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Model for capping derived from inhibition of surface receptor capping by free fatty acids

(membrane structure/membrane–cytoskeleton interactions/membrane-bound calcium)

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ABSTRACT **When low concentrations (2–5 mole %) of *cis* unsaturated free fatty acids (group A) are intercalated into lymphocyte plasma membrane, capping is inhibited. No effect is seen with *trans* unsaturated or saturated fatty acids (group B). The capping inhibition is reversible with increasing doses of extracellular calcium. Fluorescence photobleaching recovery has shown that the group A free fatty acids do not inhibit the receptor immobilization associated with patch formation, but inhibit the final energy-dependent movement of the patched receptors into a cap. We have also shown that the group A free fatty acids cause a shift in membrane-bound calcium to the lipid phase from probable protein-associated sites. We have incorporated these findings into a model for capping and membrane–cytoskeletal interactions.**

Proteins are capable of lateral movement in the plane of the membrane (1). Particular attention has been paid to the movement and internalization of surface receptors (2, 3). This process has been most extensively studied in lymphocytes where a series of surface typography changes accompanies the crosslinking of surface receptors by ligands (4). Here the crosslinked receptors form aggregates called patches, which then coalesce to form a polar cap (5). The final cap formation requires metabolic energy and involves cytoskeletal elements (6–8). However, the molecular details of this process are not understood. Aside from questions concerning molecular mechanisms, it is still unclear what, if any, is the physiologic function of the aggregation and internalization process.

We have presented data on the interaction of free fatty acids (FFA) with biological membranes (9). Our major conclusions were that FFA could be divided into two groups: group A, with *cis* double bonds, and group B, with *trans* unsaturated or no double bonds. We showed that group A FFA partition preferentially into fluid-phase lipid whereas group B FFA preferentially partition into gel-phase lipid. Using this information we were able to obtain evidence for structurally distinct lipid domains in plasma membranes. In this paper we show how, based on these observations, FFA can be used to perturb membrane lipid structure and block capping of surface immunoglobulin (SIg) on lymphocytes. From the results of this study, we propose a molecular model of capping and the interaction of surface receptors with the cytoskeleton.

MATERIALS AND METHODS

Cells and Capping. All cells were obtained fresh by mechanical disruption of AJ mouse spleens. Erythrocytes were destroyed by hyposmotic lysis; lymphocytes were maintained in Hanks' buffered salt solution, which contained 1.2 mM Ca^{2+}. Cells were incubated for 20 min at 4°C and labeled with 100 μg of fluorescein-conjugated rabbit anti-mouse gamma globulin per ml (Cappel, Cochranville, PA) at 4°C for 20 min. The cells were washed once at 4°C and allowed to cap at 22°C. After 30 min, the cells were fixed by addition of 2% formaldehyde and examined with a Leitz Ortholux fluorescence microscope.

Cell membranes were isolated by a modification of the technique of Lemmonier *et al.* (10). According to this, the cells were lysed by 25 sec of sonication with the probe of a Braunsonic sonicator set at 20 W. This resulted in greater than 99% cell lysis with minimal nuclear lysis. The membranes were prepared in 10 mM Tris·HCl/120 mM NaCl/0.2% Na azide/3 mM ethylene glycolbis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetate (EGTA) (pH 7.4).

Free Fatty Acids. All FFA were obtained from Sigma and were maintained in stock solutions in ethanol. Dilutions were made into cell suspensions while mixing on a Vortex to give final concentrations of FFA of 10–20 μg/ml and ethanol concentrations of 0.1–0.5% when the cell concentrations were between 5 and 10×10^6/ml. Cells were incubated with the FFA for 20–60 min at room temperature and washed once before any measurements were made. Control cells were treated with equivalent amounts of ethanol.

Fluorescence Studies. 8-Anilino-1-naphthalene sulfonate (ANS) was obtained from Sigma, made up fresh each day in distilled water at 0.1 mM, and protected from light. Suspensions of lymphocytes at 1×10^7 cells per ml were labeled with ≈1 μM ANS to give a membrane phospholipid:probe ratio of ≈50:1. Polarization measurements were made with an SLM model 4800 fluorescent lifetime machine; readings were corrected for scatter by measuring the polarization of the cells without ANS.

Chlortetracycline (Sigma) was made up fresh in ethanol at 50 mM concentrations. This was diluted 1:1000 into cell or membrane suspensions, which were incubated for 1 hr at 37°C with the probe and then washed once with buffer. Spectra were recorded with a Perkin-Elmer MVF-2a fluorometer with λ_{exc} = 380 nm.

Fluorescence Photobleaching Recovery. After incubation with the fatty acids and washing, the spleen cells were resuspended in 100 μl of phosphate-buffered saline and incubated with 5 μl of rhodamine-conjugated goat anti-mouse IgG or the Fab fragment for 10 min on ice. Cells were then washed and suspended in 20 μl of phosphate-buffered saline, and 1 μl was deposited on a microscope slide. The cells were allowed to settle onto the slide, and fluorescence photobleaching recovery measurements were performed as described elsewhere (ref. 11 and unpublished data). Briefly, a 530.9-nm laser beam from

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Abbreviations: FFA, free fatty acids; SIg, surface immunoglobulin; EGTA, ethylene glycolbis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetate; ANS, 8-anilino-1-naphthalene sulfonate.

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a krypton ion laser was focused down to a small (0.60 μm radius) spot on the cell surface through the vertical illumination system of a Zeiss universal microscope. By use of a highly attentuated beam, the fluorescence from the illuminated spot was monitored. The beam intensity was then increased 500-fold for 50–100 msec, irreversibly bleaching a fraction of the fluorophore in the illuminated region. The beam was then attenuated again and the reduced fluorescence intensity from the bleached spot was recorded. If the fluoroescent label was free to move in the plane of the membrane, the fluorescence signal recovered as unbleached fluorophore moved into the illuminated region. The rate and extent of recovery of fluorescence permit calculation of a diffusion coefficient and mobile fraction of the fluorescence probe (refs. 11 and 12; unpublished data).

RESULTS

Capping Inhibition by FFA. Incubation of murine splenic lymphocytes with linoleic acid inhibits capping of SIg in a dose-dependent manner (Fig. 1*A*). At the concentrations shown, the linoleate did not alter cell viability as measured by trypan blue exclusion. Similar experiments with other FFA at similar doses showed that oleic and arachidonic acids also were able to inhibit capping, whereas stearic, elaidic, and nonadecanoic acids had no inhibitory effect (Fig. 1*B*). Studies using ^{14}C-labeled fatty acids showed no difference in the binding of any of the fatty acids to cell membranes. The inhibition of capping could not be prevented by addition of 1 mM α-tocopherol or 0.1 mM butylated hydroxytoluene, suggesting that photooxidative products of the fatty acids were not responsible for the capping inhibition. Also, addition of 2 μM indomethacin did not alter capping inhibition, suggesting that the cyclooxygenase pathway of prostaglandin synthesis is not involved. We have established, under the conditions of short-term exposure of the cells and membranes to FFA in our experiments, that greater than 90% of the FFA remain unesterified and in the plasma membrane (9).

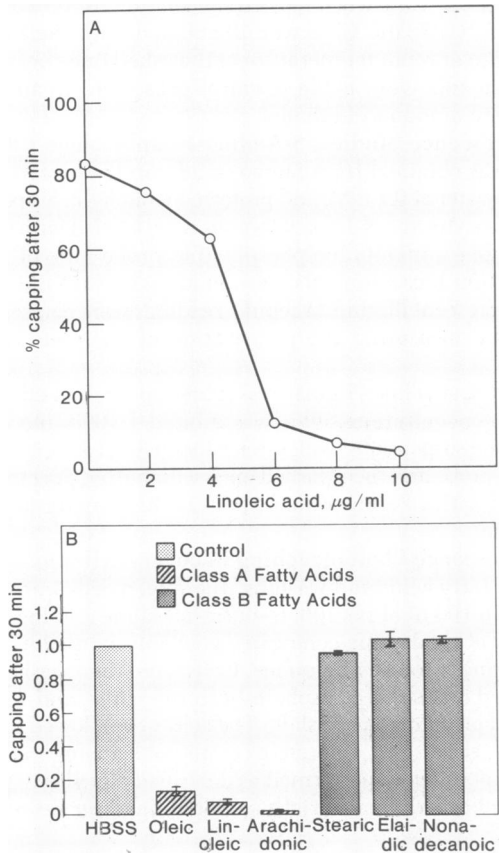

FIG. 1. (*A*) Inhibition of capping of SIg by addition of increasing doses of linoleic acid to suspensions of 8×10^6 cells per ml at 22°C. (*B*) Inhibition of SIg capping by different FFA; 10μg of each FFA per ml was added to 8×10^6 cells per ml at 22°C. Shown is the fraction of cells capped relative to control cells in Hanks' solution (HBSS); SEM is given.

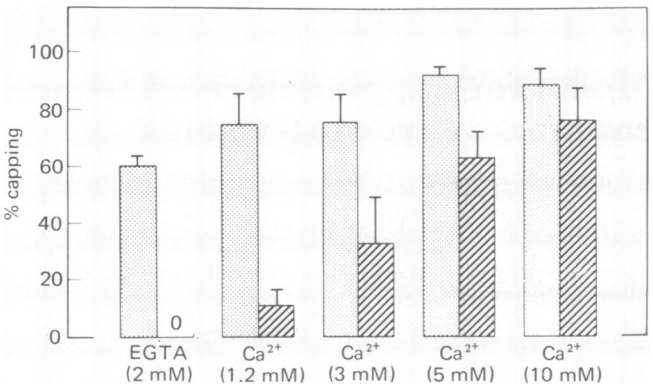

FIG. 2. Reversal of SIg capping inhibition by increasing doses of external Ca^{2+}. SEM are shown. ▣, Control; ▨, linoleic acid (10 μg/ml).

The inhibition of capping by the *cis* unsaturated fatty acids could be reversed in a dose-dependent fashion by addition of extracellular calcium (Fig. 2). Extracellular magnesium had no effect. These levels of extracellular calcium had no effect upon the cell surface binding of ^{14}C-labeled fatty acids.

The structural effects of these FFA upon cell membranes have been studied in this laboratory by fluorescent probe techniques (9). We used two probes, diphenylhexatriene and ANS, which were chosen because they are located at different depths in the bilayer: ANS is at the polar–nonpolar interface and diphenylhexatriene is localized to the acyl chain interior (13, 14). All of the FFA studied for their effect upon capping were also examined for their ability to alter the fluorescence polarization of these two probes in biological membranes. Fluorescence polarization reflects the environmental constraints upon the probe's rotation and thus reflects the structural packing of the lipids around the fluorophore. The results are shown in Table 1. On the basis of these structural changes, we divided the fatty acids into two classes: group A (*cis* unsaturated), which decreased diphenylhexatriene polarization and increased ANS polarization, and group B, which had no effect on diphenylhexatriene polarization and also increased ANS polarization. These changes were seen at the same concentrations examined in the capping studies. We have also shown that

Table 1. Classification of FFA

Group	Δ Polarization: Diphenylhexatriene	Δ Polarization: ANS	Partition into
A: Oleic	↓	↑	Fluid
Linoleic	↓	↑	Fluid
Arachidonic	↓	↑	Fluid
B: Elaidic	—	↑	Gel
Stearic	—	↑	Gel
Nonadecanoic	—	↑	Gel

Fluorescence polarization was measured on an SLM lifetime machine modified with excitation and emission polarizers. All measurements were corrected for scatter. Partition of FFA was determined by analyzing the direction of change of the endothermic phase transition in dimyristoyllecithin bilayers. Lowered transitions signify preferential partition into the fluid phase; raised transitions indicate preferential partition into the gel phase. Complete data are given in ref. 12.

the ANS polarization changes reflect lipid, and not protein, structural perturbations (9).

We then attempted to correlate these lipid structural changes with the capping inhibition. Fig. 3 shows the ability of Ca^{2+} to reverse the increase in ANS polarization caused by the fatty acid. This reversal followed the same Ca^{2+} dose dependency as the reversal of capping inhibition. Magnesium had no effect upon ANS polarization. We were concerned that the Ca^{2+} was interacting directly with the negatively charged probe; divalent cations can increase the binding of ANS to lipid vesicles, resulting in increased fluorescence intensity (15). However, we saw a progressive decrease in fluorescence intensity with increasing external Ca^{2+} consistent with the decreased quantum yield of ANS expected as a result of decreased environmental constraints (16).

The decreases in diphenylhexatriene polarization caused by group A FFA were not reversed by Ca^{2+}. Chlorpromazine *increases* both diphenylhexatriene and ANS polarization in lymphocyte membranes and, at the drug doses that alter the polarization, chlorpromazine inhibits capping (17). Calcium, but not magnesium, reverses both the ANS polarization change and the inhibition of capping by chlorpromazine but does not affect diphenylhexatriene polarization. Thus, those agents that inhibit capping increase ANS polarization and are uncorrelated with diphenylhexatriene polarization.

The problem with attempting to correlate increased lipid head-group packing with capping inhibition is that both inhibitory and noninhibitory FFA affect ANS polarization similarly. Our previous work has shown that these two classes of fatty acids partition into structurally different lipid regions (9). Group A FFA preferentially partition into more fluid lipid domains whereas group B FFA show a marked partition preference for more rigid lipid regions. Thus, the identical alterations in head-group packing may well be occurring in different regions of the membrane (see *Discussion*).

Fluorescence Photobleaching Recovery. Because capping involves the movement of receptors laterally in the membrane, we studied whether the presence of FFA altered the motion of SIg under crosslinking conditions that normally induce capping. The lateral diffusion of SIg in the lymphocyte plasma membrane has been studied under capping and noncapping conditions by the technique of fluorescence photobleaching recovery (refs. 11 and 12; unpublished data). The ability of SIg to diffuse when crosslinked with different concentrations of rhodamine-conjugated anti-Ig (in the presence of sodium azide to inhibit capping) was compared with the capping frequency (without azide). In general, anti-Ig concentrations sufficient to induce capping also completely immobilized SIg with respect to diffusion. When these measurements were performed after treatment with FFA at anti-Ig concentrations sufficient to cap most of the SIg-bearing cells, we likewise found the crosslinked receptors to be immobilized (Table 2). Thus, despite the inhibition of cap formation by group A FFA, these FFA do not hinder the immobilization of crosslinked receptors. Therefore, group A FFA inhibit the final, energy-dependent aspect of capping that aggregates the immobilized receptors into a polar cap.

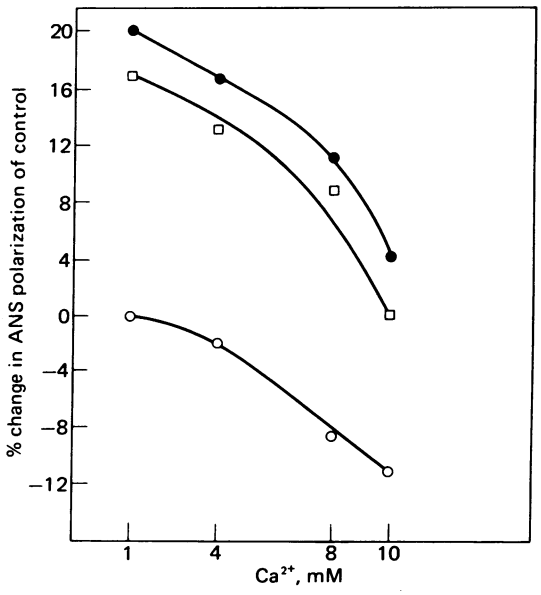

FIG. 3. Reversal of FFA-induced ANS polarization changes by Ca^{2+}. Aliquots of 500 mM $CaCl_2$ were added and the polarization was recorded. FFA (10 μg of each per ml) were added to 8×10^6 cells per ml. ○, Control; ●, linoleic acid; □, arachidonic acid.

FFA and Membrane Calcium. Although Ca^{2+} reversed the head-group structural changes reported by ANS, we felt that the reversal of capping inhibition with Ca^{2+} might reflect compensation for a perturbation of membrane Ca^{2+} homeostasis. For this reason we examined two aspects of the effects of FFA upon Ca^{2+} physiology: transmembrane Ca^{2+} flux and intramembrane Ca^{2+} binding. We found that the inhibitory FFA caused a mild increase in $^{45}Ca^{2+}$ uptake by splenic lymphocytes. This increase over control cells or cells treated with group B FFA was not reversed by raising the concentration of external Ca^{2+}. Thus, unlike capping inhibition, whatever the alteration in the cell membrane responsible for the increased Ca^{2+} flux, it was not reversed by external Ca^{2+}.

Membrane-bound Ca^{2+} was measured with chlortetracycline. This probe markedly increases its quantum efficiency in a hydrophobic environment when it forms a Ca^{2+} chelate (18). It is sensitive to phospholipid-associated Ca^{2+} but does not seem to chelate Ca^{2+} that is more tightly bound to membrane protein (19). Labeling of lymphocyte membranes with this probe and then treatment with FFA showed a 4-fold increase in chlortetracycline intensity only with group A. After preincubation of the cells with 5 mM EGTA to remove external Ca^{2+}, group A FFA still produced a 2-fold dose-dependent increase in membrane lipid Ca^{2+}, with no change occurring with group B FFA. In order to determine whether the increase in chlortetracycline intensity was due to enhanced binding of Ca^{2+} from the external medium, we looked at phosphatidylcholine and phosphatidylserine vesicles. Labeling these vesicles with chlortetracycline and FFA in Ca^{2+}-free buffer with the subsequent addition of external Ca^{2+} showed no increase in chlortetracycline intensity due to the presence of the negatively

Table 2. Effect of FFA on immobilization of SIg by anti-Ig*

FFA†	Conc., μg/ml	No. of cells	Mean mobile fraction (±SD)
None‡	—	12	0.03 ± 0.05
C-19‡	20	7	0.02 ± 0.03
Elaidic‡	20	5	0.04 ± 0.03
Linoleic	5	7	0.02 ± 0.03
Arachidonic	2	7	0.04 ± 0.07
None§	—	9	0.73 ± 0.17

Fluorescence photobleaching recovery of rhodamine-conjugated anti-IgG showing that group A FFA do not inhibit the immobilization of crosslinked receptors.

* Cells (100 μl at 2–4 × 10^4/ml) were incubated for 10 min on ice with 5 μl of rhodamine-conjugated goat anti-mouse IgG.

† Ten-minute incubations at room temperature in 1 ml of phosphate-buffered saline with 2–4 × 10^6 cells.

‡ Measurements and labeling were done in the presence of 3 mM NaN_3 to inhibit capping.

§ SIg was labeled with the rhodamine-conjugated Fab fragment of anti-Ig.

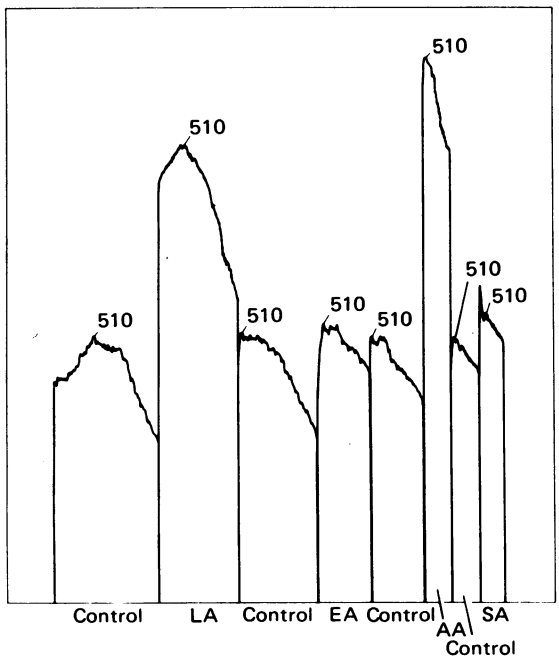

FIG. 4. Chlortetracycline fluorescence in isolated lymphocyte plasma membranes. FFA were added at 10 mole % of membrane phospholipid. LA, linoleic; EA, elaidic; AA, arachidonic; SA, stearic acid. See text for further details.

charged fatty acids in the vesicles. Finally, we looked at chlortetracycline fluorescence in isolated lymphocyte plasma membranes. These membranes were prepared in Ca^{2+}-free buffer and in the presence of 3 mM EGTA. Fig. 4 shows the effect of different FFA upon the chlortetracycline signal from these membranes. Significant increases in fluorescence intensity are seen with the group A but not with the group B FFA. Because there was no free external Ca^{2+} in this experiment, the increased chlortetracycline fluorescence must be due to shifts in intramembrane Ca^{2+} from sites inaccessible to chlortetracycline into the lipid phase (20). We interpret this to mean that the group A FFA cause a membrane protein to release Ca^{2+}, allowing the Ca^{2+} to be sensed by the lipid-bound chlortetracycline.

DISCUSSION

The movement and internalization of surface receptors are being recognized as general cell membrane events; the physiologic role remains unknown. These studies with FFA demonstrate that lipid-perturbing molecules can modulate these membrane phenomena. One of the advantages of such molecules over drugs such as Na azide is that they are not general metabolic inhibitors. Rather, we believe the fatty acids exert their effect by altering bilayer structure. Such a view supports the notion that the structure of the lipid is important in membrane function.

The particular structural change associated with capping inhibition is a difficult issue. We feel that the altered head-group packing that is monitored by ANS polarization is a good candidate largely because of the specific reversal of both the head-group disturbance and the capping inhibition by Ca^{2+}. The problem is that both the inhibitory and noninhibitory FFA affect head-group packing similarly. In another study we have documented the existence of significant lipid-site heterogeneity (which is not detectable in single-phase lipid vesicles) in plasma membranes and vesicles containing separate phase domains (9). The existence of separate, structurally distinct lipid domains was documented by the finding of heterogeneity in the nano-

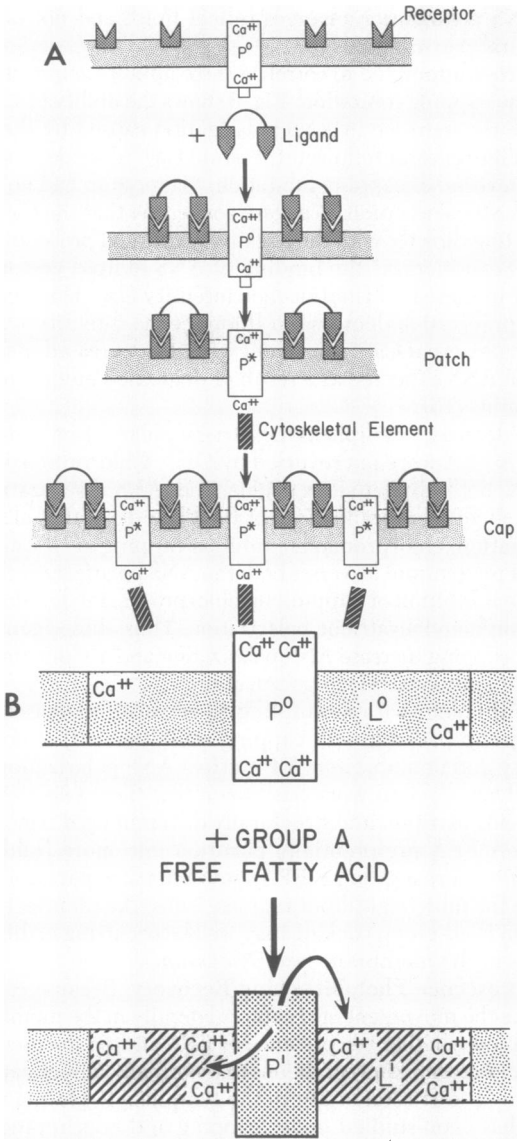

FIG. 5. (*A*) Model of receptor capping. In addition to free receptors, the membrane contains a Ca^{2+}-binding protein that is anchored to the cytoskeleton. Binding divalent ligand to the receptors initiates an association with this protein accompanied by a conformation change that makes calcium available locally to the cytoskeleton. The activated cytoskeleton then moves the receptors into a cap. (*B*) Mechanisms of inhibition of capping by group A FFA. We propose that the Ca^{2+}-binding anchoring protein sits in fluid lipid domains. Only the group A FFA have access to these. By disrupting head-group packing, the FFA cause a conformational relaxation of the protein which results in a release of its Ca^{2+}. This prevents or disrupts (or both) the association of the anchoring protein with the cytoskeleton and inhibits cap formation.

second decay of diphenylhexatriene fluorescence whether in isolated plasma membranes or mixed-phase vesicles. This heterogeneity does not exist in any single-phase phospholipid vesicle. From our previous studies with FFA we have demonstrated that the group A FFA preferentially partition into more fluid regions whereas the group B acids preferentially partition into more rigid domains. Therefore, even if the different FFA all alter head-group packing similarly, there is evidence that they exert their effect in different lipid regions (21, 22).

In attempting to interpret our results in terms of specific molecular events involved in capping, it is first useful to consider the sequential stages in the capping process. SIg is largely free to diffuse in the plane of the membrane. Crosslinking by

antibodies to the receptor causes it to aggregate into patches which can then be pulled into a cap in an energy-dependent step involving the cytoskeleton. Fluorescence photobleaching recovery measurements have shown that the receptor is immobilized under crosslinking conditions sufficient to induce capping (unpublished data). A possible interpretation of this result is that immobilization is due to binding of the receptor–ligand complex to an immobile anchoring moiety in the membrane, analogous to the X protein proposed by Bourguignon and Singer (23). There is, however, no information concerning the nature of the signal that triggers the cytoskeleton to move the patched receptors into a cap.

The role of the cytoskeleton in capping could be disturbed either by altering the spatial arrangement of the cytoskeleton, such as its linkage to the membrane, or by directly inhibiting its contractile capacity. We favor the former mechanism of action of these FFA for several reasons. (*i*) We have shown that after brief incubations, the FFA are virtually entirely associated with cell membranes (9); (*ii*) lymphocytes grown in the presence of group A FFA at concentrations used in this study can proliferate in short-term culture, suggesting that these FFA do not globally inhibit cytoskeletal function; and (*iii*) recent studies with K. Fujiwara using immunofluorescence staining have shown that the group A (but not the group B) FFA induce a marked reorganization of the lymphocyte cytoskeleton, especially with respect to the distribution of cytoskeletal elements along the plasma membrane (unpublished data).

The actual mechanism whereby the group A FFA disrupt membrane–cytoskeleton interactions is not obvious. One clue to this may be the specific reversal of the capping inhibition by extracellular calcium. Presumably, the calcium either reverses the actual inhibition by the FFA or bypasses the FFA effect. We have no evidence for the latter, but calcium is known to be able to significantly perturb bilayer lipid structure (24, 25). Furthermore, our results suggest that Ca^{2+} reverses the altered head-group packing induced by the FFA in the lymphocyte plasma membrane. The fact that both the structural reversal and the reversal of capping inhibition exhibit the same dose responses and are insensitive to magnesium supports the association of the two effects. Even if the local perturbation of membrane lipid structure is implicated in the disruption of membrane–cytoskeleton interactions, the question remains as to how this actually occurs. The chlortetracycline experiments clearly show that the group A FFA enhance the availability of Ca^{2+} to chlortetracycline in the plasma membrane. Thus, group A FFA may perturb the Ca^{2+}-sequestering sites sufficiently to allow access to chlortetracycline.

We propose a model for SIg capping consistent with all of the above results (Fig. 5). According to our proposed mechanism, the anchoring protein is either itself able to bind Ca^{2+} or is associated with a membrane Ca^{2+}-binding moiety. Furthermore, this anchoring protein is surrounded by a relatively fluid lipid domain into which group A, but not group B, FFA partition. When membrane receptors are crosslinked, they bind to the anchoring protein, thereby becoming immobilized patches. The signal to the cytoskeleton initiating contraction, and consequently capping of the aggregated receptors, is then transduced via the binding protein. Ca^{2+} is required for this step, either bound to the protein or as a mediator of a specific interaction with the cytoskeleton, triggering contraction. Group A FFA inhibit capping by altering the lipid packing surrounding the Ca^{2+}-binding protein. This perturbs its conformation sufficiently to cause it to release its bound Ca^{2+} and interrupt the interaction with the cytoskeleton. The result of this is that the capping process is frozen at the stage of patch formation and neither cap formation nor receptor internalization occurs. The reversal of capping inhibition by Ca^{2+} can be explained by the reversal of the altered head-group packing which, in turn, allows the anchoring protein to return to its Ca^{2+}-binding state.

Although this proposed mechanism for the capping process is consistent with all the data presented above, it is not a unique explanation. Nevertheless, we feel it is a useful speculative hypothesis that can be tested by further experimentation. In this regard, we have been examining the binding of Ca^{2+} to membrane proteins by examining the fluorescence energy transfer between membrane proteins and the fluorescent Ca^{2+} analog, Terbium (studies done in collaboration with A. Kleinfeld and B. Pjura). Preliminary results show that the introduction of a group A FFA into isolated lymphocyte plasma membranes indeed leads to an alteration of tryptophan–Terbium energy transfer. Finally, membrane perturbation with FFA may prove to be a useful tool for the experimental control of surface receptor aggregation and internalization.

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1. Frye, L. D. & Edidin, M. (1970) *J. Cell Sci.* **7**, 319–330.
2. Unanue, E. R., Perkinds, W. D. & Karnovsky, M. J. (1972) *J. Exp. Med.* **136**, 885–906.
3. Schlessinger, J., Schechter, Y., Cuatrecasas, P., Willingham, M. C. & Pastan, I. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5353–5357.
4. Schreiner, G. F. & Unanue, E. R. (1976) *Adv. Immunol.* **24**, 38–135.
5. Taylor, R. B., Duffus, N. P. H., Raff, M. C. & dePetris, S. (1971) *Nature (London) New Biol.* **233**, 225–229.
6. Loor, F., Forni, L. & Pernis, B. (1972) *Eur. J. Immunol.* **2**, 203–212.
7. dePetris, S. (1975) *J. Cell Biol.* **65**, 123–146.
8. Unanue, E. R. & Karnovsky, M. J. (1974) *J. Exp. Med.* **140**, 1207–1220.
9. Klausner, R. D., Kleinfeld, A., Hoover, R. & Karnovsky, M. J. (1980) *J. Biol. Chem.*, in press.
10. Lemonnier, F., Mescher, M., Sherman, L. & Burakoff, S. (1978) *J. Immunol.* **120**, 1114–1120.
11. Schlessinger, J., Axelrod, D., Koppel, D. W., Webb, W. W. & Elson, E. L. (1977) *Science* **195**, 307–309.
12. Axelrod, D., Koppel, D. E., Schlessinger, J., Elson, E. L. & Webb, W. W. (1976) *Biophys. J.* **16**, 1055–1069.
13. Podo, F. & Blaise, J. K. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1032–1036.
14. Stubbs, G. W., Litman, B. J. & Barenhenholz, Y. (1972) *Biochemistry* **15**, 2766–2772.
15. Haynes, D. H. & Chiu, V. C. K. (1978) *Ann. N.Y. Acad. Sci.* **307**, 217–220.
16. Radda, D. K. (1975) in *Methods in Membrane Biology*, ed. Korn, E. D. (Plenum, New York), Vol. 4, pp. 97–188.
17. Schreiner, G. F. & Unanue, E. R. (1976) *Clin. Immunol. Immunopathol.* **6**, 264–269.
18. Caswell, A. H. (1972) *J. Membr. Biol.* **7**, 345–364.
19. Schneider, A. S., Herz, R. & Sonenberg, M. (1978) *Biophys. J.* **21**, 120.
20. Hemminki, K. (1974) *Biochim. Biophys. Acta* **363**, 202–210.
21. Sklar, L. A., Hudson, B. S. & Simoni, R. D. (1977) *Biochemistry* **16**, 819–928.
22. Pringle, M. J. & Miller, K. W. (1979) *Biochemistry* **18**, 3314–3320.
23. Bourguignon, L. Y. W. & Singer, J. J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5031–5035.
24. Triplett, R. B., Wingate, J. M. & Carraway, K. L. (1972) *Biochem. Biophys. Res. Commun.* **49**, 1014–1020.
25. Newton, C., Pangborn, W., Nir, S. & Papahadjapoulos, D. (1978) *Biochim. Biophys. Acta* **506**, 281–287.