Receptor Mobility and the Mechanism of Cell–Cell Binding Induced by Concanavalin A

(cells on nylon fiber/receptor alignment/agglutination/lymphoma cells/lymphocytes)

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ABSTRACT The cell–cell binding induced by concanavalin A between single cells has been analyzed by use of cells attached to nylon fibers. Binding of a concanavalin A-coated cell to an untreated cell was found to a high degree between two lymphoma tumor cells, less frequently between a lymphoma cell and a normal lymphocyte, and only rarely between two normal lymphocytes. The binding was inhibited by the presence of a saccharide inhibitor of concanavalin A, but could not be reversed by addition of the inhibitor after the cells had bound to each other. Although no binding was obtained when both cells were coated with lectin or fixed with glutaraldehyde, fixation of a cell before coating with concanavalin A enhanced its ability to bind an untreated cell. The results indicate that cell–cell binding induced by concanavalin A requires short-range lateral movement of cell receptors for the lectin, that only one cell has to have mobile receptors, and that some receptors must be unoccupied by lectin molecules before cell–cell contact. Clustering of the receptors is not necessary and seems to hinder cell-cell binding. It is suggested that the short-range movement is required for alignment of individual receptors so as to form multi-point bridges between two cells by lectin molecules. The bridging is then followed by the formation of irreversible bonds between the cells. The receptors on tumor cells appear to have a greater ability than receptors on normal cells to align themselves for cell-cell binding.

The agglutination of cells by lectins has been used as a probe to study changes in the cell-surface membrane (1, 2). Agglutination is generally determined by adding the lectin to a cell suspension, shaking the suspension, and recording the degree of cell aggregation. Although this agglutination assay is rapid and convenient, it simultaneously measures the binding of lectin to the cells and of several cells to one another.

The specific attachment of cells to chemically derivatized fibers (3, 4) has been used to fractionate and manipulate normal lymphoid cells for morphological and functional studies (5–7). In the present paper, the fiber-rosette method used in antigen-binding studies (5) has been modified for the analysis of cell–cell binding induced by the lectin concanavalin A (Con A). This system allows the quantitative study of binding between single cells, in which the binding of Con A to a cell and the binding of that cell to another cell can be carried out as successive steps. This procedure has been used to study the cell–cell binding of normal lymphocytes and lymphoma tumor cells induced by Con A, and to interpret this interaction in terms of membrane structure and receptor dynamics.

MATERIALS AND METHODS

Cells. Normal lymphocytes were obtained from the lymph nodes of 6- to 8-week-old CR/RAR rats or A strain mice. Cells from the two sources contained 70–85% thymus-derived cells and gave the same results in cell–cell binding assays. Lymphocytes were obtained by teasing the cells into phosphate-buffered saline, pH 7.4 (PBS), and then washing the cell three times with PBS. Lymphoma tumor cells were obtained from a Moloney virus-induced lymphoma grown in A strain mice (8). This is a tumor of thymus-derived cells (9). Cells (10⁶) were inoculated intraperitoneally into adult mice, and the cells were harvested 10–14 days later. The cells were washed three times in PBS before they were used.

Cell–Cell Binding Assay. Nylon fibers were strung in polyethylene frames, derivatized with concanavalin A (Con A, Miles Yeda) at 0.25 mg/ml, and incubated with cells as described (3). The binding of cells to fibers by shaking at 25°C for 30 min was carried out with 2.5 × 10⁵ cells per ml in PBS. The cell–cell binding assay (Fig. 1) involved three steps: (a) binding of cells to Con A–fibers and removal of unbound cells by washing (3); (b) binding of Con A (200 µg/ml, except where noted) for 30 min at 25°C to either the fiber-bound (FB) cells or to free cells, followed by three washes with PBS; and (c) incubation of FB cells with a cell suspension (10⁶ cells per ml in PBS) at 25°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 Con A. After unbound cells were washed away, the number of cell-bound (CB) cells were counted along one edge of a 1-cm segment (Fig. 2). Because normal lymphocytes are smaller than the lymphoma cells, it was not always possible to distinguish between tumor cells bound to normal FB cells and tumor cells that may have bound directly to the fiber. This difficulty did not arise when tumor cells were fixed by glutaraldehyde so as to prevent their binding to the fiber.

Cell Fixation by Glutaraldehyde and Treatment with Metabolic Inhibitors, Trypsin, and Neuraminidase. Glutaraldehyde fixation of fiber-bound cells and free cells (5 × 10⁶/ml) was carried out with 2.5% glutaraldehyde in PBS at 4°C for 2 or 4 hr (10) or with 3% glutaraldehyde in PBS at 22°C for 2 hr (11) with identical results. The cells were then incubated in 0.2 M glycine at 4°C for 10 min, followed by three washes.

Abbreviations: Con A, concanavalin A; F-Con A, fluorescein-labeled concanavalin A; CB cell, cell-bound cell; FB cell, fiber-bound cell; PBS, phosphate-buffered saline, pH 7.4; aM, α-methyl-D-mannopyranoside.
in PBS (10). Fiber-bound or free cells were incubated with metabolic inhibitors (10 mM Na$_2$S or 1 mM 2,4-dinitrophenol) for 30 min at 25° before and during cell–cell binding. Treatment of the cells with trypsin (3× crystallized, Calbiochem; 5 $\mu$g/ml, 30 min, 25°) or neuraminidase (purified, Behringwerke AG; 25–100 $\mu$g/ml, 30–60 min, 37°) was carried out on fiber-bound and free cells before the addition of soluble Con A.

**Fluorescence Microscopy.** The distribution of Con A receptors on fiber-bound and cell-bound cells was observed in situ by fluorescence microscopy (7, 9), with 200 $\mu$g/ml of fluorescein-labeled Con A (F-Con A, Miles Yeda). F-Con A was first used to stain the FB cell and to induce cell–cell binding. F-Con A was then again added to stain the CB cell.

**RESULTS**

**Assay of Cell–Cell Binding Induced by Con A.** Fibers derivatized with Con A were used to study cell–cell binding of normal lymphocytes and lymphoma cells induced by Con A. The general procedure is summarized in Fig. 1 and illustrated in Fig. 2. When 2.5 $\times$ 10$^4$ cells per ml were incubated with Con A fibers, the same number of normal and tumor cells were bound per unit length of fiber. With 2.5 $\times$ 10$^4$ cells per ml, both cell types formed a confluent monolayer along the fiber edge (Fig. 2a). The FB cells could not be removed by subsequent incubation with the competitive inhibitor, $\alpha$-methyl-$D$-mannopyranoside ($\alpha$MM) (6). After binding of cells to the fiber, soluble Con A was added at various concentrations to allow binding of the lectin to receptors not involved in the cell–fiber linkage. The cells were washed to remove unbound Con A and the addition of free cells with receptors for Con A then resulted, in certain cell combinations, in the binding of these cells to the FB cells (Figs. 1 and 2b–d). The presence of 10 mM $\alpha$MM during the incubation of free and bound cells prevented cell–cell binding and the number of cell-bound cells was reproducible to ± 10%. Increasing the concentration of Con A produced more cell–cell binding, with a plateau at about 200 $\mu$g of Con A per ml (Fig. 3). A fiber saturated with cell-

**Fig. 2.** Photographs of fiber-bound cells and of cell–cell binding induced by Con A. (a) Lymphoma tumor cells bound to fiber. (b) Cell–cell binding between tumor cells. (c) Two tumor cells bound to the same FB tumor cell. (d) Normal lymphocyte bound to a FB tumor cell. (e) Fluorescence microscopy of a tumor cell bound to a FB tumor cell, both stained with F-Con A. (f) Fluorescence microscopy of a normal lymphocyte bound to a FB tumor cell, both stained with F-Con A. a and b $\times$332; c-f $\times$996.
TABLE 1. Cell–cell binding induced by Con A between two lymphoma tumor cells

<table>
<thead>
<tr>
<th>Fiber-bound cell*</th>
<th>Cell-bound cell*</th>
<th>Cell-cell binding (cm⁻¹)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fixation:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>T</td>
<td>3</td>
</tr>
<tr>
<td>Tc</td>
<td>T</td>
<td>359</td>
</tr>
<tr>
<td>Fixation of one cell:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tgc</td>
<td>Tgc</td>
<td>581†</td>
</tr>
<tr>
<td>T</td>
<td>Tgc</td>
<td>588†</td>
</tr>
<tr>
<td>Te</td>
<td>Tg</td>
<td>60</td>
</tr>
<tr>
<td>Tc</td>
<td>Tgc</td>
<td>18</td>
</tr>
<tr>
<td>Fixation of both cells:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tgc</td>
<td>Tg</td>
<td>4</td>
</tr>
<tr>
<td>Tg</td>
<td>Tgc</td>
<td>10</td>
</tr>
</tbody>
</table>

* T, tumor lymphoma cell; Tc, tumor cell coated with Con A; Tg, tumor cell treated with glutaraldehyde; Tgc, tumor cell treated first with glutaraldehyde and then with Con A.
† Expressed as the number of cell-bound cells observed along one edge of a 1-cm fiber segment. The standard deviation of 4 determinations was ±10%.
‡ Values are beyond the linear range of the assay. Extrapolation indicates a value of about 650 cells per cm.

bound tumor cells had 650–700 CB cells along one edge of a 1-cm fiber segment. The number of cell-bound cells was proportional to the number of free cells added, up to densities of 450 CB cells per cm. Normal lymphocytes in suspension were hardly agglutinated by Con A, so that these cells could be coated with Con A in suspension, washed, and then bound to FB cells. Similar results were obtained if either the FB or the free cells were coated with Con A (Tables 1–3). Capping of receptors was not observed at the fiber–cell or cell–cell interfaces (Fig. 2). No cell–cell binding was observed if both the free and the FB cells were not coated with Con A or if both cells were coated with Con A. The cells did not form aggregates of the type found with cells bound to beads derivatized with α-galactoside (12).

Effect of Membrane Fixation on Cell–Cell Binding. Fixation of cells inhibits Con A-induced agglutination and slightly reduces the number of Con A molecules that will bind to the cell surface (10). Fixed cells did not bind to Con A-derivatized fibers. Fixation of FB cells was, therefore, carried out after the cells were bound to the fiber. The effects of fixation on cell–cell binding are shown in Tables 1 and 2. When both the free and the fiber-bound cells were treated with glutaraldehyde, cell–cell binding was not observed. If, however, only one of the cells was fixed and then coated with Con A, its ability to bind untreated cells was even greater than that of an unixed cell coated with Con A. Similar results were obtained in solution by mixing the two cell types without further addition of soluble Con A. The coating with Con A of the fixed but not the unfixed cell was critical, in that incubation of fixed cells with unfixed Con A-coated cells gave considerably less binding. Experiments with mouse 3T3 fibroblasts transformed by simian virus 40 gave similar results to those obtained with the lymphoma cells.

Differences in the Cell–Cell Binding of Normal and Tumor Cells. At all concentrations of Con A used there was 7–10 times higher binding between two tumor cells than between two normal cells. Binding between a tumor cell and a normal cell gave intermediate values (Fig. 3). In experiments on binding to a common cell type, unfixed tumor cells gave 2.1–4.8 times more binding than unfixed normal cells, whereas fixed tumor cells gave 1.3–1.7 times more binding than fixed normal cells (Table 3).

Treatment with metabolic inhibitors can increase the agglutinability of normal and tumor cells by Con A (13, 14). Cell–cell binding between two tumor cells and between a tumor cell and a normal cell was also increased about 2-fold by the presence of NaN₃ or 2,4-dinitrophenol. Cell–cell binding between normal lymphocytes, however, was not changed by metabolic inhibitors, and this may reflect membrane changes induced by binding of the lectins to Con A fibers (7). Treatment of cells with trypsin had no effect on cell–cell binding of normal lymphocytes and only slightly increased the binding between the tumor cells; treatment with neuraminidase had no effect on the binding of either cell type.

Reversibility of Cell–Cell Binding and Agglutination. Cell–cell binding or agglutination induced by Con A after a 30-min incubation was not reversed by incubation with 0.01–0.8 M αMM for 30 min at 25°C. The formation of irreversible bonds was not prevented by the presence of metabolic inhibitors during and subsequent to cell–cell binding. Con A can bind to saccharide residues in the dextran polymer of Sephadex beads (15). These beads were agglutinated by Con A at high concentrations of lectin (1–20 mg/ml), and in contrast to cells, the agglutination of the beads was rapidly reversed by αMM.

DISCUSSION

Cells attached to nylon fibers (3, 4) have been used to analyze the mechanism of Con A-induced binding between single cells. Cell–cell binding induced by a lectin has been divided into two steps, the binding of the lectin to a cell and the binding of one cell to another cell. One or both cells can be coated with
The rotational receptors and that lateral cell surface have ability between the normal and cells in of Con cells. but lectin and binding has been binding Cells *A-binding of suspension is required for Con Tgc cell cell can be higher on normal lymphocytes than on lymphoma cells, and treatment of cells with trypsin increased this mobility in both types of cells to a similar value (18). Treatment with trypsin did not change the differences in cell–cell binding between the two cell types. Although some rotation of the receptors may be required, changes in rotational mobility over the ranges described (18), therefore, do not have a large effect on cell–cell binding.

The inhibition of cell–cell binding by fixation of both the fiber-bound and cell-bound cells indicates that cell–cell binding does require receptor mobility. Our experiments also show that fixed cells coated with Con A can bind and agglutinate with unfixed cells that have not been coated with Con A. Pre-fixed cells have been shown by electron microscopy to have a diffuse distribution of Con A-binding sites (11, 19). It therefore appears that clustering of receptors is not a requirement for cell–cell binding or agglutination. It is also unlikely that cluster formation is required after cell–cell contact, because the Con A molecules are held in a diffuse distribution by the fixed cell. The observation that prefixation of Con A-coated cells increases their ability to bind unfixed cells suggests that clustering of receptors by Con A can actually hinder cell–cell binding. This conclusion is also supported by the finding that binding was inhibited if the Con A was on the unfixed instead of the fixed cell. The enhancement of cell–cell binding by metabolic inhibitors is consistent with the suggestion that cellular ATP levels affect the stability of the plasma membrane (13, 20) and the mobility of Con A receptors (13).

The fiber experiments suggest a model for the mechanism of Con A-induced cell–cell binding (Fig. 4). This model requires short-range lateral movement of Con A receptors, over the average distance between two receptors on the same cell, so that Con A molecules bound to one cell can find unoccupied receptors on the other cell. Mobility of receptors is necessary, therefore in only one of the two cells, and at least some of the receptors must be unoccupied by lectin molecules before cell–cell contact. The speed of receptor movement is important because alignment must occur before the colliding cells separate. In contrast to previous suggestions (10, 14, 16, 21), the cluster-
ing of receptors is not required for cell–cell binding and, in fact, may hinder receptor alignment by reducing mobility (17) and blocking receptor sites. The ability of receptors on a cell to cluster after Con A binding, therefore, need not correlate with that cell’s capacity for cell–cell binding.

Our alignment model also involves the formation of irreversible bonds between the cells after alignment of receptors and multi-point bridging by Con A molecules. These irreversible bonds, which also occur between cells and fibers (6), can be formed in the presence of metabolic inhibitors. The complete dissociation of Con A-aggregated Sephadex beads by eMM suggests that multi-point bridging by itself is not sufficient to cause irreversibility. The possibility that the cell–cell binding is initiated only by these irreversible interactions induced by Con A is unlikely, because binding did not occur between two cells both of which had been coated with Con A.

Differences in the ability for the alignment of receptors can explain differences in cell–cell binding of normal and tumor cells. The fluidity of lipids in the surface membrane of the lymphoma cells is greater than that of the normal lymphocytes (22) and this is associated with a higher cholesterol to phospholipid ratio in the normal lymphocytes (23). Assuming that Con A receptors include structures that penetrate into the lipid phase of the membrane, receptor alignment could be facilitated by this higher lipid fluidity. Cell–cell binding might also be influenced by surface charge and cell size. The binding of fixed to unfixed normal lymphocytes and the lack of effect of neuraminidase treatment suggest that charge repulsion alone cannot explain the low binding obtained between normal cells. The differences in binding of normal and tumor cells was decreased by membrane fixation. This finding suggests that these differences involve receptor alignment and are not only due to the smaller size of the normal lymphocytes. It, therefore, appears that receptors on tumor cells have a greater ability than receptors on normal cells to align themselves for the formation of cell–lectin–cell bridges.

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