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Modulation of Lymphocyte Receptor Mobility by Locally Bound Concanavalin A

(cell membrane/cap formation/lectins/insolubilized concanavalin A)

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ABSTRACT **Binding of concanavalin A (Con A) to the lymphocyte surface at room temperature leads to restriction of the mobility of a variety of cell surface receptors including those for immunoglobulin (Ig), θ, H-2, β_2-microglobulin, Fc receptors, as well as receptors for other lectins. Addition of colchicine to the cell suspensions reverses this effect and allows Con A receptors as well as other receptors to form patches and caps. Capping of Con A receptors results in "co-capping" of Ig, H-2, θ, Fc receptors, and β_2-microglobulin in the absence of ligands specific for these receptors. Receptors binding Limulus hemagglutinin and wax bean agglutinin, as well as those interacting with carbohydrate-specific antibodies, were partially co-capped with Con A but receptors for wheat germ agglutinin were not co-capped, excluding the possibility that restriction of receptor mobility by Con A resulted simply from cross-linkage of mobile receptors to immobilized glycoproteins (Con A receptors). Latex beads and platelets coupled to Con A were bound to lymphocytes under the same conditions as free Con A. Binding of these particles to local regions of the cell surface resulted in restriction of the mobility of those receptors that could be co-capped with free Con A. In contrast to the findings with free Con A, however, addition of colchicine resulted in capping of the bound particles but did not cause co-capping of either the unbound Con A receptors or other receptors. These findings support the hypothesis that modulation occurs via a submembranous assembly containing microtubules, and they further suggest that the transitions of this assembly induced locally by Con A may be propagated via cooperative processes.**

Cell surface receptors that are laterally mobile in the cell membrane can be induced to cluster by multivalent ligands (1). In a metabolically active cell, these clusters or patches are subsequently collected into a cap at one pole of the cell (1). We have shown that patching (and therefore capping) of a variety of receptors and surface antigens can be reversibly inhibited by binding of concanavalin A (Con A) to the cell surface (2, 3). This inhibition can be reversed by colchicine and related drugs (4). Based on these and other findings (5, 6), we have proposed that cross-linkage of certain cell surface receptors can modulate the movement of other receptors via alterations in a cytoplasmic assembly consisting of microtubules and microfilaments (7).

Recent experiments in our laboratory have revealed that Con A bound to cells in the presence of colchicine forms caps that also contain a variety of specific receptors. This co-capping phenomenon raised the possibility that our earlier observations on restriction of receptor mobility by Con A might be explained by simple immobilization of various receptors after their cross-linkage by multivalent Con A molecules to Con A receptors that are in turn anchored to the hypothesized cytoplasmic assembly.

In order to test this possibility, we have prepared Con A bound to platelets and latex beads and have shown that local attachment of these particles to a small fraction of Con A receptor sites on the cell surface inhibits the mobility of Ig and other receptors. The attached particles were redistributed to one pole of the cell in the presence of colchicine but did not co-cap with the receptors whose mobility they originally modulated. These observations strengthen our original hypothesis that restriction of the mobility of various receptors after binding of Con A is mediated via submembranous interactions of these receptors with microtubular assemblies (7) rather than by external cross-linkage to Con A receptors. They also indicate that modulation of receptor mobility can be achieved by binding to a small portion of the cell surface, confirming previous observations (8) that cells bound at one region of their surface to nylon fibers derivatized with Con A show inhibition of receptor mobility. It is likely that the modulating effects of this local binding are amplified by a process that is either cooperative or catalytic, inasmuch as the effects of local binding are propagated throughout the cell surface.

MATERIAL AND METHODS

Mouse Splenic Lymphocytes. Spleen cell suspensions were prepared from adult NCS mice (Rockefeller University) according to methods described elsewhere (2). DBA/2J and Balb/c mice (Jackson Labs., Bar Harbor, Me.) were used as sources of spleen cells for certain of the experiments. The medium used for cell suspensions was generally phosphate-buffered saline, pH 7.4–bovine serum albumin (PBS-BSA) (2); PBS alone was used for experiments with Con A-latex beads.

Fluorochrome-Labeled Materials and Fluorescence Microscopy. Rabbit anti-mouse Ig, rabbit anti-mouse μ chain, rabbit anti-mouse γ chain, mouse anti-H-2^d (DBA/2J) from DBA/1J (H-28) × LP RIII/Sn (H-2^r) mice, anti-θ (C3H) from AKR mice, rabbit anti-dinitrophenyl bovine immunoglobulin (anti-DNP-BGG), goat anti-rabbit Ig, rabbit anti-wheat germ agglutinin, carbohydrate-specific antibodies (9), and Con A were conjugated with fluorescein isothiocyanate or tetramethylrhodamine isothiocyanate by conventional methods (2). Lectins from wheat germ, white pea, small California white beans, Idaho red beans, and wax beans as well as Limulus hemagglutinin were purified by affinity chromatog-

Abbreviations: Con A, concanavalin A; Ig, immunoglobulin; anti-Ig, rabbit Ig directed against mouse Ig; BSA, bovine serum albumin; DNP-BGG, dinitrophenylated bovine gamma globulin; fl-, fluorescein labeled; TMR-, tetramethyl rhodamine labeled; PBS, phosphate-buffered saline, pH 7.4; B-cell, bone marrow-derived lymphocyte; T-cell, thymus-derived lymphocyte.

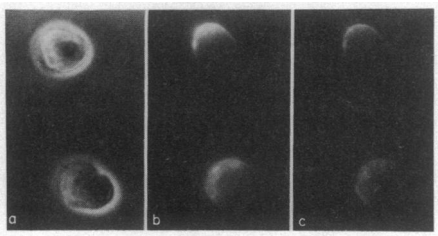

FIG. 1. Co-capping of surface Ig with Con A in the presence of colchicine. Cap formation was induced on colchicine-treated lymphocytes with fl-Con A. The lymphocytes were then stained with TMR-anti-Ig in the presence of valinomycin. a, Phase-contrast images of two lymphocytes; b, labeling with fl-Con A; c, subsequent labeling with TMR-anti-Ig.

raphy using fetuin-Sepharose columns (Sela and Edelman, manuscript in preparation). Goat antiserum to β_2-microglobulin was a gift from Dr. I. Berggård (University of Lund, Sweden). Conventional combinations of excitation filters, dichroic mirrors, and barrier filters were used for observations of fluorescein fluorescence and tetramethyl rhodamine fluorescence with a Zeiss universal microscope.

Co-Capping Experiments. The lymphocytes were treated with 10^{-4} M colchicine and then incubated with fl-Con A or TMR-Con A at 37° for 15 min in PBS-BSA. After washing to remove unbound Con A, valinomycin was added to the medium at a final concentration of 10^{-6} M in order to inhibit cap formation irreversibly without killing the cells. The cells were then incubated at 21° for 20 min with antibodies or lectins labeled with a fluorochrome different from that coupled to Con A. For each experiment, about 30 to 60 cells showing Con A caps were examined for the distribution of antibodies or lectins labeled with the second fluorochrome. The reverse co-capping experiments were carried out by incubating cells first with labeled antibodies or lectins and subsequently labeling the cells in the presence of valinomycin with Con A linked to the appropriate fluorochrome.

Con A-Platelets. Fresh human blood platelets were obtained from the New York Blood Center (New York, N.Y.). The platelets were washed three times with PBS containing 1 mM ethylenediamine tetraacetic acid and were then treated with 1% glutaraldehyde in PBS at 20° for 30 min. Glycine was added to give a final concentration of 20 mg/ml. The suspension was stirred well until the glycine was completely dissolved and it was then incubated at 20° for 15 min. After centrifugation of the platelets, this incubation was repeated once with a fresh solution of glycine. The glutaraldehyde-treated platelets were washed thoroughly with PBS and incubated with 200 μg/ml of Con A at 20° for 30 min. Unbound Con A was removed by washing with PBS and large aggregates of platelets were removed by differential centrifugation. The glutaraldehyde-treated platelets showed yellow to red autofluorescence that disturbed the observations in the fluorescence microscope, particularly when tetramethyl rhodamine was used. Using ^{125}I-labeled Con A (specific activity 4.3×10^6 cpm/mg), it was determined that on the average each glutaraldehyde-treated platelet bound 4×10^5 tetrameric Con A molecules. 2×10^8 Con A platelets labeled with ^{125}I and suspended in 1 ml of PBS-BSA released 5% of their total radioactivity into the medium during incubation at 37° for 1 hr. The released radioactivity corresponded to 0.7 μg of Con A, too low an amount of free Con A to account for the effects of Con A platelets on lymphocytes (2, 3).

TABLE 1. *Co-capping of various receptors induced by Con A in colchicine-treated lymphocytes*

Inducer of capping	Reagents used to stain lymphocytes	% Cells showing co-capping
fl-Con A	TMR-Con A	100
fl-Con A	TMR-anti-Ig	70 ± 12
fl-Con A	TMR-anti μ	75
fl-Con A	TMR-anti γ	80 ± 20
fl-Con A	DNP-BGG + TMR-anti-DNP*	97 ± 4
TMR-Con A	fl-anti-θ†	90 ± 10
TMR-Con A	Goat-anti-rabbit-β_2-microglobulin + fl-anti-goat-Ig‡	79
TMR-Con A	fl-anti-H-2^d§	100
TMR-Con A	fl-white pea lectin¶	78
TMR-Con A	fl-small California white bean lectin	70
TMR-Con A	fl-Idaho red bean lectin	80
TMR-Con A	fl-wax bean agglutinin	Partial co-capping
TMR-Con A	fl-limulus hemagglutinin	Partial co-capping
TMR-Con A	fl-carbohydrate specific antibodies	Partial co-capping
TMR-Con A	fl-wheat germ agglutinin	5 ± 5

The concentrations of fl-Con A and TMR-Con A were 200 μg/ml. The concentrations of other reagents were those which saturated the respective receptors on the cells. Where available, the experimental data are expressed as % of cells showing co-capping ± one standard error of the mean. For partial co-capping, not all of the receptors detected during the second staining were coincident with the Con A caps, indicating that some receptors were not co-capped by Con A.

* DNP-BGG was found to bind to all B-cells. Cellular receptors for DNP-BGG appear to be Fc-receptors (Yahara and Edelman, unpublished results).

† Both T-lymphocytes and thymocytes of Balb/c mice showed similar results.

‡ Goat-anti-rabbit β_2-microglobulin anti-sera and fl-rabbit anti-goat Ig were used for staining of mouse cells. Anti-rabbit β_2-microglobulin is assumed to cross-react with mouse β_2-microglobulin.

§ DBA/2J or Balb/c mice were used in this study.

¶ These lectins, agglutinins, or antibodies were isolated using affinity chromatography on Sepharose-fetuin columns (Sela and Edelman, unpublished observations).

Con A-Latex Beads. Monodisperse polystyrene latex beads (1.1 μm in diameter) were obtained from Polysciences Inc. (Warrington, Pa.) and were coated with bovine serum albumin (BSA) (10). Adsorbed BSA molecules appear to form a thin film on the surface of beads (10) and the complexes are quite stable in PBS. The BSA-coated latex beads were incubated with 2 mg/ml of Con A in PBS containing 2 mg/ml of 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (Aldrich Chemical Co., Milwaukee, Wis.). Alternatively, BSA-coated latex beads were conjugated with Con A in the presence of 1% glutaraldehyde and 0.1 M α-methyl-D-mannoside in PBS. Both methods resulted in the binding of an average of 1 to 4×10^4 Con A molecules to each bead. The carbodiimide-treated beads released about 10% of the Con A after incubation at 37° for 15 min but under identical conditions glutaraldehyde-treated beads released only 2% of the bound Con A. Thus, at most 1.3 μg of Con A were released into 1.0 ml of PBS from 2×10^9 of the carbodiimide-

treated beads. This amount of free Con A has been shown in previous studies (2, 3) to be too low to inhibit patching and capping.

RESULTS

Co-Capping of Ig and Other Receptors with Con A. Colchicine-treated cells were incubated with various doses of fl-Con A at 37° for 15 min in PBS-BSA and were washed to remove unbound fl-Con A. Aliquots of the cells were then incubated separately with TMR-Con A (200 μg/ml) and TMR anti-Ig (250 μg/ml) in the presence of 10^{-6} M valinomycin at 21° for 15 min. The labeling patterns with TMR-Con A and TMR anti-Ig were then examined on cells showing caps with fl-Con A.

At concentrations of 50 μg/ml or greater, binding of fl-Con A in the presence of colchicine redistributed all of the Con A receptors towards one pole of the cell in 40% of the total population. All of these patterns with TMR-Con A applied after capping overlapped those observed with fl-Con A; in some cells, no staining with TMR-Con A was observed on the surface after forming caps with fl-Con A. The latter finding indicates that most or all of the Con A receptors on these cells were occupied with fl-Con A before addition of TMR-Con A. Addition of fl-Con A at concentrations lower than 10 μg/ml collected a portion of the Con A receptors of individual cells into caps, but the rest of the receptors remained unoccupied and diffusely distributed over the cell surface. Intermediate doses of fl-Con A gave mixtures of the population patterns described above.

In accord with recent observations by Loor (11) and Raff and de Petris (12), addition of TMR-anti-Ig to cells that formed caps with fl-Con A produced a high frequency of cells with capped distributions of the anti-Ig stain (Fig. 1). The variation of the staining patterns of the second fluorochrome (TMR) with varying doses of fl-Con A is shown in Fig. 2. Polar staining patterns with TMR-anti-Ig were observed on some of the cells when they were incubated with as little as 10 μg/ml of fl-Con A. Inasmuch as control thymocytes treated with fl-Con A were not stained with TMR-anti-Ig, it does not appear likely that TMR-anti-Ig was bound to Con A-treated cells via already bound Con A molecules. Similar results were obtained when co-capping was examined after capping of Con A following exposure of the cells at 4° (3) in the absence of colchicine. All of these results indicate that, under appropriate conditions, surface Ig can be redistributed with Con A. In contrast to these findings, capping of Ig receptors with anti-Ig did not lead to co-capping of Con A receptors.

Various receptors other than surface Ig were also examined for co-capping induced by Con A. As shown in Table 1, Con A induced co-capping of Fc receptors, H-2 and θ-antigens, β_2-microglobulin and receptors for some lectins, as well as surface Ig, immunoglobulin μ and γ chains. It was found, however, that not all of the receptors for wax bean agglutinin and Limulus hemagglutinin and for carbohydrate-specific antibodies (9) were collected in the caps induced by Con A. Moreover, although Con A strongly inhibited capping induced by the addition of wheat germ agglutinin followed by anti-wheat germ agglutinin, the distribution of wheat germ agglutinin receptors was not affected by Con A capping induced in the presence of colchicine (Table 1).

Binding of Con A-Platelets and Con A-Latex Beads. Con A-platelets bound to mouse splenic lymphocytes suspended in PBS-BSA caused aggregation of the cells. When 2×10^6 lymphocytes were incubated with 8×10^7 Con A-platelets in 0.4 ml of PBS-BSA at 21° for 30 min, more than 80% of the lymphocytes exhibited strong binding to Con A-platelets and about 10% of the cells showed very weak binding. Binding was 95% inhibited by 0.05 M α-methyl-D-mannoside, a known competitive inhibitor of Con A. A quantitative analysis of the binding is given in Table 2. The distribution of Con A-platelets was relatively uniform over the entire cell surface on cells to which more than 10 Con A-platelets were bound (see Fig. 3a and b). No rebinding of released soluble Con A molecules to lymphocytes was detected, as shown by the fact that fl-anti-Con A stained only the Con A-platelets but not the lymphocyte surfaces. The surface areas that were not occupied with Con A-platelets had unoccupied receptors for Con A, for these portions of the cell surface could be strongly stained with fl-Con A.

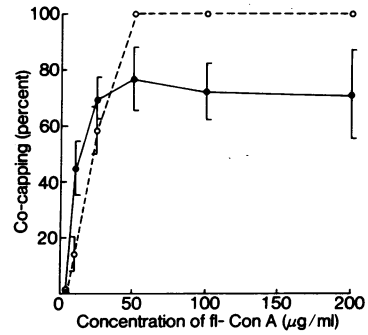

FIG. 2. Co-capping of surface Ig induced with various concentrations of Con A in the presence of colchicine. Colchicine-treated lymphocytes were incubated at 37° for 15 min with fl-Con A at concentrations indicated on the abscissa, washed, and then incubated with TMR-anti-Ig (250 μg/ml) in the presence of valinomycin. In each experiment, about 50 cells showing caps with fl-Con A were examined for staining with TMR-anti-Ig. Ordinate: (●) percentage of stained cells showing co-capping after staining with TMR-anti-Ig. (○) TMR-Con A (200 μg/ml) used instead of TMR-anti-Ig in order to determine co-capping of unoccupied Con A receptors.

Con A-latex beads were also specifically bound to lymphocytes in PBS. The number of Con A-latex beads bound to individual cells varied greatly but the binding patterns of cells with Con A-latex beads were essentially the same as those with Con A-platelets. For most cells, the Con A-latex beads were distributed randomly over the cell surface (Fig. 3e and f).

Inhibition of Patch and Cap Formation by Bound Con A Particles. The effect of binding of Con A-platelets on patch

TABLE 2. *Staining patterns with fl-anti-Ig of cells to which Con A-platelets were bound*

No. platelets per cell (n)*	No. cells	Capped	Not capped	Not stained	Undetermined†
$n > 10$‡	156	7	62	38	49
$10 > n > 5$	25	6	7	12	0
$n < 5$	19	9	6	4	0

* Number of platelets refers to platelets visible at one orientation and may represent an underestimate.

† Autofluorescence of Con A-platelets disturbed identification.

‡ Including aggregated cells with Con A-platelets.

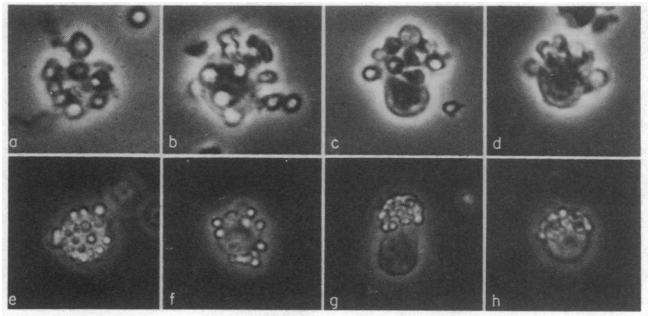

FIG. 3. Random distribution of bound Con A-platelets (a,b) and Con A-latex beads (e,f) on untreated cells. Polar distributions of Con A-platelets (c,d) and Con A-latex beads (g,h) observed when colchicine-treated lymphocytes were incubated with these particles.

and cap formation induced by fl-anti-Ig was examined in detail. Lymphocytes preincubated with Con A-platelets under the conditions described above were incubated with fl-anti-Ig (100 μg/ml) at 37° for 15 min and the frequency of patch and cap formation was determined. Among 156 cells to which more than 10 Con A-platelets were bound, only seven cells showed cap formation with fl-anti-Ig (Table 2), whereas, cell populations that were not treated with Con A-platelets showed high percentages of cap formation (Table 3). Lymphocytes that bound small numbers of Con A-platelets exhibited relatively high percentages of cap formation (Table 2); this contrasts strongly with the inhibition of cap formation induced by fl-anti-Ig on cells to which a large number ($n > 10$) of Con A-platelets were bound. A similar inhibition of patch and cap formation by Con A-platelets was observed for redistributions of H-2 antigens and Fc receptors.

When Con A-platelets and fl-anti-Ig were added simultaneously to the cells, no inhibition of patch and cap formation by Ig receptors was observed. A precise quantitative estimation of the redistribution of surface Ig could not be determined on about 30% of B lymphocytes to which very large numbers of Con A-platelets were bound because in some cases the autofluorescence of bound Con A-platelets interfered with the distinction between patches, caps, and a diffuse distribution of fl-anti-Ig. Capping induced with anti-Ig was inhibited on cells that bound Con A-latex beads, and in this case, the estimation was also somewhat biased by a small amount of fluorescent antibody that was adsorbed onto the beads. As shown in Table 3, the efficacy of inhibition was somewhat greater than that achieved by binding the cells to a Con A-derivatized nylon fiber (8); this may result from the fact that the Con A-particles form contacts with multiple points on the cell surface.

Redistribution of Con A-Platelets and Latex Beads on Colchicine-Treated Cells. In contrast to the random distribution of Con A-platelets on normal lymphocyte surfaces, colchicine-treated cells showed polar-distributions of Con A-platelets on their surfaces (Fig. 3c and d). This polar-distribution was not observed when colchicine-treated cells were incubated with the Con A-platelets in the presence of 10^{-6} valinomycin. The presence of 0.05 M NaN_3 also strongly reduced the number of cells showing polar distributions of bound Con A-platelets. Removing the NaN_3 from the medium increased the number of cells showing polar distributions but did not reduce the number of bound Con A-platelets. These results indicate that Con A-platelets were initially bound randomly to cells and were then redistributed into caps on metabolically active, colchicine-treated cells.

TABLE 3. *Inhibition of patching and capping by locally bound Con A*

Treatment	% Caps on normal B-cells	% Caps on colchicine-treated B-cells
fl-anti-Ig	90	95
Soluble Con A + fl-anti-Ig	4	40
Con A fiber + fl-anti-Ig*	30	70
Con A platelets + fl-anti-Ig	10	54
Con A latex particles + fl-anti-Ig	16	63

* From data in ref. 8 and unpublished observations (Yahara, Rutishauser, and Edelman).

Colchicine treatment of lymphocytes with bound Con A-latex beads also reversed their inhibitory effect on receptor mobility without reducing the number of Con A-latex beads bound to the cells (Fig. 3g and h). The number of cells showing polar-distributions of Con A-latex beads was greatly increased by colchicine treatment. It was found that a greater surface area was covered by Con A-latex beads in polar distributions than that covered by Con A-platelets in similar distributions. Even after colchicine treatment, few pear-shaped or hand mirror-shaped cells were observed among lymphocytes that bound Con A-latex beads.

Failure of Redistributed Con A Particles to Induce Co-Capping. Splenic lymphocytes from Balb/c mice were incubated with Con A-platelets in the presence of colchicine and the distributions of Ig, μ chains, H-2, and Fc receptors were determined on those cells showing polar distributions of Con A-platelets. Although the binding of Con A-platelets resulted in inhibition of the mobility of these receptors, it was observed that redistribution of Con A-platelets after colchicine treatment failed to co-cap the other receptors. The area of the cell surface that was packed with Con A-platelets showed autofluorescence as described above and, therefore, the staining patterns of this area could not be determined. Nevertheless, the remainder of the cell surface showed staining for Ig, μ chains, H-2, and Fc receptors when tested with the appropriate specific fluorescent reagents. This failure to induce co-capping is in sharp contrast to the results observed with free Con A.

DISCUSSION

These experiments were designed to examine the possibility that inhibition of receptor mobility by Con A results from simple cross-linkage of receptors to immobile Con A receptors. This possibility is strongly raised by the present observations that the capping of Con A receptors at 21° in the presence of colchicine is accompanied by co-capping of a variety of other receptors.

Three lines of evidence developed here tend to rule out this explanation and, therefore, tend to support our original hypothesis that modulation results indirectly from alterations in microtubule-microfilament assemblies (7). The most powerful argument in support of this hypothesis is that local attachment of cells to Con A-platelets and Con A-latex beads, or to

Con A-derivatized nylon fibers (8), inhibits receptor mobility despite the fact that the majority of Con A receptors and receptors of other specificities do not interact with these specifically derivatized solid-phase surfaces. Furthermore, the induction of capping of these bound particles in the presence of colchicine failed to induce co-capping of other receptors including the majority of Con A receptors. A second but weaker argument against direct cross-linkage as an explanation of mobility restriction is that this restriction can occur effectively at doses of Con A (2, 3) lower than those required to co-cap most other receptors. Finally, it should be noted that the mobility of receptors for wheat germ agglutinin was restricted by the binding of free Con A but these receptors were not co-capped with Con A receptors after addition of colchicine.

Fluorescence microscopy revealed that the major portion of the Con A receptors was not redistributed upon attachment of Con A-particles. Only after treatment with colchicine were the platelets or latex beads redistributed; this is in agreement with previous results using free Con A. In contrast to these results with free Con A, however, most of the Con A receptors remained diffusely distributed after "capping" of the particles. When colchicine-treated cells were incubated first with Con A-platelets and subsequently with fl-anti-Ig, the direction of capping of the Con A-platelets and anti-Ig was the same. If fl-anti-Ig was omitted, however, the surface Ig molecules remained diffusely distributed, whereas the Con A platelets assumed a polar distribution. It is interesting to note that this coincidence in the location of Con A molecules and surface-Ig-anti-Ig complexes on colchicine-treated cells was not observed when Con A fibers were used (8). In this case, the direction of cap formation induced by anti-Ig was random in relation to the region of contact of the cell with the fiber. In contrast to observations with Con A fibers, but in agreement with the present results, Loor has reported that cap formation induced by anti-Ig on cells bound to Con A-Sepharose beads was always directly towards the proximal region of contact of the cells with the beads (11). It appears that, in the presence of colchicine, cells attached to stationary, relatively massive surfaces such as Con A fibers do not rotate or slide to allow coincidence of caps formed by various receptors with the point of attachment to the fiber.

The finding that Con A capping can induce co-capping of Ig (11, 12) and of a variety of other receptors as shown here remains to be fully explained. One possibility is that multivalent Con A can cross-link various other receptors by attaching to their carbohydrate portions. It is noteworthy that although Con A does interact *in vitro* with mouse IgM and mouse H-2 antigens (Henning, Cunningham, and Edelman, unpublished observations), it does not bind to mouse IgG (Yahara and Edelman, unpublished observations). Other receptors, such as Fc receptors and θ antigen have not been investigated in this regard. At 20 μg/ml where co-capping occurs efficiently, an average of 6×10^5 molecules of Con A are bound per cell and this would be sufficient to "sample" a major subpopulation of the other receptors which occur at a density of about 10^5 receptors per cell. Moreover, electron microscopic studies of ferritin-labeled Con A indicate that at the equivalent of 20 μg/ml of active Con A, a major fraction of the cell surface would be covered by ferritin-Con A molecules (6).

An alternative explanation of co-capping is that binding of Con A followed by capping in the presence of colchicine "entrains" single receptors into the forming cap in the absence of external cross-linkage. One test to discriminate between external cross-linkage and entrainment is to check for "co-patching" of Con A and univalent Fab fragments of anti-Ig in cells that have been treated with NaN_3 and are therefore unable to cap. "Co-patching" would definitely favor the notion that external cross-linkage via Con A is the origin of co-capping. In any case, the results of the co-capping experiments reported here are in marked contrast to those (13, 14) obtained with other ligands such as anti-Ig, all of which induce independent capping of their corresponding receptors.

Whatever the explanation of co-capping, the results obtained here strongly favor the involvement of a cytoskeletal structure that functions to modulate receptor mobility (6, 7). Perhaps the most striking feature of the present experiments is the indication that modulation by such an assembly can be induced by local binding of Con A. The postulated alteration in the state of this assembly would obviously have to be global despite the fact that the original signal induced by binding is local. This suggests either that the alteration involves large, propagated, cooperative transitions in various components of the assembly, or that catalytic processes are involved. At present, we favor a cooperative mechanism. An investigation of the rates and metabolic requirements of this propagated modulation of the cell surface should clarify this issue, which has a variety of implications for our understanding of the control of cell movement, of surface-to-cytoplasmic signaling, and of cell growth.

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