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Receptor Mobility and Receptor–Cytoplasmic Interactions in Lymphocytes

(cell surface/lymphocyte receptor mobility/lectins/concanavalin A)

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ABSTRACT **An analysis of the inhibition by concanavalin A of the mobility of lymphocyte surface receptors is used to construct an hypothesis on membrane receptor–cytoplasmic interactions. It is proposed that binding of multivalent lectins alters the interaction of an assembly of colchicine-binding proteins with lectin receptors and other receptors, and reciprocally that the state of the colchicine-binding assembly alters the mobility and distribution of surface receptors on the cell membrane. Observations of the effect of colchicine and related drugs on the inhibition of receptor mobility by concanavalin A lend support to this hypothesis. The proposed model has several implications for studies of the initial events of mitogenesis in lymphocytes as well as for cell–cell interactions in general.**

One of the most important problems in the field of cell surface biochemistry is to determine how interactions of cell surface receptors with various ligands are linked to the metabolic machinery of the cytoplasm and nucleus. A description of this transduction mechanism is essential to an understanding of the differentiation and function of cells such as those of the immune system. Although lymphoid cells have several special properties related to clonal selection by antigens during the immune response, they are likely to share fundamental mechanisms of growth control with other specialized tissues. Moreover, lymphocytes are particularly suitable for studying surface interactions and growth control because they are readily available as dissociated cells, because they produce gene products of known structure as well as several other specific surface markers, and because they can be stimulated from the resting state by various mitogenic agents that bind to the cell surface.

Several recent findings (1–3) suggest that the distribution and mobility of immunoglobulin receptors and other surface receptors of lymphocytes may be under the control of specific structures in the cell. Experiments on the mobility and restriction of mobility of the surface receptors provide strong clues to the nature of these structures and their possible function in receptor anchorage at the cell surface. The purpose of this note is to correlate evidence from diverse sources in terms of an hypothesis on the interaction of lymphocyte receptors and cytoplasmic structures. Although based largely on experimental findings obtained with lymphocytes, the hypothesis has general implications for interactions of receptors in other eukaryotic cells.

Abbreviations: Con A, concanavalin A; PHA, phytohemagglutinin from *Phaseolus vulgaris*; CBP, colchicine-binding proteins.

RECEPTOR SOCIOLOGY

Lectins are particularly useful tools for the exploration of the group behavior and interactions of cell surface receptors. A clear-cut example is the use of these proteins to study mitogenesis. There is evidence to suggest that mitogenic lectins act directly at the cell surface, inasmuch as covalent coupling of concanavalin A (Con A) and phytohemagglutinin (PHA) at solid surfaces does not abolish their ability to stimulate cells (4, 5). Several lectins are not mitogenic, however, suggesting that only certain of the glycoprotein receptors on the lymphocyte surface are responsive to stimulation. Of a given population of receptors responsive to a mitogenic lectin such as Con A, as few as 6% have to be bound to induce transformation (6). One of the simplest assumptions concerning the initial step of lymphocyte stimulation is that the receptors are directly linked to structures within the cells. In any case, the mobility and attachment of the receptors must be considered in determining how the effects of their interactions might be transmitted to the interior of the cell.

Recent findings indicate that surface antigens are mobile (7) and that immunoglobulins and other receptors on the lymphocyte may be cross-linked by specific divalent antibodies and redistributed to form patches and ultimately, caps at one pole of the cell (2). Taylor *et al.* (2) have proposed that the patches are formed by diffusion but that cap formation from patches depends upon cell movement and metabolism. In addition, de Petris and Raff (8) have suggested that cell movement leading to cap formation may be under the control of structures sensitive to cytochalasin B and, therefore, that surface receptors may interact with cytoplasmic structures such as microfilaments. Experiments on the immobilization of cell receptors by binding of the mitogenic lectin Con A to be reviewed here indicate, however, that another system must interact with the receptors and modulate their anchorage, movement, and interactions before cap formation.

IMMOBILIZATION OF CELL SURFACE RECEPTORS BY CON A

Native Con A has a striking effect on the ability of the cell receptors to form patches and caps (1). If Con A is added in doses greater than 5 μg/ml to mouse splenic lymphocytes before treatment with rabbit antibodies directed against mouse immunoglobulin, both patch and cap formation of immunoglobulin receptors and Con A receptors are inhibited (1, 9) (Fig. 1). This effect may be reversed by addition of α-methyl-D-mannoside, a competitive inhibitor of Con A binding. Similar inhibitory effects are seen for capping of

θ antigens on T (thymus-derived) cells by anti-θ antibodies (I. Yahara and G. M. Edelman, manuscript in preparation). In contrast, if Con A is added to the cells at 4°, excess Con A is washed away, and the cells are brought to 37°, the bound Con A forms patches and caps with its own receptors (10). Native tetravalent Con A, therefore, has two antagonistic actions that depend upon temperature and lectin concentration. These observations imply that the mobility of cell surface receptors, including the Con A receptors themselves, is inhibited by binding a critical number of molecules of tetravalent Con A.

The chemical and three-dimensional structure of Con A has recently been determined (11), and various derivatives of the lectin with altered biological activities can be prepared. When Con A is succinylated, it dissociates from a tetramer to a dimer without alteration of its binding specificities for carbohydrates (9). Although succinyl-Con A is as mitogenic as native Con A, binding of the same number of divalent succinyl-Con A molecules at any temperature neither inhibits the mobility of the receptors nor forms caps. Equally significant is the observation that addition of antibodies against Con A to cells that have bound succinyl-Con A restores both of these activities, suggesting that the critical factors are the valence of the bound lectin and the formation of clusters of receptors (9) (Fig. 1).

From these observations, we can conclude that tetravalent Con A leads to restriction of the mobility of several receptors including its own, that this restriction is reversible, and that it depends upon the valence of the molecule. There are three mechanisms that might explain these phenomena: (*i*) the binding of a relatively small number of Con A molecules leads to a phase transition in the lipids of the membrane decreasing its fluidity and increasing its transition temperature; (*ii*) the binding of Con A leads to the formation of structures that trap other receptors or prevent their movement; (*iii*) the binding of Con A alters structures in the cell that are attached to receptors, and this binding occurs in such a way as to restrict receptor movement.

The first possibility does not seem likely, for 50% of the cells show inhibition of patching when an average of no more than 1.5×10^5 molecules are bound per cell, occupying less than 1% of the cell surface. In any case, this possibility can be tested by measurement of the relaxation time of spin labels (12) in the presence and absence of Con A. The second possibility also appears somewhat unlikely. It has been noted that binding of Con A leads to a redistribution of lectin receptors (10) as well as of intramembranous particles (13) but, given the size of the clusters formed and their number at the effective doses, it does not seem probable that they could alter the movement of the various other cell receptors. Although such clusters might "trap" other receptors, there is no evidence that they could do so efficiently or in fact, that they do so at all. For example, although Con A binds mouse IgM in free solution by binding to its carbohydrate, preliminary experiments (I. Yahara and G. M. Edelman, unpublished observations) provide no evidence that it does so on the cell surface. It seems most likely that Con A binding modifies a common cytoplasmic structure to which some of the cell surface receptors, and possibly the membrane itself, are attached.

In accord with this suggestion, we have recently found that colchicine, colcemid, vinblastine, and vincristine will partially reverse the effect of Con A on receptor mobility and thus permit the formation of Con A caps and anti-immunoglobulin caps (Fig. 1; Table 1). Colchicine does not bind to Con A nor does it cause disaggregation of its subunits. Furthermore, colchicine does not inhibit Con A–saccharide interaction or the cell-binding activity of Con A. Although it remains to

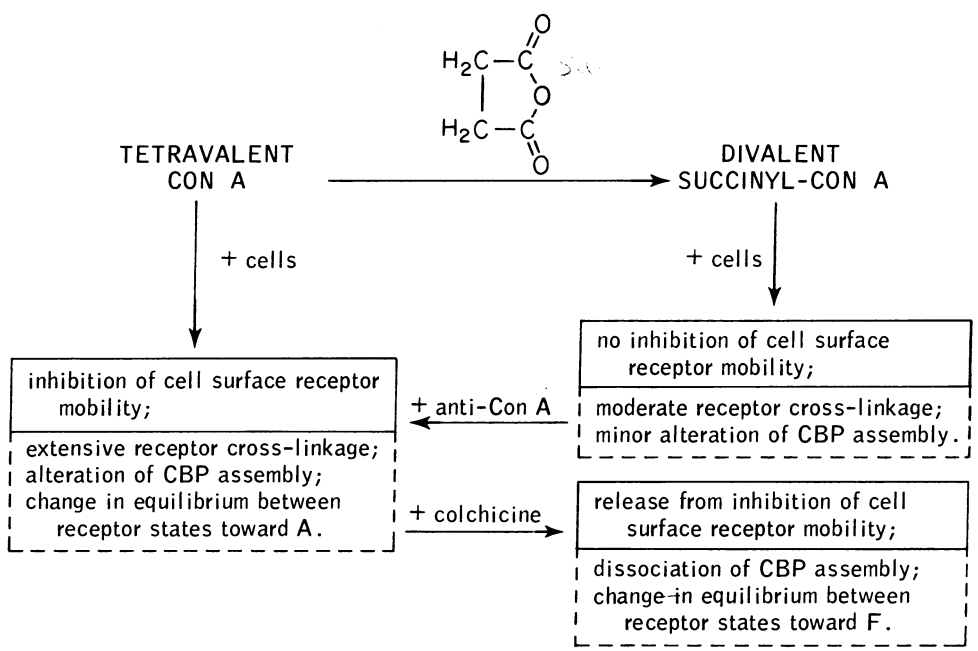

FIG. 1. Summary of the effects of binding Con A or succinyl-Con A on mouse splenic lymphocytes. The experimentally observed effect on restriction of receptor mobility is boxed by *solid lines*. The hypothesized changes in the colchicine-binding protein assembly (*CBP*) and the shift in equilibrium of receptor states between *A* (anchored to the *CBP*) and *F* (free from the *CBP*) are boxed by the *dotted lines*. The addition of anti-Con A to succinyl-Con A bound on cells mimics the effect of Con A alone. Colchicine reverses the effects of both Con A and succinyl-Con A plus anti-Con A.

TABLE 1. *Effects of various drugs on the inhibition by Con A of cap formation and on the mitogenic activity of Con A for splenic lymphocytes*

Treatment	% Cap-forming cells with fl-anti-Ig*	% Cap-forming cells with fl-anti-Ig + Con A†	% Cap-forming cells with fl-Con A‡	% of optimal mitogenic response§
Control	85	2	2	100
Colchicine¶ (0.1 mM)	87	22	31	14
Colcemid (0.1 mM)	88	25	24	—
Vinblastine¶ (0.1 mM)	91	55	42	2
Vincristine (0.1 mM)	83	15	19	15
Low temperature (4°)‖	88	30	45	—
Cytochalasin B (0.04 mM)	62	—	1	—

* In order to test for cap formation by immunoglobulin receptors, the percent of cap-forming cells obtained with fluorescein-labeled anti-immunoglobulin (fl-anti-Ig; 100 μg/ml) was measured.

† In order to test for the inhibition by Con A of immunoglobulin receptor cap formation, the percent of cap-forming cells obtained with fl-anti-Ig (100 μg/ml) was measured in the presence of Con A (100 μg/ml).

‡ In order to test for cap formation by Con A receptors, the percent of cap-forming cells obtained with fluorescein-labeled Con A (fl-Con A; 100 μg/ml) was measured.

§ Optimal mitogenic response was obtained by culturing mouse spleen cells with 3 μg/ml of Con A as described in ref. 9.

¶ Colchicine and vinblastine did not affect the amount of [^{125}I]Con A bound to splenic lymphocytes.

‖ The amount of [^{125}I]Con A bound to splenic lymphocytes at 4° was 30% of that bound at 37°.

be seen whether colchicine alters the fluidity of the lipid portion of the membrane, these observations suggest an alternative interpretation: Con A binding may affect the association–dissociation equilibrium of cytoplasmic structures, which in turn affect the mobility of cell surface receptors. Microtubular protein (14) appears to be a good candidate for this role because it has been shown to be sensitive to all four of the drugs shown in Table 1, because it has association–dissociation properties, and because it is ubiquitous in the cell. It should be noted, however, that no extensive microtubular structures have been observed directly under the plasma membrane. Nevertheless, it is intriguing to consider the possibility that Con A binding causes alterations in a common protein anchorage to which some of the cell surface receptors are attached and, therefore, that this binding causes a change in the association–dissociation behavior and mobility of the anchoring structure. We have found that cytochalasin B, which affects cap formation but not patch formation (2), has no effect on inhibition of receptor mobility by Con A. Thus, although it is possible that a filament structure other than microtubules may mediate this inhibition or that the colchicine-sensitive structure may be connected to the surface membrane by way of the microfilaments, this structure is not likely to be the same as that mediating cap formation.

A MODEL FOR RECEPTOR–CYTOPLASMIC INTERACTIONS

We propose here a model to account for various observations on the restriction of the mobility of cell surface receptors (Fig. 1; Table 1). This working hypothesis requires that some of the cell surface receptors are anchored on a common assembly that is on, in, or under the lymphocyte plasma membrane. Although the essential features of our model would hold irrespective of the exact location of this assembly, the bulk of evidence suggests that it is cytoplasmic in origin. As we have indicated, our observations implicate a protein that can be altered by colchicine and related drugs; although its identity is unknown, this colchicine-binding protein (CBP) may be related to certain of the actin-like proteins (15) or microtubules (14).

The model incorporates the following assumptions: (*i*) certain surface receptors interact reversibly with colchicine-binding proteins, possibly the microtubular assemblies of the cytoplasm. We define A as the anchored state of the receptors (attached to the CBP) and F as the free state of the receptor (not attached to the CBP); these two states exist in an equilibrium $A \rightleftharpoons F$. Through this anchorage, the distribution of the receptors on the cell surface is affected by the state of the CBP. A similar suggestion has been made by Berlin and Ukena (16, 17), who found that colchicine and vinblastine inhibited the agglutination of fibroblasts and polymorphonuclear leukocytes by Con A. (*ii*) Not only is

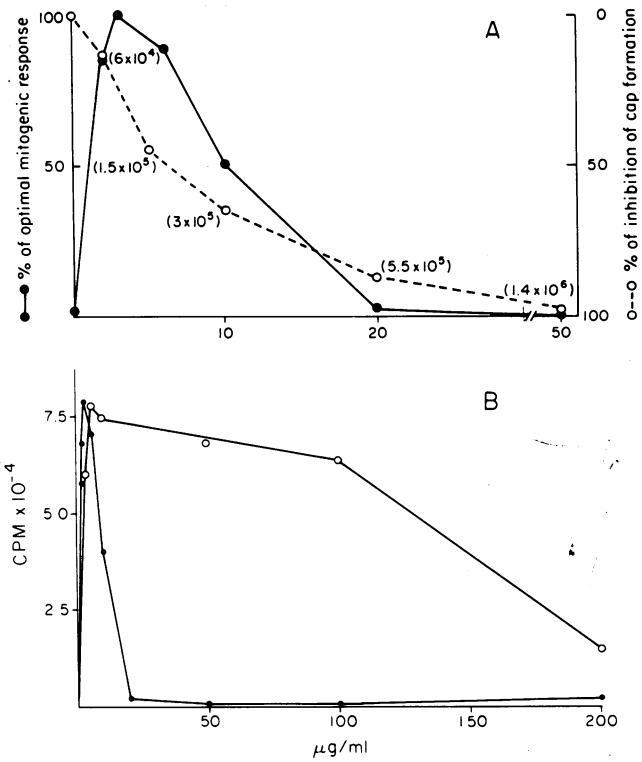

FIG. 2. (*A*) Comparison of the dose–response curve of the inhibition by Con A of anti-immunoglobulin cap formation with the dose–response curve of mitogenic stimulation by Con A of mouse spleen cells. Mitogenesis was measured by the incorporation of [^{3}H]thymidine. Cap formation was measured with fluorescein-labeled anti-immunoglobulin (100 μg/ml). Succinyl-Con A does not inhibit cap formation even at a lectin concentration of 100 μg/ml (9). The number in parentheses denotes the number of Con A subunits bound per cell at 37°. ●——●, Mitogenic stimulation; O- - -O, inhibition of cap formation. (*B*) Dose–response curves of the incorporation of [^{3}H]thymidine in mouse spleen cells stimulated by Con A (●——●) and succinyl-Con A (O——O).

the distribution of the cell receptors affected by the state of the CBP but, conversely, the mobility and state of this assembly are affected by interactions and aggregations of particular receptors. This provides the means by which receptor states can be communicated to the interior of the cell. The valence of external ligands can therefore be a critical factor in cell surface–cytoplasmic interactions. (*iii*) The mobility of the membrane or its receptors is affected by the state of the CBP so that alteration of the CBP by one set of cell surface receptors may affect the movement of the other receptors. (*iv*) Finally, the equilibrium between the two states of the receptors, *A* and *F*, is affected by cholchicine and related agents. Alteration of the equilibrium may occur either because structures such as microtubules are dissociated by these agents (18) or because receptors are released from attachment with the CBP assembly, or both. This implicitly suggests the existence of a means of coupling between certain receptors and an ordered state of the cytoplasmic CBP assembly.

It is important to consider how the cell surface receptors might be linked to this common network. Several findings may be pertinent. First, intramembranous particles of lymphocytes appear to be clustered after binding Con A (13). Second, Con A receptors bound to this lectin also appear in clusters (19). Finally, protein receptors on cell surface molecules may extend through the membrane (20). Together with our assumptions, these observations suggest two possible means of coupling—direct and indirect. Direct coupling can occur either through intramembranous particles or independently of them by extension of the lectin receptors through the membrane to interact noncovalently with proteins such as those of the CBP. Indirect coupling would act strictly via the intramembranous particles, implying that the system depends upon interaction of particles to which receptors are attached with particles to which the CBP is attached. At present, there is no direct evidence to support either of these modes of coupling but they are sufficiently specific to warrant an experimental search. An additional possibility is that lectin binding and receptor aggregation lead to a change in membrane transport or enzymatic activity, which in turn lead to alterations in the mobility of the CBP or its interaction with receptors.

According to our hypothesis, binding of tetravalent Con A at 37° to receptors in the *A* state is assumed to modify the mobility of the CBP assembly, therefore inhibiting gross mobility of the membrane (1). The valence and the formation of clusters of receptors is critical in this effect, for although divalent succinyl-Con A cannot bring it about, subsequent addition of antibodies against Con A to cells with bound succinyl-Con A restores the effect (9) (Fig. 1). It has been shown that microtubules can dissociate at low temperatures (21). Similarly, dissociation of the CBP with production of receptors in the *F* state may account for the finding that Con A can cap its own receptors after binding at 4° (10), for at that temperature it may react mainly with *F* receptors. If reassociation of the CBP assembly occurred more slowly than cap formation after returning to 37°, cap formation would be expected. Cap formation occurs in minutes (1, 2), whereas there is some evidence that microtubular reassembly can take as long as hours (22).

Several observations have been made on the temperature-dependence of Con A activity, and it is tempting to explain these observations in terms of a temperature-dependent dissociation of the CBP assembly. The interpretation is complicated, however, because it must take into account the kinetics of several complex association–dissociation equilibria involving CBP, the receptors, and Con A itself. Sachs and his colleagues (23) have noted that transformed fibroblasts and lymphocytes could be agglutinated by the mitogenic lectins Con A and PHA at 21° and 37° but not at 4°. In contrast, nonmitogenic lectins, such as the agglutinins from soybean and wheat germ, agglutinated cells at all temperatures. These workers attributed the temperature dependence of agglutination by the mitogens to some form of metabolic or enzymatic activity. The data may also be explained in part by CBP dissociation at low temperature (21), a process that might lead to redistribution of Con A and PHA receptors with diminution in their avidity. To be consistent, this interpretation would require that receptors for the nonmitogenic lectins remain unconnected to the CBP assembly.

In addition to exogenous effects such as those of Con A, several surface interactions as well as endogenous changes in the CBP may modulate the mobility of surface receptors and convert them from a relatively stable anchored state to a relatively stable mobile state. It is important to distinguish the proposed equilibrium between the *A* and *F* states of the receptors in a single cell from a population difference in cells that have a particular receptor in one state or the other. Within a given cell population, there may be a wide variety of cells with different distributions of these states. Nevertheless, the CBP may function to maintain certain receptors in stable anchored states throughout a population. In studies of phagocytosis of particles by polymorphonuclear leukocytes, for example, Ukena and Berlin (24) found that receptors mediating transport were not affected by the process of phagocytosis and inferred that the two functions were topographically separate. After treatment of the cells with colchicine, however, phagocytosis led to "internalization" of the transport receptors, possibly as a result of their conversion to the *F* state.

RECEPTOR–CYTOPLASMIC INTERACTIONS AND MITOGENESIS

Although the present model does not explicitly specify the relationship of cell surface receptor–cytoplasmic interactions to the initial events of lectin-induced mitogenesis, several experimental observations suggest that they may be related.

First, there is a strong correlation between the immobilization of the membrane receptors by Con A (Fig. 2) and the dose–response curve of lectin-induced lymphocyte stimulation. At 2–3 μg of Con A per ml, the optimal dose range for mitogenesis, about 80% of the cells showed receptor movement. At higher doses of Con A, however, both mitogenic response and cell surface receptor mobility are inhibited. Furthermore, divalent succinyl-Con A is mitogenic, does not inhibit receptor mobility, and does not induce inhibition of mitogenesis until much higher doses are reached. Inasmuch as binding of succinyl-Con A does not result in capping even after long times of observation, it appears likely that capping is not required for mitogenesis.

There also appears to be a correlation of the mitogenic activity of some lectins with the capacity to immobilize the cell surface receptors. Preliminary experiments show that

several mitogenic lectins (Con A, PHA, lentil and pea lectins, and extracts from fava beans and black turtle beans) tested in our laboratory inhibited cap formation. In contrast, nonmitogenic lectins (wheat-germ agglutinin, extracts of Idaho red beans, small California white beans, and pink beans) did not inhibit cap formation. This finding suggests the possibility that nonmitogenic lectins may attach to receptors that are not directly connected with the CBP system.

Finally, preliminary experiments have shown that the mitogenic activity of Con A is inhibited by drugs such as colchicine, vinblastine, and vincristine (Table 1), at concentrations as low as 1 μM. This effect is not attributable to inhibition of DNA synthesis, for we have found that thymidine incorporation can take place in the presence of the drugs.

A simple interpretation consistent with these observations is that mitogenic stimulation involves the formation of micropatches containing relatively mobile receptors in reversible equilibrium with the CBP assembly. The formation of these micropatches may lead to alterations in the CBP and initiate the various metabolic events in stimulation. At higher concentrations, Con A may inhibit the mitogenic response by extensive alteration of the CBP, instantaneously "freezing" the cell surface receptors, and preventing the formation of micropatches; eventually, this state would lead to cell death. A divalent lectin such as succinyl-Con A cannot immobilize the receptors and is therefore mitogenic at high concentrations without being toxic (Fig. 2). Colchicine may inhibit mitogenesis by affecting the postulated CBP assembly in such a way that, even if micropatches are formed, they cannot alter cytoplasmic function via this assembly.

Whether or not the hypothesis proposed here can be directly connected to mitogenesis, it would, if validated, have several implications for our understanding of specific cell–cell interactions, contact inhibition, and cellular motility. Although explicitly formulated in terms of lymphocytes, it may apply to various other cellular systems.

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