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CONCANAVALIN A-INDUCED MODIFICATION OF THE ELECTROPHORETIC MOBILITY OF LYMPHOCYTES

EFFECT OF COLCHICINE AND CYTOCHALASIN B

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Summary

Concanavalin A induces redistribution, i.e. 'capping' and endocytosis of its receptors, on the surface of lymphocytes. This phenomenon has been shown to alter the cellular surface charge density. Colchicine, which depolymerises cytoplasmic microtubules, and cytochalasin B, which inactivates microfilaments, were used to determine the transmembrane control of the changes in the electrophoretic mobility of mouse lymphocytes. Colchicine neither inhibited nor modified the lectin-induced changes, irrespective of whether the cells were exposed to this agent before or after the interaction with concanavalin A. Preincubation with cytochalasin B, on the other hand, inhibited the alterations induced by concanavalin A. Cytochalasin B also reversed the altered electrophoretic mobilities of concanavalin A-treated lymphocytes. These data provide further confirmation of the relationship between lectin-induced redistribution and the electrokinetic behaviour of lymphocytes and establish that 'capping' but not endocytosis of the glycoprotein receptors is responsible for the electrophoretic changes.

Introduction

The possible existence of molecular interactions between cell-surface components and intracellular mechanochemical proteins (cytoskeleton) in principle

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provides a mechanism by which certain types of extracellular signal can be transmitted to the interior of the cell. The redistribution of the surface receptors to multivalent ligands such as mitogenic lectins and anti-immunoglobulin antibody, in the case of lymphocytes, is considered as an initial step in the overall triggering process. Microtubules and microfilaments are implicated in the modulation of the mobility of surface components (reviewed in Refs. 1 and 2). This is indicated by the observations that colchicine, an alkaloid which depolymerises microtubules, and cytochalasin B, which inactivates microfilaments, enhance [1—4] and inhibit [1,2,5—8] the 'capping' of the receptors to concanavalin A and anti-immunoglobulin antibody on the surface of lymphocytes. The cells showing patched and 'capped' distribution of certain receptor have also been observed to display similar distribution of the cytoskeletal proteins such as actin and tubulin [9—11]. The demonstrations of direct associations between these proteins with the mouse histo-compatibility complex antigens (H-2) and cross-linked surface immunoglobulin on lymphocytes [12, 13] further support a role for these structures as 'connecting link' in lymphocyte activation.

Using a simple physicochemical technique of cell electrophoresis we have demonstrated that the interaction of concanavalin A with the splenic lymphocytes of AKR mice leads to biphasic changes in the electrophoretic mobility [14,15]. Existence of two behaviourally distinct sets of receptors for the lectin was thus established [14—16]. Furthermore, electrophoretic mobility may serve as a parameter for the assessment of ligand-induced redistribution. It remained to be seen, however, whether the changes in the surface change densities were also under the transmembrane control of microtubules and microfilaments. Since either 'capping' or endocytosis of the lectin-receptor complexes could have singly or together caused the alterations in the electrokinetic profiles of mouse lymphocytes, inhibitors of microtubule and microfilament function were employed to evaluate the independent contribution of these events ('capping' and endocytosis). Confirmation of these effects, if any, was obtained by fluorescence microscopy using rhodamine-labelled concanavalin A.

Materials and Methods

Suspensions of lymphocytes from spleens of healthy AKR mice were prepared in a standard saline solution (0.146 M NaCl/0.01 M KCl, pH 7.2) as described earlier [14,15]. Concanavalin A was prepared according to the methods of Agrawal and Goldstein [17] and a stock solution (100 μg/ml) was made in saline. Colchicine (Calbiochem, La Jolla, CA, U.S.A.) was used as inhibitor of microtubular activity while cytochalasin B (Aldrich Chemical Company, U.S.A.) was utilized for inactivation of microfilaments. The latter was first dissolved in a small volume of dimethyl sulfoxide and diluted with saline. Tetramethyl rhodamine isothiocyanate labelled concanavalin A ($A_{280nm}/A_{515nm} = 1.2$) was prepared according to the procedure described by de Petris [8] and used for fluorescence microscopy.

Splenic lymphocytes were treated with concanavalin A at 37°C for 1 h. Incubation with either colchicine ($1 \cdot 10^{-5}$ M) or cytochalasin B (10 μg/ml) for 2 h at 37°C was carried out either before or after the interaction with concanavalin

A. Electrophoretic mobilities were determined in standard saline at 25°C, employing a Carl Zeiss cytopherometer. The mobility of human erythrocytes was always measured as an electrophoretic standard.

Results and Discussion

The mean electrophoretic mobility of splenic lymphocytes of AKR mice was 1.19 ± 0.20 (S.D.) $\mu s^{-1} \cdot V^{-1} \cdot cm$. Incubation of these cells with colchicine or cytochalasin B or dimethyl sulfoxide did not alter this mobility ($P > 0.5$; Table I). This showed that these materials per se did not change the surface change density either by adherence or by interacting with the cytoskeletal elements of these cells. Bhisey et al. [18] have reported significant increase in the mobility of mouse peritoneal macrophages after treatment with colchicine ($1 \cdot 10^{-4}$ M). This could be due to a different cell type and high concentration of the alkaloid used in that study. In the present study a few experiments using colchicine at the concentration of $1 \cdot 10^{-4}$ M indicated microscopically discernible damage to the cell membrane.

When lymphocytes were incubated with a low concentration of concanavalin A (5 μg/ml) at 37°C for 1 h their mean mobility was significantly enhanced to −1.29 ± 0.17 (S.D.) $\mu s^{-1} \cdot V^{-1} \cdot cm$ ($P < 0.005$; Table I). Pretreatment of these cells with colchicine failed to inhibit the increase in electrophoretic mobility resulting from incubation with the lectin ($P > 0.5$; Table I). Likewise, colchicine did not prevent the subsequent reduction in the mobility of the lymphocytes caused by incubation with high (50 μg/ml) concentration of concanavalin A ($P > 0.5$; Table I).

When the cells were exposed to colchicine after treatment with either low or high concentration of concanavalin A, the alterations induced by the lectin

TABLE I

EFFECT OF PRETREATMENT WITH CYTOSKELETAL INHIBITORS ON CONCANAVALIN A-INDUCED CHANGES IN THE ELECTROPHORETIC MOBILITY OF SPLENIC LYMPHOCYTES

$5 \cdot 10^6$ lymphocytes/ml were incubated with colchicine ($1 \cdot 10^{-5}$ M) or cytochalasin B (10 μg/ml) or dimethyl sulfoxide (1 : 300, v/v) in saline for 2 h at 37°C. Incubation with concanavalin A was done at 37°C for 1 h in the presence of colchicine or cytochalasin B. Electrophoretic mobilities were measured in a Carl-Zeiss cytopherometer at 25°C using human red cells as standard. The values represent the mean mobility and standard deviation of the number of observations indicated in the parantheses, which were obtained from replicate experiments in each of which the mobilities of at least 20 cells were measured. Statistical analyses were carried out employing Student's *t*-test and *P* values for comparative treatments are mentioned in the text at appropriate places.

Pre-treatment lymphocytes	Mean mobility ± S.D. ($\mu sec^{-1} \cdot V^{-1} \cdot cm$) after subsequent treatment with		
	None	Low concn. of concanavalin A (5 μg/ml)	High concn. of concanavalin A (50 μg/ml)
1 None	−1.19 ± 0.20 (255)	−1.29 ± 0.17 (177)	−1.09 ± 0.17 (179)
2 Colchicine	−1.19 ± 0.21 (90)	−1.28 ± 0.22 (63)	−1.05 ± 0.17 (66)
3 Dimethyl sulfoxide	−1.18 ± 0.23 (90)	—	—
4 Cytochalasin B	−1.21 ± 0.18 (84)	−1.18 ± 0.17 (83)	−1.19 ± 0.17 (63)

TABLE II

EFFECT OF CYTOSKELETAL INHIBITORS OF THE ELECTROPHORETIC MOBILITIES OF CONCANAVALIN A-TREATED LYMPHOCYTES

$5 \cdot 10^6$ cells/ml were first treated with concanavalin A at 37°C for 1 h and after washing these cells were incubated with colchicine or cytochalasin B as indicated. Measurements of electrophoretic mobilities were made at 25°C in saline containing the same inhibitor as used for incubation following the treatment with concanavalin A. The values represent mean ± S.D. of the number of observations indicated in the parentheses, which were obtained from replicate experiments in each of which the mobilities of at least 20 cells were determined. Statistical analysis were carried out employing Student's *t*-test and *P* values for comparative treatments are mentioned in the text at appropriate places.

Pre-treatment of lymphocytes	Mean mobility ± S.D. ($\mu s^{-1} \cdot V^{-1} \cdot cm$) after subsequent treatment with:		
	None	Colchicine (10^{-5} M)	Cytochalasin B (10 μg/ml)
1 None	−1.19 ± 0.20 (255)	−1.19 ± 0.21 (90)	−1.21 ± 0.18 (84
2 At low concentration of concanavalin A (5 μg/ml)	−1.29 ± 0.17 (177)	−1.27 ± 0.22 (48)	−1.19 ± 0.19 (81)
3 At high concentration of concanavalin A (50 μg/ml)	−1.09 ± 0.17 (179)	−1.07 ± 0.17 (46)	−1.18 ± 0.16 (86)

remained unmodified by the microtubule depolymerising agent ($P > 0.5$; Table II). This observation was substantiated by the fluorescence microscopic data presented in Fig. 1. 34% of the lymphocytes underwent redistribution on treatment with rhodamine-labelled concanavalin A. If the cells were pretreated with colchicine this figure was 32%, whilst 37% of the lectin-treated cells still

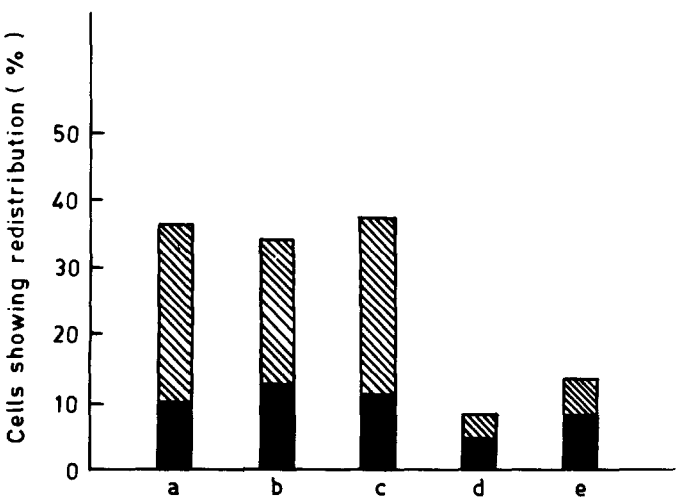

Fig. 1. 10^7 cells/ml in phosphate-buffered saline (pH 7.2) were incubated with 100 μg of the rhodamine-labelled concanavalin A at 0—4°C for 1 h, washed in the cold and then incubated further at 37°C for 1 h. They were fixed in 4% formaldehyde for 30 min and examined under a Zeiss epi-illumination microscope equipped with 50 W mercury lamp and filters optimized for rhodamine fluorescence. At least 25 fields and 200 cells were examined for every treatment. The bars show the proportion of cells showing redistribution: ▨ capping ■ endocytosis. a, Lymphocytes treated with concanavalin A alone; b, cells treated with colchicine before incubation with the fluorescent lectin; c, cells treated with colchicine after concanavalin A; d, cells treated with cytochalasin B before concanavalin A; and e, cells treated with cytochalasin B after incubation with concanavalin A.

showed redistribution, even after subsequent incubation with colchicine (Fig. 1 a—c). There was no appreciable change in the proportions of cells showing endocytosis during these experiments (Fig. 1). The colchicine neither inhibited nor enhanced concanavalin A-induced redistribution of surface receptors.

Colchicine is known to disorganise the microtubules [19]. The role of microtubules vis-à-vis 'capping' of the receptors for concanavalin A is controversial. Colchicine can inhibit the 'capping' process only in a synergistic manner with cytochalasin B [1,6,8]. However, there is considerable evidence to suggest a hindering effect of microtubules on capping [2,4,7]. Edelman and co-workers have observed that high concentrations of concanavalin A inhibit 'capping' of its own receptor on mouse spleen cells. Pretreatment of these cells with colchicine, however, abolished this inhibition, resulting in increased proportion of cells showing 'caps' [2—4]. In AKR mice, we have failed to see inhibition of redistribution by high concentrations of the lectin [14,15]. It appears, therefore, that a microtubule dependent mechanism is not primarily responsible for the displacement of the receptors for concanavalin A on the surface of AKR lymphocytes which also leads to a change in their surface charge density.

Interestingly, the effect of cytochalasin B on this system was remarkable. Prior treatment of the splenic lymphocytes with cytochalasin B completely inhibited the increase in the electrophoretic mobility of these cells induced by a low concentration of concanavalin A ($P < 0.005$; Table I). Likewise, the reduction in the mobility caused by a high concentration of the lectin was also inhibited by cytochalasin B ($P < 0.005$; Table I). The fluorescence microscopic evidence was also in agreement with this. Only 9% of these cells showed redistribution of the receptors bound to rhodamine-labelled concanavalin A when the cells were pre-incubated with cytochalasin B (Fig. 1d).

The influence of cytochalasin B on the concanavalin A-modulated lymphocyte surface was also striking. When the lectin-treated cells were further incubated with cytochalasin B, the elevated mobility at the low concentration as well as reduction in it at high concentration of the lectin were reversed ($P < 0.005$) thus restoring these cells to the electrophoretic status of the untreated cells (Table II). Moreover, only 14% of the lectin-treated cells showed redistribution after subsequent exposure to cytochalasin B (Fig. 1e). Endocytosis of receptor-ligand complexes was evident in more than half of them (Fig. 1e).

Cytochalasin B inactivates microfilaments [20]. Our data show that the changes in the electrophoretic mobility of AKR lymphocytes following the interaction with concanavalin A are under the control of microfilaments. The redistribution of surface receptors to concanavalin A is likewise dependent on the integrity of these structures, as also observed by others [5—8]. Moreover, it is clear that the cytochalasin B-sensitive structures are responsible for the maintenance of the redistributed structures once formed.

We had attributed the increase in the electrophoretic mobility of splenic lymphocytes of AKR mice at low concentrations of concanavalin A to 'capping' and endocytosis of receptor-ligand complexes and the reduction in the mobility to the post-redistributional binding of excess concanavalin A to newly emerged second set of receptors [14,15]. This has also been substantiated using the lectin preparations with two different fluorescent labels [16]. The separate contributions of 'capping' and endocytosis to the electrokinetic change were,

however, not clear. Since cytochalasin B, which inhibits as well as reverses concanavalin A-induced 'capping' of surface receptors, also inhibited as well as reversed the lectin-mediated alterations in the surface charge density of AKR lymphocytes, the increase in electrophoretic mobility at low concentration of concanavalin A can be attributed mainly to 'capping'. The same process also forms the first step in the ultimate reduction of mobility at high concentrations of the lectin [14,15]. Endocytosis of receptor-ligand complexes is not reversed by cytochalasin B and hence its contribution to the lectin-modulated electrophoretic mobility of these cells does not appear to be significant.

The observation that the incubation of lymphocytes with cytochalasin B following treatment with high concentrations of the lectin restores them to the electrophoretic status of untreated cells may suggest that out of the two types of receptor site for the ligand only the first type of receptor may be directly linked with microfilaments. Such a possibility can perhaps be verified if the cells showing redistribution of the first type of receptor can be isolated from other cells. It may be possible to achieve a charge-dependent separation of these cells. Direct association between constituents of cell surface and cytoskeletal proteins can then possibly be shown by biochemical methods. This will go a long way in the understanding of the transmembrane communication of activation and regulating signals.

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