

# CELL-TO-CELL BINDING INDUCED BY DIFFERENT LECTINS

URS RUTISHAUSER and LEO SACHS

From the Department of Genetics, Weizmann Institute of Science, Rehovot, Israel

## ABSTRACT

The cell-to-cell binding induced by concanavalin A (Con A) and the lectins from wheatgerm, soybean, and waxbean has been analyzed by measuring the ability of single cells to bind to lectin-coated cells immobilized on nylon fibers. The cells used were lymphoma, myeloid leukemia, and normal fibroblast cells. With all lectins, cell-to-cell binding was inhibited if both cells were prefixed with glutaraldehyde. However, in most cases cell-to-cell binding was enhanced when only the lectin-coated cell was prefixed. With normal fibroblasts, treatment of either one or both cells with trypsin enhanced the cell-to-cell binding induced by Con A and the wheatgerm lectin. Neuraminidase, which increases the number of receptors for soybean agglutinin, increased cell-to-cell binding only if both cells were treated. Although cell-to-cell binding induced by the lectins from soybean and wheatgerm could be partially reversed by the appropriate competitive saccharide inhibitor, binding induced by Con A could not be reversed. The experiments indicate that cell-to-cell binding induced by a lectin can be prevented by an insufficient density of receptors for the lectin, insufficient receptor mobility, or induced clustering of receptors. These effects can explain the differences in cell-to-cell binding and agglutination observed with different cell types and lectins. They also suggest that cell-to-cell binding induced by different lectins with a variety of cell types is initiated by a mechanism involving the alignment of complementary receptors on the colliding cells for the formation of multiple cell-to-lectin-to-cell bridges.

The interaction of lectins with cells has been used as a probe to study changes in the cell surface membrane associated with malignancy, mitogenesis, and differentiation (18). This interaction has frequently been evaluated by the ability of a lectin to agglutinate a suspension of cells. Although this agglutination assay is rapid and convenient, it lacks flexibility in that the binding of lectin to the cells and the binding of several cells to one another occur simultaneously, and the degree of agglutination has often been scored visually on an arbitrary scale. Improvements in the agglutination assay include quantitation by light scattering (22), and measurement of total cell protein or radioactivity of cells bound to cells that can adhere to a surface (2, 14).

In a previous paper (16), we have described a method for the analysis of cell-to-cell binding utilizing cells immobilized on derivatized nylon fibers (5). This system allows the quantitative study of binding between single cells, in which the binding of lectin to a cell and the binding of that cell to another cell can be carried out as successive steps. Any combination of cells and agglutinins can be examined, with identification of each cell type after cell-to-cell binding. This procedure was used to study the binding induced by concanavalin A (Con A)<sup>1</sup> between normal lymphocytes and

<sup>1</sup> *Abbreviations used in this paper:* Con A, concanavalin A; D, IR<sup>+</sup>D<sup>-</sup> clone 16 or IR<sup>+</sup>D<sup>+</sup> clone 21 myeloid leukemic cell; L, YAC lymphoma cell; M, IR<sup>-</sup>D<sup>-</sup> clone

lymphoma cells, and it was suggested that short-range receptor movements are required for alignment of individual receptor pairs, so as to form multiple cell-to-lectin-to-cell bridges (16).

In the present experiments, we have extended this application of the fiber assay to study differences in the cell-to-cell binding of two lines of lymphoma cells, three types of myeloid leukemia cells, and normal fibroblasts, as induced by Con A (9), wheatgerm agglutinin (WGA) (3), soybean agglutinin (SBA) (9), and waxbean agglutinin (20). The effects of neuraminidase and trypsin on cell-to-cell binding with SBA and Con A, respectively, have also been examined. The experiments indicate that the ability of a lectin to induce cell-to-cell binding is affected by lectin-induced clustering of cell surface receptors, mobility of the receptors, and the density of receptors for the lectin on the cell surface. These results suggest that the receptor alignment mechanism proposed previously for Con A (16) is generally applicable to cell-to-cell binding induced by lectins.

## MATERIALS AND METHODS

YAC lymphoma (L) cells were obtained from a Moloney virus-induced lymphoma grown in A strain mice (10). This is a tumor of thymus-derived cells (7).  $10^6$  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). L1210 lymphoma cells (21) were grown in suspension culture as described below.

Three clones of mouse myeloid leukemic cells were grown in suspension culture: clone M<sub>1</sub>, IR<sup>-</sup>D<sup>-</sup> myeloid leukemia cells (M) from SJL mice (11), and two D myeloid leukemia clones from SL mice which can be partially (clone D<sub>16</sub>, IR<sup>-</sup>D<sup>-</sup>) or completely (clone D<sub>21</sub>, IR<sup>+</sup>D<sup>+</sup>) induced to undergo normal cell differentiation (6, 11). The D<sub>16</sub> and D<sub>21</sub> clones gave identical results in cell-to-cell binding experiments. 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% fetal calf serum; the cells were subcultured every 4–5 days by seeding  $10^6$  cells/10 ml medium. The fibroblasts were dissociated by incubation with 0.02% EDTA solution (9) for 15–30 min at 22°C. All cells were washed three times

myeloid leukemic cell; PBS, phosphate-buffered saline (1,000 ml PBS contains 18 g NaCl, 0.2 g KCl, 0.2 g KH<sub>2</sub>PO<sub>4</sub>, 1.15 g Na<sub>2</sub>HPO<sub>4</sub>, 0.1 g CaCl<sub>2</sub> · 2 H<sub>2</sub>O, 0.1 g MgCl<sub>2</sub> · 6 H<sub>2</sub>O); SBA, soybean agglutinin; WGA, wheatgerm agglutinin.

in PBS and then dispersed in cell suspension in PBS before use in the cell-to-cell binding assay.

## Lectins

Purified Con A, SBA, and WGA were obtained from Miles-Yeda, Israel. Waxbean agglutinin was prepared by affinity chromatography (B. Sela, N. Sharon, and L. Sachs, manuscript in preparation). All lectins appeared homogeneous in polyacrylamide gel electrophoresis at pH 8.6. Competitive saccharide inhibitors for lectin-induced cell-to-cell binding were 0.01 M  $\alpha$ -methyl manopyranoside for Con A, 0.05 M *n*-acetylgalactosamine for SBA, and 0.001 M (*n*-acetylglucosamine)<sub>2</sub> for WGA.

## Derivatization of Fibers with Lectin

Nylon fibers were strung in polyethylene frames, washed successively with petroleum ether (30°–60°C) and carbon tetrachloride, dried, and incubated with 0.25 mg/ml solution of lectin in PBS for 30 min at 22°C (5). This procedure resulted in adsorption of the lectin to the fiber surface. The adsorption was stable in physiological medium, and the lectin solution could be reused several times. Derivatized fibers were washed three times in PBS before incubation with cells.

## Cell-to-Cell Binding Assay

The cell-to-cell binding assay (Fig. 1) involved three steps: (a) Binding of cells to Con A-derivatized fibers by incubation with  $2.5 \times 10^6$  cells/ml in PBS at 22°C for 30 min with gentle shaking and removal of unbound cells by washing as described (5). (b) Binding of lectin (Con A in Fig. 1) at the indicated concentration for 30 min at 22°C

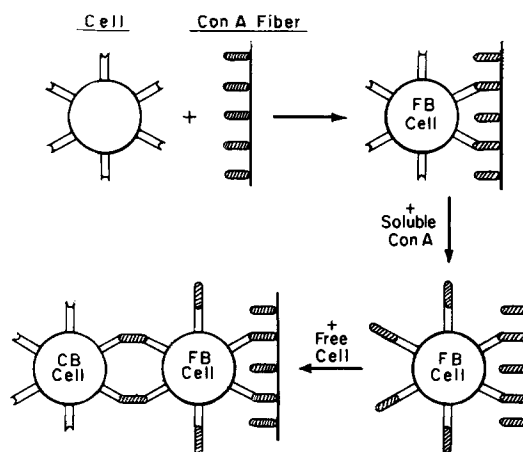


FIGURE 1 Procedure used in the cell-to-cell binding assay for the preparation of fiber-bound (FB) cells attached to Con A-derivatized fibers, and cell-bound (CB) cells. The shape and orientation of the Con A molecules and cell receptors for Con A are schematic. ⊗, multivalent Con A molecule.

to either the fiber-bound cells or free cells, followed by three washes with PBS. It should be noted that coating of the free cells with lectin was carried out only in those cases where the subsequent cell-to-cell binding assay would not be complicated by agglutination of the lectin-coated free cells with themselves. This was possible for Con A with D cells, M cells, normal lymphocytes, and normal fibroblasts, and for the lectins tested with any cells which had been prefixed with glutaraldehyde (see below). (c) Incubation of fiber-bound cells with free cells in suspension ( $10^8$  cells/ml in PBS) at 22°C for 30 min with gentle shaking, as in the binding of cells to fibers. In each step a new Petri dish was used to prevent carry-over of cells or lectin. To avoid the release of bound cells, the fibers were kept immersed in PBS during the entire procedure (5). After washing away unbound cells, the number of cell-bound cells were counted along one edge of a 1-cm segment of fiber, using an inverted microscope at 100 $\times$  magnification (Fig. 2).

#### *Cell Fixation with Glutaraldehyde*

Fixation of the cell membrane was carried out with 3% glutaraldehyde in PBS for 2 h at 22°C. The fixed cells were then centrifuged, resuspended in 0.2 M glycine in PBS, incubated for 10 min at 22°C, and washed three times with PBS (4, 8). Because fixed cells did not bind to fibers, fixation of fiber-bound cells was carried out after the cells were bound to the fibers. Membrane fixation slightly decreased the number of lectin molecules which bound to the cell surface (8).

#### *Treatment of Cells with Trypsin and Neuraminidase*

Treatment of cells with trypsin (crystallized three times, Calbiochem, San Diego, Calif.; 5  $\mu$ g/ml, 5 min, 22°C) or neuraminidase (purified, Behringwerke AG, Marburg-Lahn, West Germany; 5  $\mu$ g/ml, 30 min, 22°C) was carried out on fiber-bound or free cells before coating the cell with lectin. The treated cells were not visibly damaged and did not aggregate spontaneously. In some experiments, neuraminidase was added after fixation of the cells with glutaraldehyde.

## RESULTS

### *Assay of Cell-to-Cell Binding Induced by Lectins*

The cell-to-cell binding induced between single cells by several lectins has been analyzed using cells attached to nylon fibers. The general procedure is summarized in Fig. 1 and illustrated in Fig. 2. In the first step, cells were attached to Con A-derivatized fibers to form a confluent monolayer along the fiber edge (Fig. 2 *a*). Although binding of cells to the fiber can be prevented by the

presence of  $\alpha$ -methyl mannopyranoside, a competitive inhibitor for Con A, the bound cells could not be released by subsequent incubation with this inhibitor (15). Soluble lectin was then used to coat the fiber-bound cells or to coat the free cells in cases where agglutination would not occur in suspension. In all cases in which lectin-coated free cells would not bind to themselves, coating either the fiber-bound or free cells gave identical results in the cell-to-cell binding assay (16). The excess lectin was removed and the free cells were incubated with the fiber-bound cells. With certain combinations of lectins and cell types, single free cells became attached to the fiber-bound cells (Figs. 1 and 2). The individual cell-bound cells, that is, the number of cells in the second layer of cells shown in Fig. 2 *b*, were counted at 100 $\times$  magnification. The binding required the presence of Con A (16), SBA, WGA, or waxbean lectin on either the free or fiber-bound cells, but did not occur if both cells were coated with the same lectin. Prefixation of one or both cells with glutaraldehyde did not induce cell-to-cell binding in the absence of lectin. Cell-to-cell binding induced by lectins was prevented by the presence of a competitive saccharide inhibitor and was reproducible to  $\pm 10\%$  (Table I).

Cell-to-cell binding induced by Con A was not reversed by incubation with 0.05–0.3 M  $\alpha$ -methyl mannopyranoside for 30 min at 22°C. This irreversibility was not affected by trypsin, neuraminidase, vinblastine (100  $\mu$ g/ml), temperature (0–25°C), or milliosmolarity (150–600 mosM in NaCl). However, about 75% of cells bound to SBA-coated or WGA-coated cells were released by incubation with 0.01 M *N*-acetylgalactosamine or 0.001 M (*n*-acetylglucosamine)<sub>2</sub>, respectively, for 30 min at 22°C.

With leukemic L, L1210, D, and M cells, which are similar in size, the number of cell-bound cells induced by any lectin was proportional to the number of free cells added up to densities of 450 cell-bound cells/cm (for L cells and Con A, see Fig. 3). A fiber saturated with leukemic cell-bound cells had 650–700 cell-bound cells along one edge of a 1-cm fiber segment. With the larger normal fibroblasts, saturation was achieved at 300 cell-bound cells/cm and the assay was linear to about 200 cell-bound cells/cm. Increasing the concentration of lectin produced more cell-to-cell binding, the amount being dependent on the lectin or cell type used (Fig. 4). The assay was sensitive enough to detect cell-to-cell binding with lectin concentra-

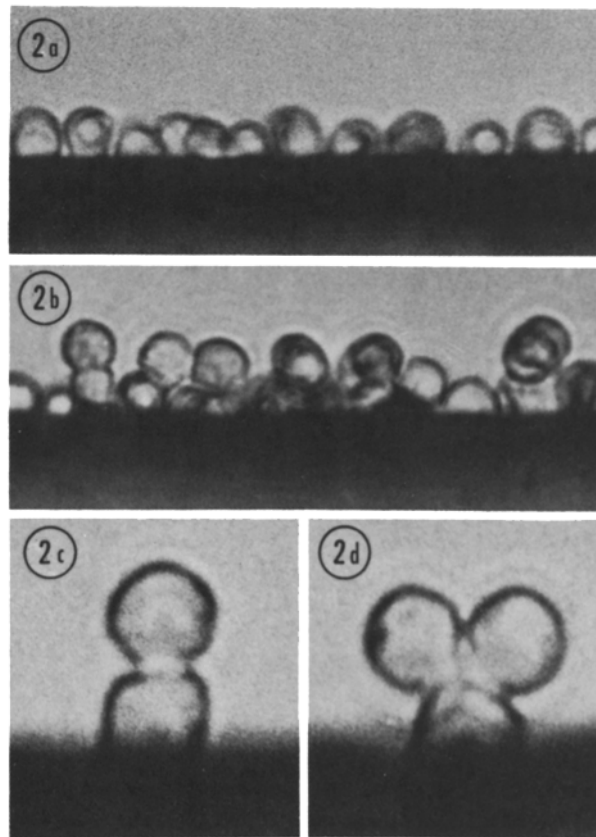


FIGURE 2 Photographs of fiber-bound cells and of cell-to-cell binding induced by lectins. *a*, L cells bound to fiber; *b-d*, cell-to-cell binding between L cells. *a* and *b*,  $\times 650$ ; *c* and *d*,  $\times 1,400$ .

tions of 1–10  $\mu\text{g}/\text{ml}$ . In general, concentrations of 100–200  $\mu\text{g}/\text{ml}$  of lectin were used.

#### *Cell-to-Cell Binding Induced by Con A and Waxbean Agglutinin*

Con A induced a high level of cell-to-cell binding between two L cells but rarely between cells from the D or M lines of myeloid leukemia or L1210 lymphoma. Binding occurred between a Con A-coated L cell and an uncoated D cell, and, to a lesser extent, an uncoated M cell, but rarely between a Con A-coated D or M cell and an uncoated L cell (Table II). All the leukemic cells bound about  $5 \times 10^6$  Con A molecules per cell, measured with  $^{125}\text{I}$ -labeled Con A as described in reference 22.

As reported for normal lymphocytes (16), prefixation of D and L1210 cells (Table II) and normal fibroblasts (Table III) before coating with Con A resulted in a high degree of binding to

untreated cells. Prefixation of a Con A-coated L cell enhanced its cell-to-cell binding to a lesser extent, so that the 10-fold difference in binding of unfixed Con A-coated L and D cells to untreated L cells was reduced to less than twofold when prefixed Con A-coated cells were compared (Fig. 5). Prefixation of M cells did not enhance their ability to bind untreated M cells (Table II). Mixed binding between fixed and unfixed L, D, and M cells indicated that, in many cases, M cells could be induced to bind with a low efficiency to D and L cells. With all cell types, little binding was obtained between a Con A-coated cell and a fixed cell, and no binding was obtained between two fixed cells (Table II).

Although normal fibroblasts agglutinate poorly in solution with Con A, brief treatment with trypsin greatly enhances their agglutinability by this lectin (9). Similarly, untreated normal fibroblasts gave little binding with Con A in the fiber assay, but pretreatment of either or both the

TABLE I  
Cell-to-Cell Binding Induced by Con A

Fiber-bound cell*	Free cells added*	Cell-bound cells
		<i>cm<sup>-1</sup></i>
L	L	3 ± 3†
Lc	L	369 ± 26
Lc	L (+ α-MM)§	12 ± 5
Lc	L (+ D-Gal)§	352 ± 23
Lgc	L	520 ± 29
L	Lgc	489 ± 17
Lc	Lgc	17 ± 8
L	Lg	11 ± 6

\* L, YAC lymphoma cell; Lc, cell coated with Con A (200 μg/ml); Lgc, cell fixed with glutaraldehyde and then coated with Con A.

† Standard deviation in four experiments.

§ 0.01 M α-methyl-D-mannopyranoside (α-MM), which binds to the Con A saccharide binding site, or 0.01 M D-galactose (D-Gal), which is not bound by Con A, was present during incubation of free cells with fiber-bound cells.

|| Lgc cells do not aggregate in solution.

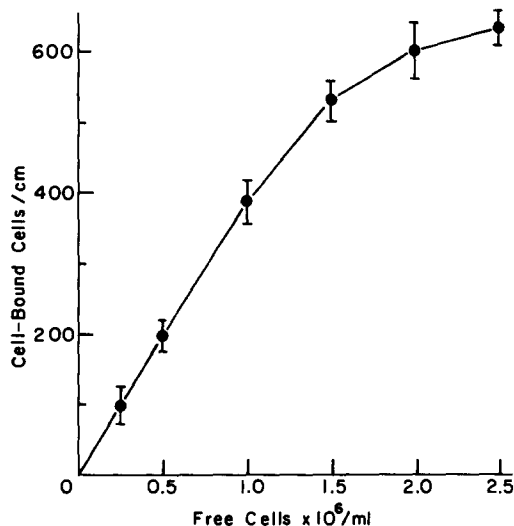


FIGURE 3 Effect of the number of free L cells in solution on the number of cell-bound L cells in cell-to-cell binding induced by Con A. The standard deviation was derived from four determinations.

fiber-bound and free cells with trypsin enhanced the cell-to-cell binding (Table III). Trypsinization did not have a large effect on the cell-to-cell binding of L, L1210, D, and M cells with any of the lectins studied.

The results obtained with waxbean agglutinin

generally resembled those shown for Con A. Cell-to-cell binding induced by waxbean agglutinin, however, was consistently higher with all cell types than that induced by the same concentration of Con A (for L cells, see Fig. 4). Unlike the results obtained with Con A, binding between M cells was enhanced by prefixation of the waxbean agglutinin-coated cell.

#### Cell-to-Cell Binding Induced by WGA

WGA induced cell-to-cell binding between D, M, and L1210 cells but not between L cells (Table IV). WGA-coated D or M cells bound uncoated L cells, but WGA-coated L cells rarely bound uncoated D or M cells. As with L cells and Con A, the cell-to-cell binding of D, M, and L1210 cells with WGA was enhanced by prefixation of the WGA-coated cell, decreased by prefixation of the uncoated cell, and prevented by fixation of both cells. Unfixed L cells bound well to fixed WGA-coated M or D cells, whereas fixed WGA-coated L cells bound unfixed M or D cells to a lesser extent. As with M cells and Con A, prefixation of a WGA-coated L cell did not enhance its ability to bind untreated L cells.

WGA induced weak cell-to-cell binding between

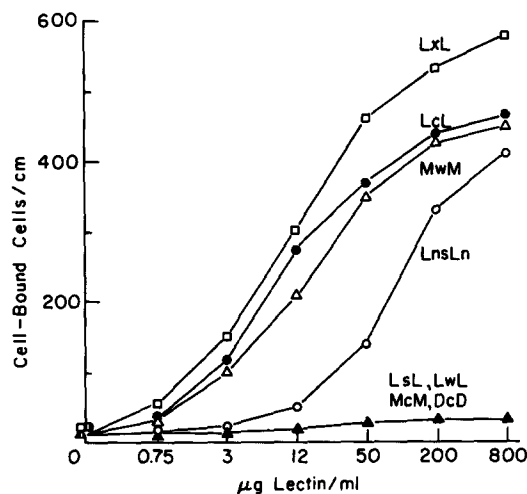


FIGURE 4 Effect of lectin concentration on the number of cell-bound cells. LxL, binding induced between L cells by waxbean agglutinin; LcL, binding induced by Con A; MwM, binding induced between M cells by WGA; LnsLn, binding induced between neuraminidase-treated L cells by SBA; LcL and LwL, binding induced between L cells by SBA and WGA, respectively; McM and DcD, binding induced by Con A between two M cells and two D (clone D<sub>16</sub>) cells, respectively.

TABLE II  
Cell-to-Cell Binding of L, D, and M Cells Induced by Con A

Fiber-bound cell*	Cell-bound cell*	Cell-to-cell binding $cm^{-1}$
Binding between two unfixed cells		
Lc	L	358
Lc	D	320
Lc	M	104
Dc	L	46
Dc	D	21
Dc	M	13
Mc	L	23
Mc	D	13
Mc	M	18
Binding between a fixed and an unfixed cell		
Lgc	L	498
Lgc	D	420
Lgc	M	115
Dgc	L	421
Dgc	D	398
Dgc	M	120
Mgc	L	239
Mgc	D	220
Mgc	M	15
Lc	Lg	60
Dc	Dg	23
Mc	Mg	12
Binding between two fixed cells		
Lgc	Lg	8
Dgc	Dg	6
Mgc	Mg	9

L1210 lymphoma cells gave results similar to those shown for D cells.

\* L, YAC lymphoma cell; D, clone D<sub>16</sub> myeloid leukemic cell; M, clone M<sub>1</sub> myeloid leukemic cell. The lower case letters refer to treatments of the cell before cell-to-cell binding; c, cell coated with Con A (200  $\mu$ g/ml); g, cell fixed with glutaraldehyde; gc, cell fixed and then coated with Con A.

two normal fibroblasts unless the cells were pretreated with trypsin. Prefixation of a WGA-coated fibroblast did not increase its ability to bind untreated fibroblasts.

### Cell-to-Cell Binding Induced by SBA

SBA did not induce cell-to-cell binding between L, D, M, L1210 cells or normal fibroblasts even if the SBA-coated cell was prefixed with glutaraldehyde. Cell-to-cell binding (100–250 cell-bound cells/cm) was achieved with all these cell types, however, if both the fiber-bound and the free cells were pretreated with neuraminidase. Treatment of only one cell did not induce cell-to-cell binding (Table V). With L and L1210 cells, binding between two neuraminidase-treated cells was enhanced by prefixation of the SBA-coated cell. This

TABLE III  
Effect of Trypsin and Fixation with Glutaraldehyde on Cell-to-Cell Binding of Normal Fibroblasts Induced by Con A

Fiber-bound cell*	Cell-bound cell*	Cell-to-cell binding $cm^{-1}$
Nc	N	79
Ntc	N	194
Nc	Nt	166
Ntc	Nt	226
Ngc	N	227

\* N, normal hamster fibroblast; c, cell coated with Con A (200  $\mu$ g/ml); t, cell treated with trypsin; tc, cell first trypsinized and then coated with Con A; gc, cell fixed with glutaraldehyde and coated with Con A.

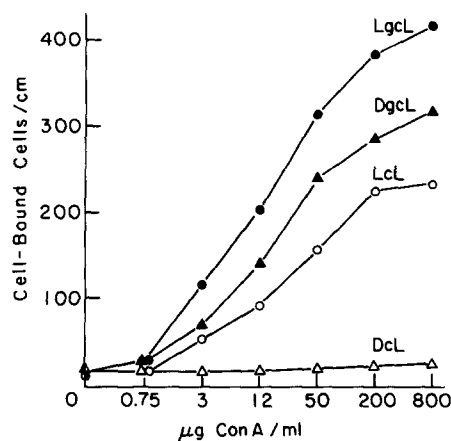


FIGURE 5 Cell-to-cell binding induced by Con A between fixed and unfixed L or D (clone D<sub>16</sub>) cells which have been coated with Con A, and untreated L cells. LgcL and DgcL, binding between a fixed Con A-coated L or D cell and an untreated L cell; LcL and DcL, binding between an unfixed Con A-coated L or D cell and an untreated L cell.

TABLE IV  
Cell-to-Cell Binding of L, D, and M cells Induced by WGA

Fiber-bound cell*	Cell-bound cell*	Cell-to-cell binding
		$cm^{-1}$
Binding between two un-fixed cells		
Lw	L or D	10
Dw	L	176
Dw	D	340
Binding between a fixed and an un-fixed cell		
Lgw	L	18
Lgw	D	256
Dgw	L or D	534
Lw	Lg	8
Dw	Dg	46
Binding between two fixed cells		
Lgw or Dgw	Lg or Dg	6

M cells and L1210 lymphoma cells gave results similar to those shown for D cells.

\* See Table I for abbreviations of cell types. The lower case letters refer to treatment of the cell before cell-to-cell binding: w, cell coated with WGA (100  $\mu$ g/ml); g, cell fixed with glutaraldehyde; gw, fixed and then coated with WGA.

enhancement did not occur with D cells, M cells, or normal fibroblasts. In all cases, binding was prevented by fixation of the uncoated cell or of both cells. Neuraminidase treatment was equally effective when carried out before or after fixation of the cell to be coated with SBA (Table V).

#### Agglutination of Cells in Suspension with Soluble Lectins

In those cases where comparable experiments could be carried out, results of cell agglutination in solution were usually qualitatively the same as those obtained in the cell-to-cell binding assay. Con A induced moderately strong agglutination of L cells but only weakly agglutinated D, M, L1210 cells or normal fibroblasts. WGA strongly agglutinated D, L1210, and M cells, but weakly agglutinated normal fibroblasts. Neuraminidase greatly increased the agglutination of all cell types with SBA. In those cases where cell-to-cell binding

was enhanced by prefixation of only the lectin-coated cell (Tables III-V), this effect was also observed in the agglutination which resulted from mixing fixed lectin-coated cells in suspension with untreated cells in suspension. Large discrepancies were found between the fiber and solution assays in the effect of WGA on L cells and the effect of SBA on M and D cells. Although WGA caused a strong agglutination of free L cells, a fiber-bound L cell which had been coated with WGA did not bind untreated L cells. If a WGA-coated L cell was prefixed, however, it did not bind untreated L cells in neither the fiber nor agglutination assays. Similarly, M or D cells agglutinated with SBA but did not bind to SBA-coated M or D cells, and prefixation of a SBA-coated M or D cell did not increase its ability to bind or to agglutinate with un-fixed M or D cells.

#### DISCUSSION

The cell-to-cell binding induced by various lectins between several types of cells has been analyzed using cells attached to nylon fibers. The fiber assay allows the study of binding between single cells of the same or different type, and separates the binding of lectin to a cell and the attachment of that cell to another cell into successive steps. We have previously used this assay (16) to analyze the binding induced by Con A between lymphoma cells and normal lymphocytes. This study suggested that the requirement for receptor mobility

TABLE V  
Cell-to-Cell Binding with SBA: Effect of Neuraminidase and Fixation with Glutaraldehyde

Fiber-bound cell*	Cell-bound cell*	Cell-to-cell binding
		$cm^{-1}$
Ls	L	10
Lgs	L	23
Lns	L	20
Ls	Ln	32
Lns	Ln	241
Lngs	Ln	401
Lgns	Ln	375
Lns	Lng	28
Lngs	Lng	3

\* L, YAC lymphoma cells; similar results were obtained with the L1210 cell line; s, cell coated with SBA (100  $\mu$ g/ml); n, cell treated with neuraminidase; g, cell fixed with glutaraldehyde. Multiple treatments (Lgs, Lns, Lngs, Lgns) were carried out sequentially from left to right.

in the agglutination of cells by Con A (4, 8, 12) involves movement of specific cell-surface receptors in order to align receptors on one cell with complementary receptors on another cell (16). As discussed previously (16), the requirement for mobility does not appear to involve capping of receptors, or receptor rotation as detected by fluorescence polarization. Identical cell-to-cell binding results were obtained with both clones of D cells although one of them (clone 16 IR<sup>+</sup>D<sup>-</sup>), forms caps with Con A at a much lower frequency than the other (clone 21 IR<sup>+</sup>D<sup>+</sup>) (18). This suggests that the inability of these cells to cell-to-cell bind with Con A is also not related to their ability to cap with the lectin.

In the model proposed for Con A-induced cell-to-cell binding, the complementary receptors are Con A-occupied saccharide receptors on the surface of one cell and unoccupied saccharide binding sites for Con A molecules on another cell. According to this model, stable cell-to-cell binding occurs provided a sufficient number of receptor pairs align, and multiple cell-to-Con A-to-cell bridges are formed before the colliding cells can separate. The bridging by Con A is then followed by the formation of bonds between the cells which, as in previous experiments (14, 16), cannot be reversed by a competitive inhibitor for Con A. The present studies indicate that the Con A-induced cell-to-cell binding also cannot be reversed by trypsinization or changes in milliosmolarity. In contrast to Con A-induced binding, however, we have found that cell-to-cell binding induced by SBA and WGA can be dissociated by addition of a competitive inhibitor for the saccharide binding site on the lectin.

Except for this difference in dissociation after cell-to-cell binding between Con A and SBA and WGA, the present studies suggest that the proposed alignment mechanism for cell-to-cell binding is applicable to a variety of lectins and cell types. Cell-to-cell binding induced between the leukemic L, L1210, D, and M cells and normal fibroblasts by Con A, WGA, SBA, or the waxbean lectin can be completely prevented by prefixation of both the fiber-bound and the cell-bound cell or by coating both cells with the same lectin. The finding that cell-to-cell binding can occur between a prefixed lectin-coated cell and an untreated cell indicates that mobility of receptors for these lectins is required in only one of the two cells. It has been shown that receptors for a multivalent lectin on the cell surface are randomly distributed,

but can be induced to aggregate into clusters upon binding of the lectin. This clustering can be prevented by fixation of the cell with glutaraldehyde or formaldehyde before addition of lectin (4, 8, 12, 13). The observation that the cell-to-cell binding of an untreated cell to a prefixed lectin-coated cell is, in most cases (Table VI), higher than that between an untreated cell and an unfixed lectin-coated cell, therefore, indicates that clustering of receptors is not required and may hinder cell-to-cell binding. The conclusion that prefixation enhances cell-to-cell binding by preventing lectin-induced clustering is supported by the result that there was almost no binding between a fixed cell and an unfixed lectin-coated cell. The strong inhibition of cell-to-cell binding observed by coating the unfixed cell suggests that lectin-induced clustering may reduce the number of available binding sites, steric accessibility, or mobility of cell-bound Con A molecules, so that they are incapable of forming a sufficient number of bonds with immobilized saccharide receptors on the fixed cell.

The results of experiments on the effect of trypsin and neuraminidase on cell-to-cell binding are also compatible with a receptor alignment model. The action of these enzymes may expose new receptors or alter their mobility. If cell-to-cell binding involves the alignment of cell-bound lectin on one cell with unoccupied receptors for the lectin on other cells, the exposure of receptors would be most effective when both cells are treated, whereas changes in mobility can effect cell-to-cell binding even if only one cell is treated. In the present experiments, neuraminidase, which greatly increases the number of receptors for SBA (22), enhanced cell-to-cell binding only when both the fiber-bound cell and the cell-bound cell had been treated. The observation that neuraminidase treatment was equally effective with fixed or unfixed cells suggests, that the increase in SBA-induced cell-to-cell binding caused by treatment with neuraminidase was not only the result of changes in the mobility of receptors for SBA.

It has been inferred from electron microscopy studies (12, 13) that trypsin can increase the mobility of Con A receptors on normal fibroblasts without increasing the number of Con A receptors (1). Trypsinization of only the fiber-bound or cell-bound normal fibroblasts increased cell-to-cell binding almost as much as pretreatment of both cells. This is consistent with a requirement for mobility in only one of the two cells. A similar



effect was obtained with WGA. Increased Con A-induced binding of trypsinized fibroblasts to untreated fibroblasts has also been observed in another cell adhesion assay (14).

Our results indicate that the critical parameters in cell-to-cell binding induced by lectins include a balance between the density of receptors for the lectin, the mobility of receptors on the cell surface, and the lectin-induced clustering of receptors on an unfixed cell (Table VI). The decrease in cell-to-cell binding caused by interaction of a lectin with receptors on an unfixed cell was particularly evident with D and L1210 cells and Con A, in that appreciable cell-to-cell binding only occurred if the lectin-coated cell was prefixed. The difference in the cell-to-cell binding properties of L and D cells, as shown previously for L cells and normal lymphocytes (16), may be almost entirely due to a difference in lectin-induced clustering of receptors. On the other hand, the enhancement of cell-to-cell binding with normal fibroblasts either by trypsinization or by fixation of the lectin-coated cell suggests, that cell-to-cell binding with these cells can be facilitated by either increased receptor mobility on the trypsinized cells or by the greater steric accessibility of diffusely distributed Con A molecules on the fixed cell. It should be noted that in the cell-to-cell binding assay, where the lectin can interact with cell-surface receptors for 30 min

but cell-to-cell contact is very brief, receptors which have sufficient mobility for lectin-induced clustering may not have a rapid enough mobility for the proposed receptor alignment.

In contrast to D cells, the low levels of cell-to-cell binding obtained with M cells cannot be explained only by lectin-induced clustering of receptors. Prefixed M cells coated with Con A did not bind M cells, although their ability to bind L and D cells was increased. Furthermore, Con A-coated L cells, with or without prefixation, bound fewer M cells than L or D cells. M, D, and L cells all have a similar number of receptors for Con A per cell. These results suggest that the cell-to-cell binding of M cells was hindered not only by receptor clustering but also by a low mobility of receptors.

The binding of fiber-bound L cells coated with WGA to other cells also appears to be influenced by both receptor clustering and a low receptor mobility. This is suggested by the observation that little cell-to-cell binding was obtained between an untreated L cell and a fixed or unfixed L cell coated with WGA, whereas binding of M or D cells to a WGA-coated L cell was enhanced by prefixation. In contrast to the results with cell-to-cell binding, L cells were strongly agglutinated in solution by WGA. This difference between the fiber and solution assays can be explained by

TABLE VI  
Summary of Cell-to-Cell Binding Induced by Lectins

Cell-to-cell binding between two:	Con A			WGA			SBA		
	Untreated	Trypsin	One cell fixed*	Untreated	Trypsin	One cell fixed*	Untreated	Neuraminidase	Neuraminidase One cell fixed
L cells (YAC)	Med (clustering)†	Med	High	Low (low mobility)	Low	Low	Low (few receptors)	Med (clustering)	High
L1210 lymphoma	Low (clustering)	Low	High	Med (clustering)	Med	High	Low (few receptors)	Med (clustering)	High
D (clone D <sub>1a</sub> )	Low (clustering)	Low	High	Med (clustering)	Med	High	Low (few receptors)	Med (low mobility)	Low-Med
M cells	Low (low mobility)	Low	Low	Med (clustering)	Med	High	Low (few receptors)	Med (low mobility)	Low-Med
Normal hamster fibroblasts	Low (low mobility, clustering)	High	High	Low (low mobility)	High	Low	Low (few receptors)	Med (low mobility)	Low-Med

Low, intermediate (med), and high refer to the relative number of cell-bound cells per centimeter. For L, L1210, D, and M cells: low = 0-75, med = 150-350, and high = 400-600 cell-bound cells/cm. For normal fibroblasts: low = 0-75, med = 100-175, and high = 200-275 cell-bound cells/cm.

\* Prefixation of the lectin-coated cells.

† The terms given in parentheses indicate the proposed reason for which a high level of cell-to-cell binding between the homologous cells was not obtained: clustering indicates inhibition of cell-to-cell binding caused by lectin-clustering of receptors; low mobility indicates an insufficient mobility of receptors for alignment during cell-to-cell contact; few receptors indicates that the cells do not have sufficient exposed receptors to allow formation of multiple cell-to-lectin-to-cell bridges.

changes in the behavior of receptors for WGA induced by the fiber or differences in the dynamics of the cell-to-cell interaction in the two assays. The agglutination of L cells with WGA is more sensitive to membrane fixation than with Con A or SBA (8). We have also found that untreated L cells do not agglutinate in solution with prefixed WGA-coated cells. These experiments suggest that the mobility of receptors for WGA on L cells is marginally sufficient for alignment in agglutination assays and therefore is affected by mild fixation or fixation of only one cell. In such a case, reduction of receptor mobility on a fiber-bound cell, which has been observed with immunoglobulin receptors on fiber-bound lymphocytes (17), or the shorter collision time between cells in the cell-to-cell binding assay, could have a large effect on the efficiency of receptor alignment. The observation that D and M cells, but not L cells, bind to WGA-derivatized fibers (Rutishauser and Sachs, manuscript in preparation) also suggests that the dynamics of the cell-to-fiber or cell-to-cell collision are critical in this case. The behavior of M cells, D cells, and normal fibroblasts with SBA, which is similar both on fibers and in solution, indicates that with this lectin a low mobility of receptors on these cells inhibits cell-to-cell binding.

Differences in the agglutination of a variety of cell types, most notably between normal and malignant cells, have been observed using a number of lectins (3, 9, 18–20). Our experiments suggest that these differences can result from the relative ability of a cell-bound lectin molecule on one cell and an unoccupied receptor on the other cell to align themselves for the formation of cell-to-lectin-to-cell bridges. With cells which cannot be induced by a lectin to bind other cells (Table VI), this alignment may be inhibited either by a low density of receptors for the lectin, a low receptor mobility, or by lectin-induced clustering of receptors.

This work was supported by contract NO1-CP-42341 with the Virus Cancer Program of the National Cancer Institute, United States Public Health Service. U. Rutishauser is a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

Received for publication 19 July 1974, and in revised form 6 December 1974.

## BIBLIOGRAPHY

1. BEN-BASSAT, H., M. INBAR, and L. SACHS. 1971. Changes in the structural organization of the surface membrane in malignant cell transformation. *J. Membr. Biol.* **6**:183–194.
2. BERLIN, R. D., and T. E. UKENA. 1972. Effect of colchicine and vinblastine on the agglutination of polymorphonuclear leucocytes by concanavalin A. *Nat. New Biol.* **238**:120–122.
3. BURGER, M. 1969. A difference in the architecture of the surface membrane of normal and virally transformed cells. *Proc. Natl. Acad. Sci. U. S. A.* **62**:994–1001.
4. DE PETRIS, S., M. C. RAFF, and L. MALLUCCI. 1973. Ligand-induced redistribution of concanavalin A receptors on normal, trypsinized and transformed fibroblasts. *Nat. New Biol.* **244**:275–278.
5. EDELMAN, G. M., and U. RUTISHAUSER. 1974. Fractionation and manipulation of cells on chemically derivatized fibers and surfaces. *Methods Enzymol.* **34**:195–225.
6. FIBACH, E., M. HAYASHI, and L. SACHS. 1973. Control of normal differentiation of myeloid leukemic cells to macrophages and granulocytes. *Proc. Natl. Acad. Sci. U. S. A.* **70**:343–346.
7. INBAR, M., H. BEN-BASSAT, and L. SACHS. 1973. Difference in the mobility of lectin sites on the surface membrane of normal lymphocytes and malignant lymphoma cells. *Int. J. Cancer.* **12**:93–99.
8. INBAR, M., C. HUET, A. R. OSEROFF, H. BEN-BASSAT, and L. SACHS. 1973. Inhibition of lectin agglutinability by fixation of the cell surface membrane. *Biochim. Biophys. Acta.* **311**:594–599.
9. INBAR, M., and L. SACHS. 1969. Interaction of the carbohydrate binding protein concanavalin A with normal and transformed cells. *Proc. Natl. Acad. Sci. U.S.A.* **63**:1418–1425.
10. KLEIN, E., and G. KLEIN. 1964. Antigenic properties of lymphomas induced by the Moloney agent. *J. Natl. Cancer Inst.* **32**:547–568.
11. LOTEM, J., and L. SACHS. 1974. Different blocks in the differentiation of myeloid leukemia cells. *Proc. Natl. Acad. Sci. U. S. A.* **71**:3507–3511.
12. NICOLSON, G. L. 1973. Temperature-dependent mobility of concanavalin A sites on tumor cell surfaces. *Nat. New Biol.* **243**:218–220.
13. ROSENBLITH, J. Z., T. E. UKENA, T. E. YIN, R. D. BERLIN, and M. K. KARNOVSKY. 1973. A comparative evaluation of the distribution of concanavalin A binding sites on the surfaces of normal, virally-transformed and protease-treated fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* **70**:1625–1629.
14. ROTTMAN, W. L., B. T. WALTHER, C. G. HELLERQUIST, J. UMBREIT, and S. ROSEMAN. 1974. A quantitative assay for concanavalin A-mediated cell agglutination. *J. Biol. Chem.* **249**:373–380.
15. RUTISHAUSER, U., P. D'EUSTACHIO, and G. M. EDELMAN. 1974. Immunological function of lymphocytes fractionated with antigen-derivatized fibers. *Proc. Natl. Acad. Sci. U. S. A.* **70**:3894–3899.
16. RUTISHAUSER, U., and L. SACHS. 1974. Receptor mobility and the mechanism of cell-cell binding

- induced by concanavalin A. *Proc. Natl. Acad. Sci. U. S. A.* **71**:2456-2460.
17. RUTISHAUSER, U., I. YAHARA, and G. M. EDELMAN. 1974. Morphology, motility and surface behavior of lymphocytes bound to nylon fibers. *Proc. Natl. Acad. Sci. U. S. A.* **70**:1149-1153.
  18. SACHS, L. 1974. Regulation of membrane changes, differentiation and malignancy in carcinogenesis. *Harvey Lect.* **68**:1-35.
  19. SELA, B., H. LIS, N. SHARON, and L. SACHS. 1970. Different locations of carbohydrate containing sites in the surface membrane of normal and transformed mammalian cells. *J. Membr. Biol.* **3**:267-279.
  20. SELA, B., H. LIS, N. SHARON, and L. SACHS. 1973. Isolectins from waxbean with differential agglutination of normal and transformed mammalian cells. *Biochim. Biophys. Acta.* **310**:273-277.
  21. SHEVACH, E. M., J. D. STOBO, and I. GREEN. 1972. Immunoglobulin and  $\theta$ -bearing murine leukemias and lymphomas. *J. Immunol.* **108**:1146-1151.
  22. VLODAVSKY, I., and L. SACHS. 1974. Lectin receptors on the cell surface membrane and the kinetics of lectin-induced agglutination. *Exp. Cell Res.* In press.