Cell Surface Interactions with Concanavalin A: Determination by Microhemadsorption[1] (36429)

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It is well known that many tumor cells, unlike normal cells, are readily agglutinated by concanavalin A (Con A) and other plant lectins (1–4). The molecular basis for the large difference in agglutinability is not known, but it has recently been shown that this difference is not due to variation in the number of binding sites since both tumor and normal cells bind similar amounts of radioactively labeled lectins (5–7).

We described here a method which distinguishes between the simple binding of Con A and its availability for agglutination. The method is based on the adherence of red blood cells to test cells (3T3 or SV3T3), by pretreatment with Con A of either the red cells, or the test cells, or both. The test cells can be scored individually, while attached to a substrate, without need for the extensive manipulation required by agglutination assays.

Materials and Methods. Cells and media. Balb/3T3 (3T3) and Balb/3T3 cells transformed by simian virus 40 (SV3T3) were obtained from Dr. S. Aaronson. The cells were grown in 60 mm plastic tissue culture dishes (Falcon Plastics) in Dulbecco's modified Eagle's Medium supplemented with 15% calf serum (Microbiological Associates) and antibiotics in 10% CO_2-air. The cultures were checked periodically for mycoplasma contamination by cultivation on PPLO agar medium (Difco), and were found to be negative.

Agglutination assay. Exponentially growing cells were removed from the culture dishes with 0.02% EDTA in calcium–magnesium-free phosphate buffered saline (CMFPBS) at 37°, washed twice with CMFPBS, and suspended in the same buffer at a concentration of 3 to 10 $\times$ 10^5 cells/ml. Aliquots of 0.5 ml were mixed with 0.5 ml of the appropriate concentration of Con A (3 times crystallized, Miles-Yeda, Ltd.) in phosphate buffered saline (PBS) in a 35-mm plastic petri dish (Falcon Plastics) and rocked gently at 22° for 10 min. The degree of agglutination was then observed with an inverted phase microscope.

Microhemadsorption assay. Outdated units of human type O blood or freshly drawn blood of the same type were used as a source of red blood cells (rbc's). The cells were washed 3 times with PBS immediately before use and suspended at a final concentration of 2% by volume in PBS. "Indicator-rbc's" were prepared by incubation of the washed rbc's with the appropriate concentration of Con A at 37° for 10 min. These cells were then washed three times with PBS and dispersed by pipette to give a single cell suspension. Although cells prepared in this manner and stored at 4° gave consistent results for at least 1 week, they were freshly prepared for all experiments reported here.

The test cells, 3T3 and SV3T3, were removed from the tissue culture dishes with trypsin-EDTA, and 200–500 cells were seeded into the individual wells of Microtest Plates (Falcon Plastics). After incubation for 24–48 hr, the medium was removed and the cells were washed 2–3 times by flooding the entire plate with PBS.

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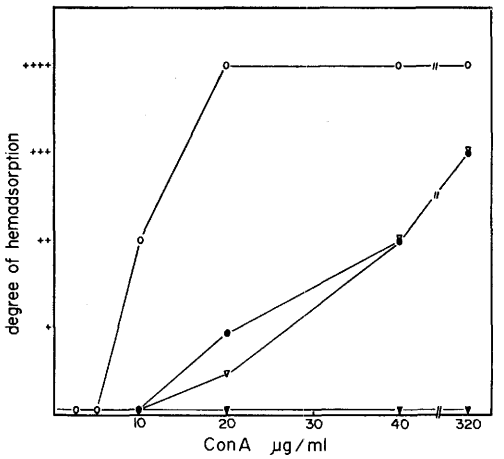

FIG. 1. Determination by microhemadsorption of the interaction of tissue culture cells with Con A. Assays were performed and scored as described in the text. O—O, SV3T3 cells incubated with Con A coated indicator rbc's; ●—●, SV3T3 cells treated with Con A and then with uncoated rbc's; ▽—▽, 3T3 cells treated with Con A coated indicator rbc's; ▼—▼, 3T3 cells treated with Con A and then with uncoated rbc's.

In order to assess the ability of these test cells to bind Con A, indicator rbc's were added to fill each well, and the plates were incubated at 37° for 10–20 min. Alternatively, the test cells were first treated with Con A at the appropriate concentrations at 37° for 10 min and washed, and untreated rbc's were then added to each well. After incubation, unadsorbed rbc's were removed by washing with PBS, and this was facilitated by tapping the sides of the plate against a hard object. The test cells were then scored under phase optics for adherence of rbc's as previously described (8).

Occasionally, the rbc's were suspended in PBS containing 0.1% bovine serum albumin (Fraction V, Pentex) in order to stabilize the cells and enhance their removal from the microtest plate. This addition had no effect on the degree of rbc adsorption to test cells.

Trypsin Treatment. Test cells in the wells of Microtiter Plates were incubated with 0.001% trypsin (crystalline, Schwarz–Mann Biochemical Co.) for 2 min at 22°. The reaction was terminated by washing the plates with PBS containing 0.02% soybean trypsin inhibitor (Chromatographically Pure, Sigma Biochemical Co.) followed by washing twice with PBS alone. The cells were then tested by microhemadsorption as described above.

Results. Characteristics of the assay. With the microhemadsorption assay as described above, the degree of adherence of Con A indicator rbc's to SV3T3 cells increased directly with the concentration of Con A used to coat the rbc's, between 5 and 20 μg/ml. As reported by others (4–7), the concentration of Con A required to produce half-maximal agglutination of SV3T3 cells in suspension was about 20–40 μg/ml (Fig. 1, 2). As expected, 3T3 cells were not agglutinated in suspension by as much as 640 μg/ml of Con A. On the other hand, the adsorption of Con A coated indicator rbc's (see Materials and Methods) was only 2–4 times greater to SV3T3 cells than to 3T3 cells, at equivalent Con A concentrations. Similarly, the concentration of Con A on the rbc's required for equivalent reactivity was only 2–4-fold more for 3T3 than for SV3T3 cells (Fig. 1).

Test cells coated with Con A. In contrast to the findings above, coating of the test cells, but not the rbc's resulted in a very marked difference in hemadsorption. For example, 20 μg/ml of Con A incubated with SV3T3 cells sufficed to produce half-maximum hemadsorption of uncoated rbc's but 3T3 cells did not absorb significant numbers of rbc's when treated with Con A at concentrations as high as 640 μg/ml.

Specificity of hemadsorption. The ability of both 3T3 and SV3T3 cells to adsorb Con A coated indicator rbc's was completely inhibited by addition of α-methyl-D-mannopyranoside to the wells at a final concentration of 50 m*M*. α-Methyl-D-glucopyranoside was inhibitory at 100 m*M* and slightly inhibitory at 10 m*M*. These results are consistent with those reported for inhibition of agglutination and binding in suspension (4, 6, 7).

Inhibition of hemadsorption by coating both rbc's and test cells. Although 3T3 and SV3T3 cells adsorbed indicator rbc's coated with Con A, if the test cells were first coated with a high concentration of Con A (160 μg/ml), hemadsorption with the same indicator rbc's was nearly completely inhibited. This result strongly suggests that adsorption

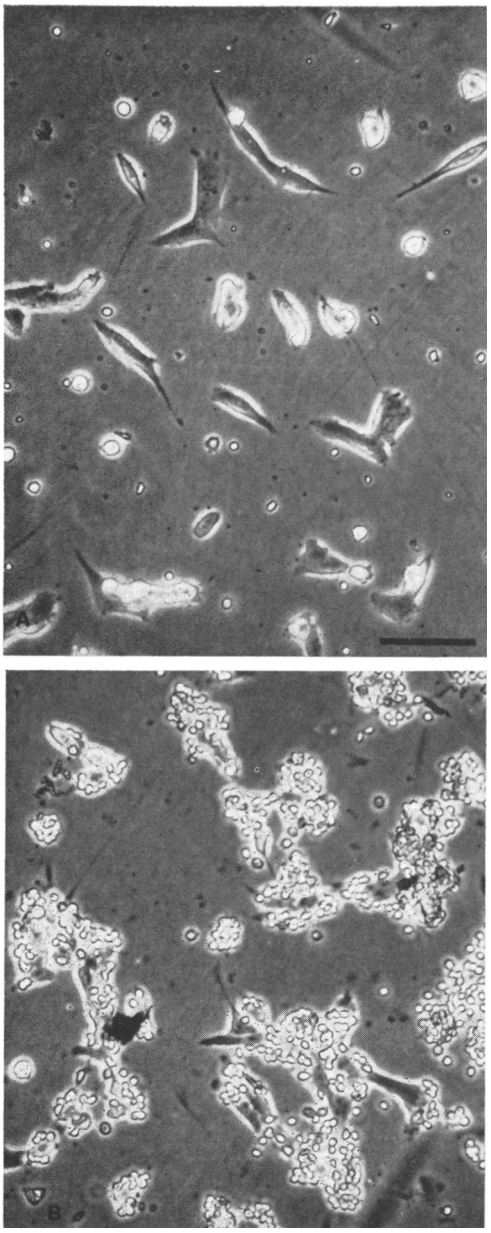

FIG. 2. Adherence of Con A coated indicator rbc's to SV3T3 test cells. (A) rbc's with no Con A, nonreactive; (B) rbc's treated with 20 μg/ml Con A, reactive. Scale represents 100 μ.

of Con A coated cells requires the availability of the specific Con A binding sites on the test cells.

Effects of trypsin. It has been reported previously that trypsin treatment renders normal cells agglutinable by Con A and other plant lectins (3, 4). With the microhemadsorption assay, incubation of 3T3 cells with 0.001% trypsin for 2 min at 22°, followed by treatment of these cells with Con A and addition of uncoated rbc's, resulted in adherence with a half-maximum reaction at only 80 μg/ml of Con A. SV3T3 cells, treated in a similar manner, did not differ from untreated cells in the degree of adsorption. Trypsin treatment of both 3T3 and SV3T3 cells slightly enhanced adsorption of Con A coated indicator rbc's.

Discussion. In contrast to other methods for studying the interaction of plant lectins with cell surfaces (3–7, 9), the microhemadsorption assay described here can be performed on single cells which remain attached to their substrate. We find this rapid and extremely reproducible. Although the method lacks the precise quantitation of radioactive binding studies, it enables determination of both Con A binding and the availability of the bound Con A for agglutination. We have also used the assay to detect small numbers of SV3T3 cells grown in an excess of 3T3 cells.

Our results confirm that Con A molecules on the coated red cells have a binding site available for attachment to receptor sites on other cells (10). Hence, adherence of Con A coated rbc's to 3T3 or SV3T3 cells reflects the binding of the lectin to the surface of these test cells. On the other hand, adherence of uncoated rbc's to Con A coated test cells measures the ability of the bound Con A to participate in agglutination (with red cells). These results also agree with the reported observations that although 3T3 and SV3T3 cells differ greatly in agglutinability, no difference is noted in the binding of lectin to these cells (5–7).

Other recent experiments have demonstrated, by use of ferritin and fluorescein labeling, the presence of Con A on the surface of treated cells in the absence of agglutination (11, 12). We find, moreover, that when 3T3 cells are treated with low concentrations of Con A, washed, and incubated with antiserum prepared against Con A, they become agglutinated.

If receptor sites on both red cells and test cells are occupied by Con A, agglutination is inhibited (see Results). Hence, it is probable that Con A and its specific binding sites participate directly in agglutination, and unlikely that Con A induces agglutination only by altering cell surfaces (7).

While the experiments described here were in progress, a report appeared on the detection of wheat germ agglutinin receptors on human tumor cells by mixed hemadsorption of cells in suspension (13).

Summary. A microhemadsorption method is described for measuring the interaction between concanavalin A and single transformed or untransformed cells without removing them from the substrate. When indicator red blood cells were coated with Con A, their adherence to SV3T3 cells was only 2–4-fold greater than to 3T3 cells. On the other hand, when the two cell lines were coated with Con A, there was a very marked difference in the ability of the lectin on each to adsorb uncoated red cells.

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