LIGAND-INDUCED MOVEMENT OF LYMPHOCYTE MEMBRANE MACROMOLECULES

III. RELATIONSHIP BETWEEN THE FORMATION AND FATE OF ANTI-IG-SURFACE Ig COMPLEXES AND CELL METABOLISM*

BY EMIL R. UNANUE,† MORRIS J. KARNOVSKY, AND HOWARD D. ENGERS§

(From the Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115)

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Many of the molecules identified on the surface of both bone marrow-derived (B) and thymus-derived (T) lymphocytes can be displaced from their original position on the surface membrane after reaction with one or more ligands. The displaced molecules can form multiple complexes on the membrane or can agglutinate into a single compact mass at one pole of the cell, the cap. For example, the surface immunoglobulin (Ig) on murine B lymphocytes usually agglutinates in a cap upon reaction with anti-Ig (1, 2) or specific multivalent antigen (1, 3). Other surface molecules such as the antigens that react with heterologous antilymphocyte antibodies (2), the concanavalin A (Con A) receptors (2), histocompatibility antigens (4), and the theta isoantigen (1) are also redistributed after reaction with appropriate ligands.

Several factors in the ligand-induced movement of surface molecules have been analyzed in previous studies (1, 2, 4, 5). (Usually the movement was ascertained microscopically by determining with appropriate markers whether caps formed on the surface membrane.) It was found that the movement of complexed surface molecules was dependent on:

(a) Temperature. No cap formation took place at 4°C, but caps readily developed at 37°C (1, 2).

(b) A cross-linking ligand. Caps developed with bivalent antibodies, but not with monovalent antibodies (1, 2).

(c) Amounts of ligand. Caps developed only when antibody cross-linked most of the surface molecules, presumably into a single lattice; lower concentrations of antibody led to small, disseminated complexes that were interiorized by the cell, but that did not agglutinate into a single mass on the membrane (2).

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Abbreviations used in this paper: B lymphocytes, bone marrow-derived lymphocytes; cAMP, dibutyryl cyclic AMP; Con A, concanavalin A; DMSO, dimethyl sulfoxide; FITC, fluorescein-tagged cells; RAMG, rabbit antmouse immunoglobulin; T lymphocytes, thymus-derived lymphocytes.

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The geographic disposition of the surface molecules. The distances between molecules varied and apparently determined whether cap formation would occur after direct or sandwich reaction, i.e., molecules close enough to be linked within the span of the combining sites of the ligand (usually an antibody: 120 Å) readily led to caps (i.e., Ig and Con A sites) whereas those farther apart did not yield caps unless an antibody to the ligand was brought into the reaction (total theoretical span of an antibody on another antibody is 420 Å) (2, 5, 6).

Another point to emerge from our own previous studies relates to the association between the formation of complexes with the surface molecule and the process of interiorization (2, 5). Clearly no association was evident between complex formation with or without capping, and endocytosis. Although complexes of anti-Ig-membrane Ig were interiorized (regardless of whether or not capping occurred), complexes formed with antilymphocyte antibodies (bivalent or monovalent) or with antibodies to histocompatibility antigens were interiorized slowly. Moreover, caps formed with antilymphocyte antibodies (in a sandwich-type reaction) were not interiorized within the period of several hours. These results suggested to us that there were a rapid and a slow form of endocytosis. Rapid endocytosis was conditioned not only by the property of the ligand to cross-link the surface molecule but also by the geographic disposition of the molecules on the membrane. Slow endocytosis was not related to cross-linking of membrane determinants.

In the present paper we report studies on the relationship of viability and metabolic activity of the lymphocytes to both surface movement and interiorization of Ig, anti-Ig being used as the ligand. Movement was ascertained by microscopic examination of cells exposed to fluorescein-tagged (FITC) anti-Ig. Interiorization was determined in part by ultramicroscopic examination, but mainly by determining whether the anti-Ig labeled with radioiodine was degraded. Previous studies have shown that the interiorized anti-Ig is partially degraded by lymphocyte hydrolases (7).

Materials and Methods

Cells.—Cells were harvested from spleens of A/St mice by conventional methods using Eagle's minimal essential medium (Grand Island Biological Co., Grand Island, N. Y.) with 10% fetal calf serum. The mice, 8-12 wk old, of either sex, were obtained from West Seneca Labs, Buffalo, N. Y. In most experiments the cells were centrifuged on a Ficoll-Hypaque gradient in order to separate the live cells from dead cells, erythrocytes, and debris (8). After centrifugation the live cells (>99% viable) were washed three times and were used in the various experiments.

Effects of Drugs on Movement of Anti-Ig-Surface Ig Complexes.—The spleen cells at a concentration of 5 × 10^6, usually in 100-200 µl of medium, were first incubated with the drugs (in 100 µl) for various periods of time, usually 10-30 min at room temperature. The cell suspension was then placed in an ice bath at 4°C and incubated with 10 µg of fluorescein-conjugated rabbit antismouse Ig (FITC-RAMG) for 30 min. The cells were spun, washed three times, resuspended in about 500 µl of medium, placed in a 37°C incubator (containing 5% CO₂ in air), and examined for periods of up to 6 h (in most experiments the cells were examined at 5, 10, and 20 min after warming). The movement of the surface complexes was evidenced by the formation of the cap, i.e. the concentration of the fluorescence reaction at one pole of the cell, usually in an area encompassing a third of the cell surface. In our hands, cap forma-
tion on mouse B lymphocytes is very rapid in that about 80–95% of fluorescent spleen lymphocytes cap after 1–5 min incubation at 37°C. (About 45–50% of spleen lymphocytes have surface Ig detectable by FITC-RAMG.)

The anti-Ig used in these experiments was polyvalent and precipitated with all mouse Ig and with Fab determinants. It was the same anti-Ig employed in the experiments reported previously, for which specificity controls have been fully detailed (2, 5, 9).

The following drugs were employed: Na azide, dinitrophenol (Eastman Chemical Products, Inc., Rochester, N. Y.; it was recrystallized twice with ethanol and resuspended in phosphate saline at pH 7.2), oligomycin (Sigma Chemical Co., St. Louis, Mo.), NaCN, NaF, iodoacetamide, 2-deoxyglucose, cytochalasin B (ICI Research Lab, Cheshire, England), colchicine, dibutyryl cyclic AMP (cAMP), actinomycin D, cycloheximide, and puromycin (the last five drugs were obtained from Sigma Chemical Co.). All solutions of drugs were freshly prepared and used immediately.

Effects of Drugs on the Catabolism of [125I]Anti-Ig by B Lymphocytes.—Previous studies indicated that the complexes of anti-Ig-surface Ig on B lymphocytes were readily interiorized in small vesicles (2, 5) and that once interiorized, the anti-Ig was partially degraded by the B lymphocytes (7). The process of interiorization and degradation was tested by labeling the anti-Ig molecule with 125I and following the fate of the label after binding of the antibody to the surface of the B lymphocyte. In the present experiments, the influence of various drugs on the catabolism of [125I]anti-Ig was investigated. The rabbit IgG with anti-Ig activity, RAMG, was labeled with 125I (125I[RAMG]) by the lactoperoxidase method (10) to a specific activity of 1 µCi/µg. In all experiments 20 µg of 125I[RAMG] were incubated with 10⁷ spleen cells at 4°C for 30 min, after which the cells were washed five times, resuspended in 1 ml of medium, and placed in culture 1–6 h. At these times, the supernatant fluid was separated from the cells by centrifugation. The cell pellets were lysed in 1 ml of 0.5% (IGEPAL CA-630, a polyethyleneoxy derivative) (GAF Corporation, New York) in phosphate-buffered saline at room temperature for 15 min. The intact nuclei were removed by centrifugation at 1,500 g for 20 min. Samples of the culture supernatants and cell-associated samples were tested for total radioactivity and were precipitated in 12% TCA. Hence radioactivity was measured in supernatants and cell-associated fractions as precipitated, protein-bound 125I, and not precipitated, non-protein-bound 125I.

As in the previous experiments, the cells were incubated with drugs for various periods of time before and after the reaction with 125I[RAMG]. The spleen cells used in these experiments were incubated on plastic dishes in order to remove macrophages before testing with 125I-RAMG: 3 × 10⁷ spleen cells (after centrifugation through a Ficoll-Hypaque gradient), in 3 ml, were incubated on 50 x 25 mm dishes (Falcon Plastics, Oxnard, Calif.) for 1 h at 37°C. The cells that did not attach were incubated on dishes for a second period of 1 h. Further details on these procedures have been given in reference 7.

Ultrastructural Analysis of B Lymphocytes.—Ultrastructural studies were done on spleen B lymphocytes treated with metabolic inhibitors at 4°C, then incubated with ferritin-conjugated RAMG, washed, and then incubated at 4 or 37°C for various periods of time. The distribution on the surface of the anti-Ig-Ig complexes was studied by freeze-etching methods, previously described in detail (5). Conventional thin section studies were also done. The ferritin-conjugated RAMG was the same used in our previous study (5); the ratio of ferritin to Ig was 0.5; the dose of conjugate was 20 µg/10⁷ spleen cells.

RESULTS

Cap Formation

Effects of Metabolic Inhibitors.—Table I summarizes a series of experiments in which different inhibitors, at different doses, were used. Inhibitors of oxida-
tive phosphorylation and of glycolysis inhibited the movement of surface Ig molecules into a cap. In most of the experiments the cells were incubated with the inhibitors for 10 min, washed, incubated with FITC-RAMG, and warmed.

**TABLE I**

**Drugs That Inhibit Capping**

| Drug          | Dose         | Inhibition of capping |
|---------------|--------------|-----------------------|
| Na azide      | $3 \times 10^{-3}$ M | 0                     |
|               | $10^{-2}$ M   | 30                    |
|               | $10^{-1}$ M   | 100*                  |
| Dinitrophenol | $5 \times 10^{-3}$ M | 90-95*               |
|               | $5 \times 10^{-4}$ M | 32                   |
|               | $5 \times 10^{-5}$ M | 0                     |
| Oligomycin    | 100 µg       | 100                   |
|               | 10 µg        | 100                   |
|               | 1 µg         | 100                   |
| NaCN          | $10^{-5}$ M  | 100                   |
|               | $10^{-4}$ M  | 57-80                 |
| NaF           | $10^{-5}$ M  | 15                    |
|               | $10^{-3}$ M  | 0                     |
| Iodoacetamide | $10^{-2}$ M  | 100                   |
|               | $5 \times 10^{-3}$ M | 97-100             |
|               | $10^{-3}$ M  | 40-65                 |
| 2-Deoxyglucose| $10^{-3}$ M  | 30-50-59              |
|               | $10^{-5}$ M  | 0                     |
|               | $10^{-4}$ M  | 0                     |

This table summarizes several different experiments. Cell death in all experiments was about 5%. Groups marked with * denote cell death in the range of about 25%. Usually 45-50% of spleen cells were positive for anti-Ig, and of these about 80-95% capped after warming for 10 min at 37°C. Each figure represents the results of one experiment. All drugs except 2-deoxyglucose were incubated for 10 min at room temperature before addition of FITC-RAMG. Cells were incubated with 2-deoxyglucose in saline for 30 min before incubation with FITC-RAMG; incubation at 37°C also took place with cells suspended in phosphate-buffered saline.

Cells were examined for periods of up to 6 h. The pattern of fluorescence in the inhibited cells changed from a finely linear reticulated type pattern when examined at 4°C to a coarse pattern, occasionally with a beady appearance, after several hours at 37°C in presence of the inhibitors. (The presence or
absence of the inhibitor during the incubation at 37°C did not influence the results.) The appearance suggested a coarser accentuation of the original pattern of distribution.

### TABLE II

**Drugs That Did Not Affect Capping**

| Drug              | Dose  | Inhibition of capping |
|-------------------|-------|-----------------------|
|                   | µg    | %                     |
| Cytochalasin B    | 10    | 0                     |
|                   | 30    | 23*                   |
|                   | 60    | 30*                   |
| Colchicine        | 10    | 0                     |
|                   | 50    | 0                     |
|                   | 100   | 0                     |
| Actinomycin D     | 0.5   | 0                     |
|                   | 5.0   | 0                     |
| Puromycin         | 0.1   | 0                     |
|                   | 0.5   | 0                     |
| Cycloheximide     | 0.2   | 0                     |
|                   | 0.4   | 0                     |
| cAMP              | 10^-4 | 0                     |
|                   | 10^-6 | 0                     |
| EDTA              | 0.01  | 0                     |

This table summarizes several different experiments. Cell death in all experiments was about 5%. Groups marked with * denote cell death in the range of about 25%. Usually 45-50% of spleen cells were positive for anti-Ig, and of these about 80-95% capped after warming for 10 min at 37°C. Lymphocytes were incubated with actinomycin D, puromycin, or cycloheximide for 2 h before reaction with FITC-RAMG. With the remaining drugs, the lymphocytes were just incubated for 10 min.

In all the experiments reported in Table I, the cells were checked for viability by the trypan blue exclusion test. With some concentrations of inhibitors, cell viability was impaired. In these cases high cell mortality could account for the inhibitory effects (i.e., azide at 10^-1 M, dinitrophenol at 5 × 10^-3 M). Indeed, other experiments clearly established that dead lymphocytes would not develop cap formation: for example, in one experiment we tested, for capping of anti-Ig, lymphocytes that had been incubated with FITC-RAMG, washed, and then incubated for 18 h in phosphate-buffered saline at 4°C. After 18 h most cells were dead and did not cap upon warming. In contrast,
the control group lymphocytes incubated overnight in medium were viable and readily developed caps after warming at 37°C. Other, more drastic methods for killing, such as incubation in 0.001% merthiolate, also led to similar effects.

**Effect of Inhibitors of Protein Synthesis.**—10 million spleen cells were incubated for 2 h at 37°C in 1 ml of medium containing actinomycin D (0.02-5.0 µg), puromycin (0.1 and 0.5 µg), and cycloheximide (0.2 and 0.4 µg), after

| TABLE III |
| Effects of Drugs on Catabolism of \[^{125}\text{I}]\text{Anti-Ig} |
| Culture supernatants | Cell-associated |
|-----------------------|-----------------|
|                       | Total \[^{125}\text{I}]| PB-[^{125}\text{I}]| NPB-[^{125}\text{I}]| Total \[^{125}\text{I}]| PB-[^{125}\text{I}]| NPB-[^{125}\text{I}] |
| Control               |                 |
| 37°C (3)              | 32.5            |
| 0°C (2)               | 2.8             |
| Idoacetamide          |                 |
| 10 \(^{-2}\) M (2)    | 11.4            |
| 10 \(^{-4}\) M (1)    | 18.9            |
| 10 \(^{-6}\) M (1)    | 29.1            |
| Dinitrophenol         |                 |
| 5 \times 10 \(^{-3}\) M (1) | 13.0          |
| 5 \times 10 \(^{-4}\) M (1) | 30.0          |
| 5 \times 10 \(^{-5}\) M (1) | 31.0          |
| NaCN                  |                 |
| 10 \(^{-3}\) M (1)    | 12.5            |
| Oligomycin            |                 |
| 2 µg/ml (1)           | 15.4            |
| 10 µg/ml (1)          | 15.6            |
| Cycloheximide         |                 |
| 0.4 µg (1)            | 43.0            |
| Cytochalasin B        |                 |
| 10 µg in 10 µl of DMSO (1) | 23.1         |
| 10 µl of DMSO; no cytochalasin B (1) | 24.9         |
| Colchicine            |                 |
| 100 µg (1)            | 19.2            |
| 2-Deoxyglucose        |                 |
| 10 \(^{-7}\) M (1)    | 35.6            |

10⁷ nonadherent spleen cells incubated with 20 µg \[^{125}\text{I}]\text{RAMG} in the presence of inhibitor for 30 min at 4°C, washed five times, and then cultured for 120 min at 37°C in the absence of inhibitor. ND = not tested. Figures represent the percent distribution after 2 h of the \[^{125}\text{I}] bound after 30 min of incubation at 4°C. PB-[^{125}\text{I}]:[^{125}\text{I}] radioactivity precipitated in 12% TCA; NPB-[^{125}\text{I}]:[^{125}\text{I}] radioactivity soluble in 12% TCA. Figures in parentheses in column 1 represent number of experiments. When more than one experiment was done, the results have been averaged.
which they were incubated with FITC-RAMG at 4°C, washed, and warmed. There was no inhibition of cap formation at the doses used (see Table II).

![Graph](image)

**Fig. 1 a.** The release of total radioactivity during the 4.5-h period of incubation. 100% radioactivity is that bound to the cells after 30 min of incubation with [125I]RAMG at 4°C.

![Graph](image)

**Fig. 1 b.** The release of radioactivity soluble in TCA. 100% radioactivity is that bound to the cells after 30 min of incubation with [125I]RAMG at 4°C.

**Effects of Cytochalasin B and Colchicine.**—Spleen cells (5 × 10⁶) were incubated first with 10, 30, and 60 μg of cytochalasin B in 100 μl for 15 min; FITC-RAMG was added to the tube, and the cells were incubated for 3.5 h, after which they were washed three times in medium containing the appropriate amount of cytochalasin B. Control cells were treated with appropriate concen-
trations of dimethyl sulfoxide (DMSO). At concentrations of 30 and 60 μg/ml there was some inhibition of capping (23 and 30%, respectively). At these concentrations many cells appeared enlarged and swollen, and about 25% of them were dead. At a concentration of 10 μg there was no inhibition of capping.

Two different batches of cytochalasin were tested, with identical results. Both batches appeared to be active since they were effective in altering some macrophage functions. To test the drug, 1 million macrophages harvested from the peritoneal cavity of mice that had received an intraperitoneal injection of 0.5 ml of 10% proteose peptone (Difco Laboratories, Inc., Detroit, Mich.) 3 days previously, were planted in Leighton tubes in 1 ml of medium for 2 h. At that time the macrophages were well spread on the glass. 1 μg of cytochalasin B added to the culture rapidly reversed the spreading and caused the cells to round up. This effect was rapidly reversible by replacing the medium containing the drug with fresh medium.

In other experiments, which will be detailed in the future, such macrophages did not phagocytose sheep erythrocytes opsonized with rabbit antierthrocyte antibodies, although they did pinocytose radioactive proteins such as hemocyanin.2

Colchicine at a concentration of up to 100 μg did not reduce the formation of caps. (For these results, see Table II.)

Other Treatment.—Cap formation was not affected by cAMP at concentrations of 10⁻⁴–10⁻⁸, or by EDTA at 0.01 M. (See Table II.)

Catabolism of [¹²⁵I]RAG

Table III summarizes the results of several experiments. Usually in 2 h the spleen lymphocytes released 26–42% of the amount of ¹²⁵I bound initially (i.e., just after incubation at 4°C). 63–80% of this released ¹²⁵I was not precipitable in TCA. (It was previously shown that the radioactivity soluble in TCA represents ¹²⁵I bound to amino acids and peptides [7]. The protein-bound ¹²⁵I released early in culture includes monomeric Ig, partially degraded Ig, and Ig probably complexed to surface Ig [about ½ of total protein bound released] [7].) On the other hand after 2 h of incubation at 37°C, one-fourth

2 Unanue, E. R., and M. J. Karnovsky. Unpublished experiments.
Fig. 4. Similar to Fig. 2, except the cells were incubated with ferritin-labeled rabbit anti-mouse immunoglobulin for 30 min at 4°C, washed, and then incubated at 37°C for 4 h. There is marked clumping of the label into large aggregates, leaving broad expanses of bare, unlabeled membrane. × 45,000
Fig. 5. Similar to Fig. 4.
to one-half of the cell-associated $^{125}$I did not precipitate in TCA. Note that iodoacetamide, NaCN, dinitrophenol, and oligomycin readily inhibited the degradation of $[^{125}]$RAMG. On the other hand, cycloheximide, cytochalasin B, colchicine, and 2-deoxyglucose did not impair the process.

Figs. 1 a and 1 b show the results of one experiment in which the spleen cells with bound $[^{25}]$RAMG were cultured for 1, 3, and $4^{1/2}$ h after treatment with oligomycin and iodoacetamide. The figures show the release of $^{25}$I in the culture fluids. The inhibition of the catabolism of $[^{25}]$RAMG during this entire period is clearly demonstrated.

**Ultrastructural Analysis**

30 million spleen cells were incubated with $5 \times 10^{-3}$ M iodoacetamide for 10 min at $37^\circ$C, then washed and resuspended in 400 $\mu$l containing 80 $\mu$g of ferritin-conjugated RAMG. After $1/2$ h, the cells were washed and incubated at $37^\circ$C for 30 min and for 4 h. Also, samples of cells treated with the drug were maintained at $4^\circ$C for 4 h.

The pattern of distribution by freeze-etching methods of ferritin-RAMG on iodoacetamide-treated B lymphocytes after 30 min at $4^\circ$C was the same as detailed before; it consisted of a fine, random network of mostly interconnected patches over the entire surface of the cells (Fig. 2). Cells that were incubated at $4^\circ$C for 4 h showed, at the most, a slight accentuation of the patches, i.e., no high degree of aggregation took place at $4^\circ$C within the 4-h period (Fig. 3). Cells incubated with iodoacetamide for 4 h at $37^\circ$C showed the presence of molecules on the surface. These were distributed again in a random pattern, but now in the form of coarse networks consisting of large, patchy aggregates (Figs. 4, 5). Considerable expanses of bare membrane were observed between the labeled areas. (The results on cells not treated with the drug were reported in our previous study [5, 6]; normal untreated cells showed concentration of the molecules in one pole [cap formation] within the first 20 min, in essence leaving a surface bare of Ig molecules.) Thin sections of the cells treated with iodoacetamide disclosed the presence of many ferritin molecules on the surface and a few small endocytic vesicles containing the conjugate.

**DISCUSSION**

Our studies have related the movement and interiorization of surface complexes to an active metabolic process and have given precise information on the disposition of immune complexes on lymphocyte surfaces in metabolically inhibited cells. We surmise that the combination of an antibody with an antigen on the cell surface can lead to different phenomena that are not necessarily interrelated: (a) the formation of single or multiple complexes occupying many zones on the cell surface, (b) the formation of a single compact mass at one pole of the cell, the cap, and (c) the interiorization of the surface complexes.
The requirements for multiple aggregation of surface complexes over the entire surface and for cap formation do not run parallel, and one does not per se result in the other. Cap formation, apart from the factors analyzed earlier (bivalency of ligand, sufficient amount of ligand, sites within the combining sites of the ligand), requires an actively metabolic cell. A dead lymphocyte or one whose metabolism is inhibited will not develop caps. Clearly, inhibitors of glycolysis or of oxidative phosphorylation inhibited cap formation.

Results similar to these with selected inhibitors have been previously reported (1, 11). Hence cap formation does not result from the passive condensation of the antigen-antibody complexes into one tight mass, but requires that the cell in some active way propel the whole complex toward a zone of the surface, usually opposite the Golgi region. It is most likely that this process takes place as a result of increased mobility of the cell surface with or without concomitant cell movement. Indeed, the freeze-etch studies on membranes in cells exhibiting caps disclosed what appeared to be undulations or waves of the whole cell surface (5). Also, lymphocytes in the process of capping adopted a pear shape, the nucleus at one zone being followed by a long tail at the end of which the immune complex was observed (5). One should predict, therefore, that cells that are slow or sluggish in movement would exhibit cap formation poorly; and to test this, studies are now being conducted in our laboratories.

An alternative explanation is that the cells are not sluggish in movement, but rather that the surface movement that is required for cap formation must be unidirectional rather than random (in the latter case, one would expect multiple complexes at different sites). Studies in progress do suggest differences in capping among cells that may not be explained entirely on the basis of the density of surface molecules. For example, histocompatibility antigens cap well on some tumor cells, but not on immature sperm cells.2 (However, quantitative data on density of sites are not yet available.)

One might have to reconcile the present hypothesis with the results obtained with cytochalasin B, in which no clear inhibition of capping was observed. The effects of cytochalasin B on cells are multiple and difficult to interpret (12, 13), but it has been observed that the drug inhibits cell movement in some systems. There is no indication that this drug necessarily affects membrane movement per se.

The multiple complexes that formed over the entire surface membrane of metabolically inhibited lymphocytes were large, with clumps and thick strands of molecules; clearly, this formation of multiple surface complexes is unrelated to cell metabolic activity. The question arises whether the formation of caps in normal cells is a stepwise process involving, first, the formation of multiple complexes disseminated throughout, which then coalesce to form the cap. Our previous studies on normal cells with the freeze-etching method, the best method available to date, failed to disclose any readily discernible two-step
process in the mouse B lymphocytes. Loor, Forni, and Pernis (11) do interpret their fluorescence data as suggesting that multiple complexes did precede capping, but this interpretation is limited by the resolution of the immunofluorescence method.

Clearly, in the case in which there is formation of multiple small complexes, such as occurred in our past study with small doses of anti-Ig on B lymphocytes or with antibodies to histocompatibility antigens on thymic cells, the tendency was for these complexes not to eventuate into a single mass, the cap (2). In our studies, it was only when the B lymphocyte was covered with sufficient antibody to form a lattice covering the entire cell that the whole complex readily moved into a single, tight, cap zone.

For the above reasons, we conclude that for formation of a cap one must link together those cell surface antigens that are within the span of the ligand forming a single, large lattice over the entire cell membrane; this lattice is then rapidly moved by the cell. The reasons why most cells having small multiple complexes do not end up having a single complex as caps are not clear. Perhaps variations in the degree of membrane movement with formation of small complexes, or competition between dissociation and interiorization of the complexes and the movement that leads to cap, or other factors that restrict the displacement of surface molecules are important.

Inhibitors of metabolism also led to suppression of interiorization and catabolism; and in this context, the metabolic needs for both capping and endocytosis appeared similar. Also, the formation of multiple complexes at 37°C in inhibited cells is in agreement with current concepts on the fluidity of the membrane lipid layer. However, generalizations on the metabolic requirements (or lack thereof) for multiple complex formation of other receptors must be made with caution, since surface Ig molecules may have properties different from those of other surface molecules.

Finally, a brief comment on the methodology of studies on geographic distribution of surface molecules is warranted. Our studies clearly show that there was minimally, if any, significant aggregation of the anti-Ig-Ig lattice when B cells were incubated at 4°C even for as long as 4 h. Hence the method of incubating cells at this temperature for ½ h with an appropriate ligand, if carried out under conditions of strict temperature control, appears to give a reasonable representation of the true surface distribution of molecules at a given time. Obviously, we are aware that the use of a bivalent ligand may bring closer receptors that are within the span of the ligand, although the extent of this approximation, if indeed present, is probably not enough to distort the general geographical picture.

SUMMARY

Spleen lymphocytes were studied for the movement and interiorization of complexes of anti-Ig-surface Ig. The movement of the complex into a small,
compact zone of the cell membrane (forming a cap) was inhibited by drugs that inhibited glycolysis and oxidative phosphorylation, but not by drugs that affected protein synthesis. Dead lymphocytes did not form caps. Freeze-etching techniques revealed that inhibited lymphocytes showed formation of multiple small complexes over the entire cell surface. Inhibitors of glycolysis and of oxidative phosphorylation also inhibited the interiorization and catabolism of radiiodinated anti-Ig. We hypothesize that cross-linking of all the surface Ig triggers the membrane movements that are required to pull the lattice into one zone of the cell.

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