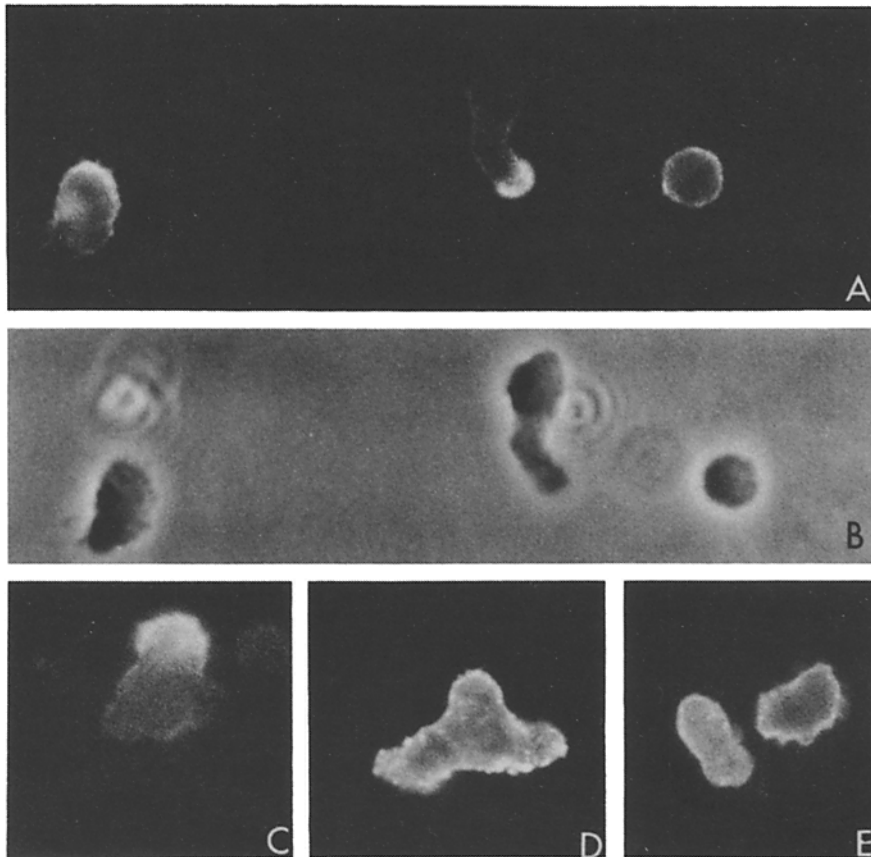


FIG. 1. Figs. 1 A and 1 B are micrographs of B cells first cultured 24 h in LPS and then for 20 min for the test period. Fig. 1 A shows the distribution of surface Ig: the long cell in the center has Ig exclusively in one short segment; in the cell to the left, Ig is mostly concentrated in a pole, but there is a pale trail of fluorescence around the rest of the cell; the round cell to the right has Ig all over the surface. Fig. 1 B shows the same fixed cells photographed under phase contrast. Fig. 1 C shows Ig in a cap in a cell first cultured in trypsin for 24 h. Figs. 1 D and 1 E show a diffuse distribution of Con A-binding sites and H-2 sites in a motile cell.

Comparable results were observed in cultures of lymphocytes transformed by exposure to trypsin (Fig. 1 C). Spontaneous capping was also seen in normal B cells stimulated to move by a 10 min exposure to 10^{-9} M carbamylcholine (7). This study was made in cells previously cultured for 2 days without any stimuli or cells not cultured at all. In this situation the number of motile cells was not as extensive as in the cultures of transformed B cells, yet spontaneous caps were found in about one-half of the motile cells (experiment 3 of Table I). Very few of the B cells not cultured with LPS or trypsin or not exposed to carbamylcholine exhibited spontaneous motility.

Spontaneous capping thus far has been unique to surface Ig. Neither Con A-binding sites nor histocompatibility antigens were rearranged on the cell surface during the course of cell movement. Thus, lymphocytes cultured in LPS or trypsin, fixed, and then stained with fluorescein-labeled Con A or sandwich-

TABLE I
Relationship of Cell Capping to Motility

Exp.	Initial culture	Test period		Motile forms	Caps	Caps in motile cells
		Time	Treatment			
		<i>min</i>		%	%	%
1	LPS for 24 h	0	None	0	0	
		2	None	6	0	0
		3	None	16	4	24
		4	None	26	11	44
		6	None	32	18	56
		20	None	44	34	77
2	LPS for 24 h	90	None	50	43	85
		90	Cyanide (10^{-3} M)	0	0	0
		90	Oligomycin (10 μ g/ml)	12	12	100
		90	Cytochalasin B (20 μ g/ml)	12	8	66
		90	Chlorpromazine (10^{-4} M)	4	0	0
		90	Colchicine (10^{-4} M)	50	50	100
		90	Dibutyryl cyclic AMP (10^{-4} M) and theophylline (10^{-4} M)	4	4	100
		90	A23187	12	7	66
3	48 h	60	Carbamylcholine (10^{-9} M)	20	16	80
	9 h	60	Carbamylcholine (10^{-9} M)	11	5	45

Spleen cells were examined for motility (the test period) after culturing for a day in LPS (exps. 1 and 2) or as in exp. 3 after 2 days in culture or immediately after harvesting from the spleen. Motile forms refer to cells exhibiting an ameboid or pear-shaped morphology (Fig. 1 B). Percent caps refers to caps among all the cells examined. All spontaneous caps were found in cells with ameboid morphology; the percent of cells with motile morphology showing surface Ig in a cap is shown in the last vertical column.

labeled with anti-H-2 and fluorescein-labeled anti-Ig showed completely dispersed staining over both round and ameboid cells (Figs. 1 D and E). Attempts to detect Fc receptor were unsuccessful in fixed cells.

Discussion

The spontaneous capping of surface Ig involves the transport of a specific set of membrane proteins to a discrete location on the surface of a moving lymphocyte. Con A-binding sites and H-2 remained dispersed over the surface of the motile cell. The spontaneous Ig capping was dependent upon and subsequent to the spontaneous initiation of motility. We did not observe spontaneous capping in round cells, as would be expected if capping preceded motility. Furthermore, in timed studies, the number of motile cells that redistributed surface Ig only gradually increased with time, consistent with spontaneous capping as an effect rather than a cause of motility. In addition, agents that inhibited lymphocyte motility were powerful suppressors of spontaneous Ig capping. These same agents did not have the same effects in anti-Ig-induced caps in which motility followed capping (5, 7, 8).

At least three explanations for the spontaneous Ig capping are possible, two of which can be readily discarded. The first is that all membrane proteins, which have a lower diffusion rate than lipids in a fluid membrane (9), trail the forward flowing lipid components of an advancing pseudopod. This possibility is readily eliminated by the lack of redistribution of Con A-binding sites and H-2 proteins. The second explanation is that Ig is somehow being cross-linked in our experimental culture conditions, this leading to capping. This is highly unlikely given the absence of patches or caps on round cells, which one would expect to find if

the proteins were being cross-linked before fixation. (After fixation, surface proteins are not capped by ligands; furthermore, the results with a monovalent antibody excludes the remote possibility that the bivalent anti-Ig was responsible for the phenomenon of spontaneous capping.) Although we have no data on the interaction of LPS with surface Ig, the observations that trypsin-transformed cells or cells stimulated by a cholinergic agent also segregate surface Ig, further eliminates cross-linking as a mechanism. It should be stressed that transformation was relevant to spontaneous capping only by virtue of stimulating movement. Basically, about the same percent of ameboid cells displayed spontaneous Ig caps regardless of cell size or previous exposure to LPS, trypsin, or carbamylcholine.

A third possibility is that surface Ig is segregated in the moving cell because it selectively interacts directly or indirectly with contractile elements of the cell cortex activated during motility. On the basis of the effects of Ca^{2+} on the formation and disruption of caps, we previously had hypothesized that ligands bound to surface Ig would induce a reversible association between surface Ig and cytoplasmic contractile elements (5). We now assume that there may be an equilibrium state between activated microfilaments and surface Ig in the absence of external ligands. In a microfilament-dependent function such as motility, then, microfilaments would anchor surface Ig from the flow of other membrane elements at the points of activation. The relationship between contractile elements and surface Ig is being pursued at present. Regardless of the explanation, the phenomenon of spontaneous capping may represent one example of the control exerted by the cytoplasm on the topography of surface molecules.

Previously published papers have mentioned in passing the segmental distribution of some surface molecules. For example, de Petris and Raff observed that the theta antigen was excluded from the uropod of an occasional cell, although it was diffuse on round cells (10). Tao et al. noted surface Ig in a segmental distribution on some chicken B cells and interpreted it to represent a focal secretion of Ig (11). Ryan et al. noted that some fluorescein-labeled surface proteins were displaced to the trailing portion of a neutrophil despite the apparent absence of cross-linking (12). It is most likely that some of these examples are analogous to the phenomenon of spontaneous capping described herein. It would be of interest to determine in moving cells which surface molecules are spontaneously segregated and which are not, inasmuch as this information could give some idea of the mode of anchorage to the membrane and/or association with the cell cortex.

Summary

This paper reports that B cells undergoing translatory motion spontaneously segregate their surface Ig to one portion of their plasma membrane. The spontaneous redistribution of surface Ig was found to be: (a) selective, concanavalin A-binding sites and H-2 antigens were not redistributed; and (b) absolutely dependent on translatory motion and energy metabolism. Abundant B cells undergoing motility were found after cultures in lipopolysaccharide or trypsin, or after brief exposure to cholinergic drugs.

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