

RECEPTOR MOBILITY AND THE BINDING OF CELLS TO LECTIN-COATED FIBERS

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ABSTRACT

The ability of cells to bind to nylon fibers coated with lectin molecules interspaced with varying numbers of albumin molecules has been analyzed. The cells used were lymphoma cells, normal lymphocytes, myeloid leukemia cells, and normal and transformed fibroblasts, and the fibers were coated with different densities of concanavalin A or the lectins from soybean or wheat germ. Cells fixed with glutaraldehyde did not bind to lectin-coated fibers. The number of cells bound to fibers could be increased by increasing the density of lectin molecules on the fiber, the density of specific receptors on the cell, or the mobility of the receptors. It is suggested that binding of cells to fibers involves alignment and binding of specific cell surface receptors with lectin molecules immobilized on the fibers, and that this alignment requires short-range rapid lateral mobility (RLM) of the receptors. The titration of cell binding to fibers coated with different densities of lectin and albumin has been used to measure the relative RLM of unoccupied cell surface receptors for the lectin. The results indicate a relationship of RLM to lectin-induced cell-to-cell binding. The RLM of receptors for concanavalin A (Con A) was generally found to be higher than that of receptors for the lectins from wheat germ or soybean. Receptor RLM could be decreased by use of metabolic inhibitors or by lowering the temperature. Receptors for Con A had a lower RLM on normal fibroblasts than on SV40-transformed fibroblasts, and trypsinization of normal fibroblasts increased Con A receptor RLM. Normal lymphocytes, lymphoma cells, and lines of myeloid leukemia cells that can be induced to differentiate had a high receptor RLM, whereas lines of myeloid leukemia cells that could not be induced to differentiate had a low receptor RLM. These results suggest that the RLM of Con A receptors is related to the transformation of fibroblasts and the ability of myeloid leukemia cells to undergo differentiation.

The interaction of lectins with carbohydrate-containing receptors on the surface of cells has been used as a probe to study membrane changes associated with malignancy, mitogenesis, and differentiation (20, 21). Differences in the effects of various lectins on the same cell, or of a particular lectin on different cell types can involve both the number and mobility of lectin-specific receptors.

Movements of receptors on the surface membrane include three types of mobility: large lateral movements as in cap formation, molecular rotation, and small lateral movements. The detection and measurement of these mobilities are of considerable importance in analyzing lectin-cell interactions. Although capping can be observed directly (5, 13, 20, 24) and molecular rotation of receptors can be

measured by fluorescence polarization (10), the existence of short-range lateral movements has been deduced only from their proposed role in ligand-induced receptor clustering (2, 8, 16, 17, 20). Furthermore, in all three cases the detection procedure requires the use of a probe, such as a fluorescent lectin, which may alter the behavior of the receptor itself.

We have previously postulated that lectin-induced binding between cells involves the alignment of complementary receptors, and that this alignment requires mobility of the receptors on at least one of the cells (18, 19). The present studies were undertaken to develop a system in which to titrate the type of mobility required for receptor alignment. These experiments involve the titration of cell binding to nylon fibers (3, 4) coated with lectin molecules interspaced with varying numbers of albumin molecules. The results suggest that this procedure can measure the relative mobility of receptors whose behavior has not been altered by attachment of a specific probe. The nature of this receptor mobility is discussed in relation to lectin-induced cell-to-cell binding and the biological properties of various types of cells.

MATERIALS AND METHODS

Cells

YAC lymphoma cells (L cells)¹ and EL4 lymphoma cells were obtained from a Moloney virus-induced lymphoma grown in A-strain mice and from a lymphoma grown in C57/B1 mice, respectively (11, 23). Both of these lines are thymus-derived cells (7, 23). 10^6 cells were inoculated intraperitoneally into adult mice, and the cells were harvested 10–14 days later by aspiration of the peritoneum with phosphate-buffered saline, pH 7.4 (PBS). Normal lymph node lymphocytes, 70–80% of which are thymus-derived cells, were obtained from CR/RAR rats by teasing the lymph nodes in PBS, and aggregates were removed by low-speed centrifugation.

All other cell types except fibroblasts were grown in suspension cultures. The cultured cells used were from

two independently isolated clones of IR⁻D⁻ myeloid leukemia cells (which will be referred to as M cells) from SJL mice (14), two D myeloid leukemia clones from SL mice which can be partially (clone D₁₆, IR⁺D⁻) or completely (D₂₁, IR⁺D⁺) induced to undergo normal cell differentiation (which will be referred to as D cells) (6, 14), L1210 lymphoma cells (23), SV40-transformed golden hamster fibroblasts, and normal fibroblasts. The normal fibroblasts were obtained from secondary cultures of golden hamster embryos. Cells were cultured in 100-mm plastic Petri dishes in Eagle's medium with a fourfold concentration of amino acids and vitamins (H-21, Grand Island Biological Co., Grand Island, N.Y.) and 10% serum. Horse serum was used for D and M cells, while fetal calf serum was used for L1210 cells and fibroblasts. The cells were subcultured every 4–5 days by seeding 2×10^6 cells (10^6 for L1210 cells) per 10 ml medium. The fibroblasts were dissociated by incubation with 0.02% ethylenediaminetetraacetic acid (EDTA) solution (9) for 15–30 min at 22°C. To prepare trypsinized fibroblasts, the cells were dissociated with 0.25% trypsin (Difco Laboratories, Detroit, Mich., 1:300) for 15 min at 37°C. All cells were washed three times in PBS and then dispersed into a single cell suspension in PBS before use in the cell-to-fiber binding assay.

Lectins

Purified concanavalin A (Con A), soybean agglutinin (SBA), and wheat germ agglutinin (WGA) were obtained from Miles-Yeda, Israel. All lectins appeared homogeneous in polyacrylamide gel electrophoresis at pH 8.6.

Coating of Fibers with Protein

Nylon fibers were strung in polyethylene frames, washed successively with petroleum ether (30–60°C) and carbon tetrachloride, dried, and incubated with the indicated protein solution in PBS for 30 min at 22°C. This procedure resulted in strong adsorption of the protein to the fiber surface, which was stable in PBS for at least 8 h. Protein solutions could be reused several times. Lectin-coated fibers were washed three times in PBS before incubation with cells (3, 4). For routine binding assays, fibers were incubated with lectin alone at 500 µg/ml. To prepare fibers with different densities of adsorbed lectin, a series of protein solutions containing lectin and bovine serum albumin (BSA) (Armour Pharmaceutical Company, Phoenix, Ariz.) were used in which the concentration of lectin was decreased by factors of two from 500 µg/ml to 4 µg/ml and enough BSA was added to obtain a total protein concentration of 500 µg/ml. The density of lectin and/or BSA on the fibers was determined by using ¹²⁵I-labeled proteins and counting the derivatized fibers directly in a gamma counter. In some experiments, bovine hemoglobin (Worthington Biochemical Corp., Freehold, N. J.) was compared with BSA for use as a spacer molecule for the lectin. Similar lectin densities, determined as above, were

¹ Abbreviations used in this paper: BSA, bovine serum albumin; Con A, concanavalin A; D cells, IR⁺D⁻ clone 16 or IR⁺D⁺ clone 21 myeloid leukemia cells; Dnp, 2,4-dinitrophenol; FBC, fiber-bound cells; L cells, YAC lymphoma cells; M cells, IR⁻D⁻ clone 1 myeloid leukemia cells; PBS, phosphate-buffered saline (1,000 ml PBS contains 18 g NaCl, 0.2 g KCl, 0.2 g KH₂PO₄, 1.15 g Na₂HPO₄, 0.1 g CaCl₂·2H₂O, 0.1 g MgCl₂·6H₂O); RLM, short-range rapid lateral mobility of cell surface receptors; SBA, soybean agglutinin; WGA, wheat germ agglutinin.

obtained with both proteins when used at the same weight concentration.

Binding of Cells to Fibers

4 ml of a cell suspension in PBS was incubated with lectin-coated fibers at 22°C for 30 min with gentle shaking as described previously (3, 4). Unbound cells were removed by washing in a series of vessels containing PBS, and the cells attached along both edges of a 1-cm fiber segment were counted *in situ* at a magnification of 100 (3, 4). The standard deviation of four independent cell-to-fiber binding experiments was $\pm 10\%$ over a range from 100 to 800 cells/cm. Below 100 cells/cm, the standard deviation increased up to $\pm 25\%$. Binding of cells to lectin-fibers in the presence of a competitive inhibitor for the lectin was less than 10 cells/cm.

For comparison of cells in their binding to fibers, two types of experimental conditions were used. To measure the binding of cells to fibers coated with lectin alone, each cell type was used at a concentration of 5×10^6 cells/ml or 2.5×10^6 cells/ml and the number of bound cells was counted. This number varied from about 10 to 800 cells/cm. To titrate the ability of a cell to bind to fibers coated with varying ratios of lectin and BSA, the concentration of each cell type was adjusted (from 1.25×10^6 to 2×10^6 cells/ml) so that a fiber coated with lectin alone bound 500–800 cells/cm. This procedure minimized a bias which might result from differences in the density of bound cells. The binding was then evaluated in terms of the percentage of fiber-binding cells (FBC) obtained with various ratios of lectin to BSA on the fiber, relative to the number which bound to a fiber coated with lectin alone.

Bovine hemoglobin was compared with BSA as a spacer in fibers coated with different densities of Con A. In experiments carried out with EL4 lymphoma cells and normal lymphocytes, the same Con A-specific binding (inhibited by 0.01 M α -methyl mannopyranoside) was obtained with both types of molecules as a spacer. Since hemoglobin compared to BSA gave a two- to threefold higher nonspecific binding at Con A concentrations below 2.5 ng, BSA was generally used as a spacer in all the experiments.

Treatment of cells before incubation with fibers included fixation with glutaraldehyde (2, 8) (3%, 2 h, 22°C), neuraminidase treatment (purified, Behringwerke AG, Marburg-Lahn, W. Germany; 50 U/ml, 30 min, 22°C), or trypsinization (3-times crystallized trypsin, Calbiochem, San Diego, Calif., 5 μ g/ml, 5 min, 37°C). The treated cells were not visibly damaged and did not aggregate or bind nonspecifically to fibers. Fixation of cells decreased the number of receptors for each lectin by about 25% (8). Fibroblasts dispersed by trypsinization, as described above under "Cells," were not treated further with trypsin. In some experiments binding was carried out at 0°C; in others, 0.001 M dinitrophenol, 0.002 M glucose, or 1–100 μ g/ml vinblastine were present during

the incubation. The cells were also incubated with these compounds for 30 min before incubation with fibers.

RESULTS

Interspacing of Lectin Molecules on Fibers with BSA

The weight of a protein adsorbed per unit length of fiber reflected its concentration in the derivatization mixture. For all three lectins and BSA, the weight ratio of protein in solution to the amount of protein adsorbed was nearly identical (Fig. 1). Because the BSA concentration was increased to compensate for a decrease in the lectin concentration, the total amount of fiber-bound protein was therefore constant. This indicates that the average density of lectin molecules can be controlled by interspacing lectin molecules with BSA molecules. Given the number of protein molecules of known size adsorbed to a fiber with a given surface area, the average distance between adjacent molecules can be estimated. Assuming that the fiber is a cylinder with a diameter of 0.125 mm, and that Con A and BSA molecules can be represented by spheres with diameters of 64 Å and 56 Å, respectively, 1 cm of fiber has enough surface area to be coated by approximately 17 ng of BSA or 20 ng of Con A. The amount of Con A and/or BSA actually bound per centimeter of fiber was near these values (Fig. 1), so it appears that essentially the entire surface of the fiber is covered by Con A and/or BSA. Given this high density and the stability of the protein-fiber bond (3), it is unlikely

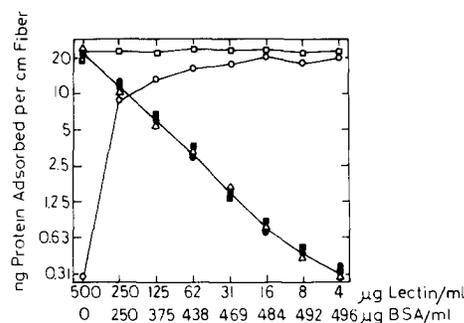


FIGURE 1 The average density of Con A (●), SBA (Δ), WGA (■), BSA (○), and total protein (□) adsorbed to a fiber incubated with a protein solution containing 500 μ g protein/ml composed of different weights of lectin and BSA. Both axes are expressed on a log scale. 125 I-labeled BSA and/or lectin were used to determine the amount of each protein bound and the total amount of protein.

that fiber-bound lectin molecules are able to move along the fiber surface. In cell binding experiments, fibers that have been coated with lectin alone will be referred to as high density lectin-fibers (20 ng lectin/cm). Lowering the density of fiber-bound lectin will refer to the interspacing of lectin molecules with increasing numbers of BSA molecules to give from 10 to 0.3 ng lectin per centimeter of fiber.

In studies with different lectins, the fibers were coated with equal weight densities of each lectin. The molecular weights of Con A, SBA, and WGA at pH 7.4 are 108,000, 110,000, and 35,000, respectively. Con A has four binding sites per molecule; SBA and WGA have only two binding sites (12, 15). It is therefore possible that the average and/or local density of binding sites on the fibers differed by a factor of two to three for the different lectins.

Binding of Cells to Lectin-Coated Fibers

Under the conditions used in these studies, cells shaken together with lectin-coated fibers bound upon collision with the sides of the fiber, but did not bind extensively to the top or bottom faces of the fiber (Fig. 2 *a*). The bound cells were counted by focusing on both sides of the fiber. Although appearing to lie next to each other along the edges, these cells are actually spread out over an area about five times wider than a single cell (Fig. 2 *b*). Cells covering half of the fiber edges (300–400 cells/cm for fibroblasts, 700–800 cells/cm for other cell types) therefore occupied only about 10% of the available binding surface, and the number of bound cells was still proportional to the number of cells in suspension.

Binding of cells to lectin-coated fibers was measured by counting the number of cells bound to a high density lectin-fiber, and to fibers having lower lectin densities. With all the lectins and cells studied, the number of fiber-bound cells (FBC) decreased with decreasing lectin density. With high density lectin-fibers, binding is given in terms of FBC per centimeter in Table I and in the inset boxes of Fig. 3–7. The decrease in binding of cells to fibers with lower lectin densities is expressed in Figs. 3–7 as percent FBC in which 100% FBC is the number of bound cells obtained with high density fibers.

Although BSA has a relatively high net negative charge at pH 7.4, several observations indicate that differences in cell-to-fiber binding reflect the

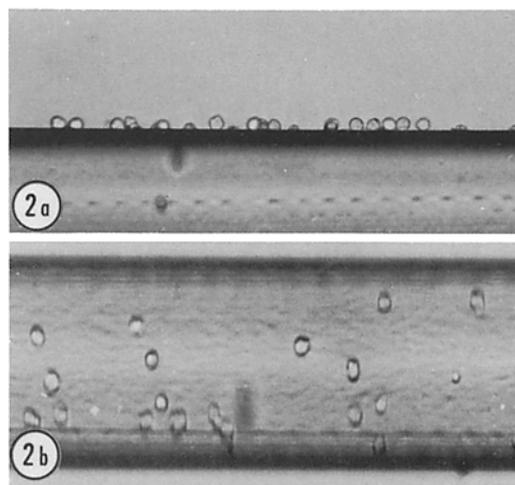


FIGURE 2 Cells bound to lectin-coated fibers. (*a*) Normal orientation of the fiber with focus on the fiber edge. This represents a fiber with 800 cells bound along both edges of a 1-cm fiber segment. (*b*) Rotation of fiber 90° so that the bound cells from one edge are visible on the face of the fiber. The density of cells is also about 800 cells/cm. The cells visible along the edge in (*a*) are spread out over a large portion of the fiber surface and only a small portion of the effective binding surface is covered by cells.

properties of the receptor, the lectin, or the treatment of the cells, rather than changes in the surface charge of the lectin-BSA fibers. The use of hemoglobin, which has a low net charge at pH 7.4, instead of BSA did not change the amount of specific binding to fibers at all lectin densities. Pretreatment of cells with neuraminidase to reduce negative charges from the cell surface also did not affect cell-to-fiber binding to Con A-BSA fibers.

All the cell types used in this study have cell surface receptors for Con A, WGA, and SBA (7, 20, 26), and with most of the cell types studied these receptors resulted in binding of cells to high density lectin-fibers (Table I). Although prefixation decreased the number of receptors for these lectins only by about 25% (reference 8 and unpublished results), fixed cells did not bind to lectin-coated fibers of all densities. Binding of cells to lectin-fibers was also prevented by the presence of a specific competitive inhibitor of the saccharide binding site of the lectin: 0.01 M α -methyl-mannopyranoside for Con A (9), 0.01 M acetylgalactosamine for SBA (22), and 0.001 M (*n*-acetylglucosamine)₂ for WGA (1). Differences in

the level of binding of various cell types to high density fibers was greater with WGA and SBA than with Con A (Table I). With all the cell types used, treatment with neuraminidase, which greatly increases the number of receptors for SBA (26), enhanced the level of binding to SBA fibers. L lymphoma cells, which have a large number of receptors for WGA (26), were unable to bind to WGA fibers.

Binding of L, M, D, and Normal Lymphocyte Cells to Con A-Coated Fibers

L lymphoma cells, M and D myeloid leukemia cells, and normal lymphocytes all bound to high density Con A fibers (Table I). The binding obtained with the M cells was two to four times lower than that obtained with the other cell types. In addition, the decrease in percent FBC caused by lowering of the Con A density was much greater with M cells than with L or D cells (Fig. 3). The decrease in percent FBC of normal lymphocytes and D cells with decreasing Con A density was slightly less than with L cells.

Binding of Normal and Transformed Fibroblasts to Con A-Coated Fibers

Transformed fibroblasts are readily agglutinated by Con A, whereas normal fibroblasts are only weakly agglutinated. Trypsinization of the normal fibroblasts, however, can greatly increase their agglutinability with Con A (9) without necessarily increasing the number of receptors for the lectin (20). This effect of trypsin on fibroblasts has been attributed to an increase in the mobility of the Con A receptors (16, 17, 20) which enhances the ability of complementary receptors on colliding cells to align for the formation of multiple cell-lectin-cell bridges (18, 19). The binding of transformed fibroblasts to high density Con A fibers was two to four times greater than that of normal fibroblasts (Table I). The decrease in percent FBC of transformed fibroblast binding caused by lowering of the Con A density was also less than with normal fibroblasts (Fig. 4). After trypsinization, the ability of the normal cells to bind to Con A fibers of all densities was higher than that of the untreated transformed cells. Trypsinization of L, M, D, or normal lymphocyte cells did not greatly affect either their agglutination, cell-to-cell binding (18, 19), or fiber-binding efficiency with any of the lectins tested.

Comparison of Cell Binding to Fibers Coated with Different Lectins

By comparing the ability of a particular cell type to bind to fibers having decreasing densities of lectin, differences between Con A, WGA, and SBA were detected. Although L1210 cells bound well to fibers having a high density of Con A,

TABLE I
Binding of Cells to Lectin-Coated Fibers

Cell type at 5×10^5 cells/ml	Fiber-binding cells/cm*		
	Con A†	WGA†	SBA†
L (YAC)	693	9	211
EL4	713	244	767
L1210	618	990	645
D ₃₁ (D ⁺ IR ⁺)	502	443	263
D ₁₄ (D ⁻ IR ⁺)	489	244	253
M (D ⁻ IR ⁻)	263	856	782
Normal lymphocyte	605	317	384
Transformed fibroblast	502	110	19
Normal fibroblast	127	55	12

* Values represent the number of cells bound to both edges of a 1-cm fiber segment. The standard deviation of four determinations was $\pm 10\%$ for values above 100 FBC/cm. In the presence of a competitive inhibitor for the lectin, binding was less than 10 FBC/cm.

† Fibers coated with 20 ng of lectin per centimeter.

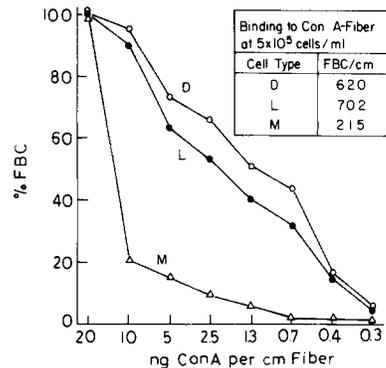


FIGURE 3 Binding of D, L, and M cells to Con A-BSA fibers. The number of cells bound per centimeter of high density Con A fiber (20 ng Con A/cm) at 5×10^5 cells/ml is shown in the inset box. The decrease in binding of cells to fibers with lower lectin densities is expressed as percent FBC in which 100% FBC is the number of bound cells obtained with high density Con A fibers. See Fig. 1 for Con A densities on Con A-BSA fibers. Normal lymphocytes gave results similar to those obtained with D cells.

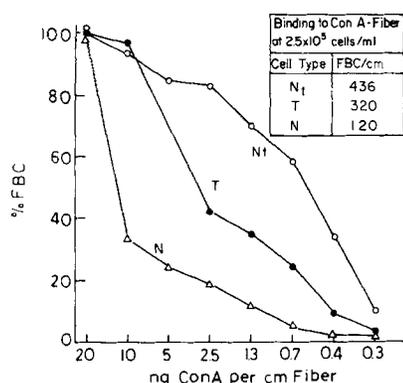


FIGURE 4 Binding of fibroblasts to Con A-BSA fibers. *N*, normal fibroblast; *T*, transformed fibroblast; *N*₁, trypsinized normal fibroblast. The inset shows the number of fiber-bound cells/cm obtained with high density (20 ng/cm) Con A-fibers at 2.5×10^5 cells/ml. See Fig. 3 for definition of percent FBC.

WGA, or SBA (Table I), the decrease in percent FBC of these cells with fibers having lower lectin densities was considerably more with WGA or SBA (Fig. 5). Increasing the number of receptors for SBA by treatment with neuraminidase enhanced the binding of the L1210 cells to SBA fibers of all densities, but the decrease in percent FBC caused by lowering the SBA density was still greater than that obtained with Con A fibers. Results obtained with normal lymphocytes, EL4, and D cells were similar to those shown for L1210 cells.

The Effect of Dinitrophenol and Temperature on Fiber to Cell Binding

Metabolic inhibitors are known to increase the Con A-induced agglutination of several cell types (25). The presence of 0.001 M 2,4-dinitrophenol (Dnp) considerably diminished the fiber-to-cell binding with all the cell types and lectins studied (Table II). Dnp also increased the drop in percent FBC of L cell binding to Con A fibers caused by lowering the Con A density (Fig. 6). The effect of Dnp appears to involve the metabolism of ATP in that other inhibitors of ATP production, 0.01 M NaN₃, 5 μ M oligomycin, and 0.001 M *n*-ethyl maleamide, had the same effect. The effects of Dnp could be almost completely reversed by the presence of 0.002 M glucose (Fig. 6), which restores the ATP levels in cells to normal levels by

glycolysis (25). This concentration of glucose does not significantly affect the binding of L cells to Con A fibers.

With all cell types used, binding to high density Con A and WGA fibers was lower at 0°C than at 22°C. However, binding of these cells to high density SBA fibers, with or without neuraminidase treatment, was either not affected or slightly enhanced at 0°C (Table III). With Con A fibers and L cells, the drop in percent FBC caused by decreasing the Con A density was not different at 0°C and 22°C (Fig. 7).

Vinblastine (1–100 μ g/ml), which disrupts microtubular structures and can alter the behavior of receptors for Con A on normal lymphocytes and D

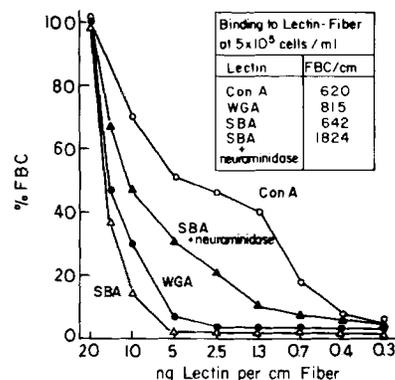


FIGURE 5 Binding of L1210 cells to Con A-BSA, SBA-BSA, and WGA-BSA fibers. The inset shows the number fiber-bound cells per centimeter obtained with high density (20 ng/cm) lectin fibers at 5×10^5 cells/ml. See Fig. 3 for definition of percent FBC. The results shown for neuraminidase-treated cells are extrapolated from experiments at 2.5×10^6 cells/ml.

TABLE II
Effect of 2,4-Dinitrophenol on Binding of L1210 cells to Con A-, SBA-, and WGA-Coated Fibers

Lectin on fiber*	Fiber-bound cells/cm†	
	PBS	0.001 M Dnp
Con A	555	18
WGA	750	25
SBA	587	21

Similar results were obtained with all the cell types used.
* Fibers coated with 20 ng of lectin per centimeter.
† Cells incubated with fibers at 4×10^5 cell/ml. Numbers represent bound cells along both edges of a 1-cm fiber segment.

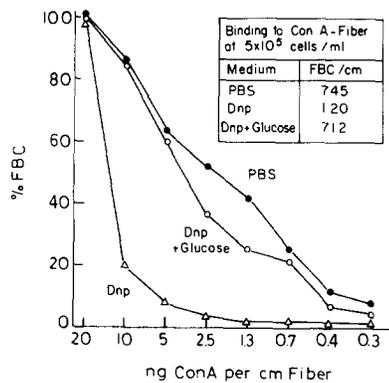


FIGURE 6 Binding of L cells to Con A-BSA fibers in the presence of 0.001 M Dnp or Dnp + 0.002 M glucose. Binding to PBS containing glucose alone gave results similar to those shown for PBS. The inset shows the number of fiber-bound cells per centimeter obtained with high density (20 ng/cm) Con A-fibers at 5×10^5 cells/ml. See Fig. 3 for definition of percent FBC.

TABLE III
Binding of L1210 cells to Lectin-Coated Fibers at 0°C and 22°C

Lectin on fiber*	Fiber-bound cells/cm†	
	22°C	0°C
Con A	510	25
WGA	649	29
SBA	660	812
SBA§ (+ neuraminidase)	776	945

Similar results were obtained with L, D, M, and normal and transformed fibroblast cells.

* Fibers were coated with 20 ng of lectin per centimeter.

† Values represent the number of cells bound to both edges of a 1-cm fiber segment. Cells were incubated with fibers at 4×10^5 cells/ml; with neuraminidase-treated cells, 2×10^6 cells/ml were used.

§ Cells were pretreated with neuraminidase before incubation with fibers.

cells (5)², did not have a significant effect on the binding of normal lymphocytes or D cells to Con A fibers of all densities.

DISCUSSION

The binding of cells to a lectin-coated fiber has been titrated as a function of the density of lectin molecules on the fiber. In all cases, the number of cells bound decreased as the density of the lectin on the fiber decreased. The shape of the titration

² I. Vlodaysky, J. Lotem, and L. Sachs. 1974. Unpublished observations.

curve, however, was found to depend on the lectin, cell type, and treatment of the cells. One of the purposes of the present paper is to interpret these titration curves in terms of differences in the ability of lectin receptors to move on the surface of the cell membrane.

Fixation of cells with glutaraldehyde inhibits the mobility of membrane receptors including the mobility of microvilli. This fixation completely inhibited the ability of cells to bind to lectin-coated fibers, but did not greatly affect their ability to bind soluble lectin. As previously concluded for agglutination (2, 8, 16) and cell-cell binding (18), this indicates that cell-fiber binding requires mobility of cell-surface receptors for these lectins. We have postulated that the requirements for receptor mobility in cell-cell binding and agglutination involves alignment of complementary receptors for the formation of multiple cell-lectin-cell bonds (18, 19). In applying this alignment mechanism to cell-to-fiber binding, the analysis of receptor mobility is simplified in that the lectin molecules are immobilized on the fiber surface, the density of lectin molecules on the fiber can be controlled, and cells are not treated with soluble lectin.

A schematic representation of the requirements for receptor alignment in cell-to-fiber binding are shown in Fig. 8. Fibers having a high density of lectin molecules will even bind cells having relatively immobile receptors (Fig. 8 a). When the lectin molecules are interspaced with BSA molecules, however, effective alignment can be achieved only with cells having receptors with higher mobility (Fig. 8 b). The spacing of lectin by BSA can be

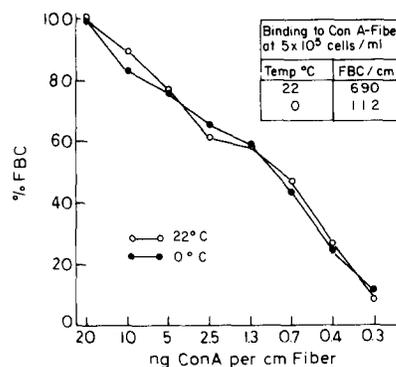


FIGURE 7 Binding of L cells to Con A-BSA fibers at 0°C and 22°C. Inset shows the number of fiber-bound cells with high density (10^{11} molecules per centimeter) Con A-fibers at 5×10^5 cells/ml. See Fig. 3 for definition of percent FBC.

compensated by a higher density of receptors (Fig. 8 c), but even this is inadequate for alignment if the receptors are immobilized by fixation with glutaraldehyde (Fig. 8 d). It can be predicted from this model that the titration to fiber-to-cell binding on fibers with decreasing densities of lectin will measure the relative ability of cell-bound receptors to align with the immobilized fiber-bound lectin molecules. Cell types of similar size can have little or not differences in the number of receptors for a particular lectin on the cell surface (20, 26), and it can be concluded from this model that the major factor in alignment is the mobility of individual receptors.

Three types of receptor mobility have been described: large lateral movements as observed in cap formation (24); molecular rotation as detected by fluorescent polarization (10); and small lateral movements that can result in clustering of receptors (2, 8, 13, 16, 17). Receptor movements associated with capping or molecular rotation do not correlate with cell-to-cell binding (18, 19) and they also do not appear to be limiting factors in cell-to-fiber binding. About 5% of clone D₁₆ and 50% of clone D₂₁ myeloid leukemia cells form caps with Con A (20)², but the two cell types bind equally well to Con A-coated fibers at all densities. The differences reported for molecular rotation of Con A-receptor complexes between normal lymphocytes and L lymphoma cells and the increase in receptor rotation caused by trypsinization of these cells (10) were also not reflected by differences in binding to Con A-coated fibers.

It can be suggested from the model in Fig. 8 that cell-to-fiber binding requires short range lateral mobility of cell surface receptors. The spacing of molecules on the fiber surface indicates that the average distance between saccharide binding sites on the fiber is about 30–3,000 Å for fibers with 20–0.2 ng of lectin per cm. In view of the short duration of cell-with-fiber collisions, we will refer to the mobility required for receptor alignment as short range rapid lateral mobility or RLM. Receptor alignment occurs before lectin-to-receptor binding, so that this mobility is that of the unoccupied receptor. Cell surface receptors for a lectin are likely to be heterogeneous (26) in their structure and mobility, and the RLM of the saccharide moieties on these receptors can include both their intrinsic flexibility and movement of the entire receptor. The mobility of receptors may also be influenced by the movements of microvilli and the elastic properties of the cell surface membrane.

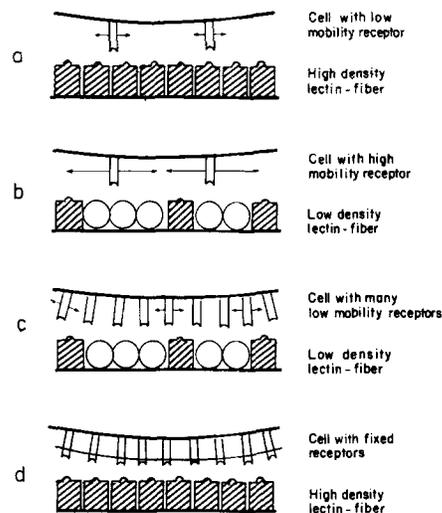


FIGURE 8 Schematic representation of different parameters in cell-to-fiber binding. Cell-to-fiber binding will occur in (a), (b), and (c), but not in (d). □, Cell surface receptor; ▨, lectin molecule immobilized on fiber; ○, BSA spacer molecule immobilized on fiber. Arrows represent the range of lateral receptor mobility.

In previous studies on cell-to-cell binding induced by Con A (18, 19), it was found that there were only low levels of cell-to-cell binding between normal lymphocytes, D cells, or M cells. Prefixation of Con A-coated lymphocytes and D cells greatly increased their ability to bind to unfixed cells, so that it was concluded that the poor binding obtained with these cells was caused by Con A-induced receptor clustering and not by insufficient mobility of the receptors. Prefixation did not enhance the cell-to-cell binding of M cells, and it was therefore suggested that these cells, which have a size and number of Con A receptors similar to those of D cells, have low mobility receptors for Con A. The cell-to-fiber binding results reported here and their interpretation in terms of receptor RLM are consistent with these conclusions, in that the Con A receptors on normal lymphocytes and D cells have a higher average RLM than those on M cells (Table IV). Cell-to-cell binding induced by Con A between transformed fibroblasts is higher than between normal fibroblasts (19), and this also correlates with a higher RLM of Con A receptors on the transformed than on the normal fibroblasts. Trypsinization of normal fibroblasts increased both Con A receptor RLM and Con A-induced cell-to-cell binding (Table IV).

Lectin-induced cell-to-cell binding has been

TABLE IV
The Relationship of Con A-Receptor RLM to Cell-to-Cell Binding Induced by Con A

Cell Type	RLM* of receptors	Cell-to-Cell Binding†	
		Unfixed cells§	One cell fixed
YAC lymphoma (L)	High	Med	High
L1210 lymphoma	Med-high	Low (clustering)	High
Normal lymphocyte	High	Low (clustering)	High
IR ⁺ myeloid leukemia (D)	High	Low (clustering)	High
IR ⁻ myeloid leukemia (M)	Low	Low	Low
Transformed fibroblast	Med-high	Med	High
Normal fibroblast	Low-med	Low (clustering)	High
Trypsinized normal fibroblast	High	High	High

* RLM: rapid lateral mobility of unoccupied cell surface receptors for Con A. Low, medium, and high refer to the relative receptor mobility as determined in Figs. 3-6.

† Binding of a Con A-coated cell to an untreated cell in which one cell is first immobilized on a nylon fiber (18, 19). Low, medium, and high refer to the relative number of cell-bound cells per centimeter obtained by this method.

§ Cases in which cell-to-cell binding is strongly inhibited by Con A-induced receptor clustering are indicated. In other cases, low and medium binding resulted from a low RLM and/or a weaker inhibition by receptor clustering (see Discussion).

|| Prefixation with glutaraldehyde of the cell to be coated with Con A in the cell-to-cell binding assay. This prefixation immobilizes receptors on the cell and prevents Con A-induced receptor clustering (see Discussion).

shown to depend on the lectin used (19), and binding of the same cell type to Con A, WGA, and SBA fibers also shows differences between lectins. Although the density of lectin on the fibers in these comparisons may differ by a factor of two or three, it is unlikely that this can entirely account for the large differences observed in cell-to-fiber binding with these lectins. The results suggest that the average RLM of receptors for SBA and WGA is considerably lower than that of receptors for Con A. A low RLM of WGA receptors is consistent with the high sensitivity of WGA-induced agglutination to membrane fixation (8). A low RLM of WGA receptors may also account for differences between the fiber and agglutination assays, which differ in their collision dynamics (19), and the finding that binding to WGA fibers is much more temperature-sensitive than WGA-induced agglutination of cells in suspension (20, 26). The sharper drop in binding obtained in titrations with WGA and SBA fibers than with Con A fibers may mean that the receptors for WGA and SBA are more homogeneous in their RLM than those for Con A.

The results on cell-to-fiber binding also suggest that a decrease in ATP level reduces receptor RLM. Although both Dnp and low temperature inhibit metabolism, they differ in their effect on cell-to-fiber binding. Dnp reduced binding to Con A, SBA, and WGA fibers, but low temperature did not affect cell binding to SBA fibers. In addition,

Dnp, but not low temperature, increased the drop in percent FBC caused by lowering the Con A density. The effects of Dnp and low temperature are complex, but it can be suggested that changes in ATP levels affect structures which preferentially modulate rapid receptor movements, whereas low temperature reduces binding perhaps by changing the fluidity of the whole membrane.

In the series of cell types tested, our results indicate two correlations between receptor RLM and the biological behavior of cells. With fibroblast cells, which grow as a solid tissue in vivo, malignant transformation was associated with an increase in the RLM of Con A receptors. YAC lymphoma cells and normal lymphocytes, cells which exist in vivo either as free cells or in a semisolid lymphoid organ, both have a high Con A receptor RLM. The second correlation was with the ability of myeloid leukemia cells to undergo differentiation. D cells, which can be induced to express IR differentiation markers on their surface (14), have a higher Con A receptor RLM than M cells, which could not be induced to express these markers (14).

Rapid movements of cell surface components are likely to be of importance in a number of biological functions. Receptor RLM is likely to play a role in surface recognition between cells, especially those which require multiple cell-to-cell bonds. The redistribution and/or clustering of

receptors, which have been suggested as possible primary events in the activation of lymphocytes by lectins (5, 20), would also be facilitated by receptor RLM. The present assay may therefore be of general use in evaluating the properties of receptors involved in cell-to-cell interactions, differentiation, and mitogenesis.

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