**Monoclonal Antibodies Block Cell-Cell Adhesion in Dictyostelium discoideum**

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Of 39 monoclonal antibodies that bind the cell surface of aggregating *Dictyostelium discoideum*, 4 block 78-98% of cell-cell adhesion measured in an *in vitro* assay. The active antibodies all bind in the range of 10⁶ antigens/cell surface and react with more than one material on nitrocellulose blots prepared after polyacrylamide gel electrophoresis of whole aggregating cells in sodium dodecyl sulfate. Active antibodies can be grouped into two classes, each with two very similar members. Class I binds several molecules that are prominent in aggregating cells but scarce or undetectable in vegetative cells, blocks cell adhesion only in the presence of EDTA, and has no detectable effect on cell morphology. Class II binds a wide range of molecules present in both vegetative and aggregating cells, inhibits adhesion as well in the absence as in the presence of EDTA, and reversibly alters cell shape.

Cellular association is believed to be mediated by specific molecules on and between cells. Attempts to identify them often begin by raising antisera against crude cellular antigens in the hope of obtaining one that blocks cell-cell adhesion (1). Such antisera are the basis of an assay for cell adhesion molecules, operationally defined as those that neutralize its anti-adhesion activity.

Much of the work with this approach has been done with the cellular slime mold *Dictyostelium discoideum*, as reviewed recently (2). One major finding is that few antibodies directed against cell surface antigens block cell adhesion, so that those that do may well react with molecules that play a direct role in the adhesion process. The substances identified as neutralizers of adhesion blocking antisera include glycoproteins with M, 80,000 (1, 3), 95,000 (4, 5), and 150,000 (6), and a pronase-resistant glycoconjugate fraction of high molecular weight (7).

An important limitation of this approach is that the complex antisera initially used for screening cannot be used as affinity absorbants to purify the presumed cell adhesion molecules, since they also react with so many irrelevant cellular components. In practice then, purification is done by conventional biochemical techniques with successive enrichment for materials that neutralize the adhesion-blocking antiserum. What this selects for is the molecule richest in those antigens that have this neutralizing property. With this approach, there is a danger of discarding scarcer molecules that also neutralize the antiserum and that could be the true cell adhesion molecules.

In principle, monoclonal antibodies might have advantages for such work, since they could be used not only for initial identification but also for subsequent purification of cell adhesion molecules. However, it is easy to conceive of limitations. For example, many monoclonal antibodies that react with antigenic determinants on a molecule involved in cell adhesion might have no functional effect, while the complex antiserum might have many antibodies directed against the same molecule, thus ensuring both specificity and biological efficacy. Indeed, several monoclonal antibodies that react with the glycoprotein with M, 80,000, strongly implicated in cell adhesion (1), are inactive (3). We here describe 4 monoclonal antibodies that inhibit cell-cell adhesion extensively. These preliminary results, therefore, establish the feasibility of using monoclonal antibodies to identify cell adhesion molecules.

**MATERIALS AND METHODS**

**Preparation of Monoclonal Antibodies**—The 8-azaguanine-resistant murine myeloma cell line P3-NS/1/AG 4.1, obtained from Ivor Royston of the Veterans’ Administration Medical Center, San Diego, CA, was maintained in RPMI 1640 (Irvine Scientific Co., Santa Ana, CA) supplemented with 10% calf serum and 2 mM glutamine. Hybridomas secreting monoclonal antibodies (9) were produced by fusion (9) of the myeloma cells with spleen cells from immunized BALB/c mice. Each mouse was injected subcutaneously with 10⁷ aggregating *D. discoideum* cells in complete Freund’s adjuvant followed by two biweekly subcutaneous injections in incomplete Freund’s adjuvant. One week thereafter, 10⁸ cells were injected into the tail vein. Four days later, the spleen was removed for fusion (9). Supernatants from resulting hybridomas were screened for binding to living aggregated *D. discoideum* cells immobilized on glass filters (10) using a Microfold immunofiltration system (V&P Scientific, San Diego, CA). Antibody bound to cells was detected using horseradish peroxidase conjugated affinity purified goat anti-mouse IgG² (Tago, Inc., Burlingame, CA) and 2,2-azino-di-[3-ethyl-benzothiazoline-sulfonate(6)] (Litton Bionetics, Inc., Kensington, MD) as substrate. Hybridomas found positive in this initial assay were cloned by plating at limiting dilutions, then incubated for several days and the tight aggregate stage, as described previously (11).

**Cell-Cell Adhesion Assays**—*Dictyostelium discoideum* NC-4 was grown and differentiated until the tight aggregate stage, as described previously (11). For adhesion assays (7, 12), the aggregates were dissociated and cell-cell adhesion was measured with an electronic particle counter after incubation of cells with or without antibodies. Assay mixtures contained 10 mM EDTA except where indicated. In most assays, intact monoclonal antibodies were used following the procedure used with intact rabbit immunoglobulins from a polyclonal antiserum (7). To prevent possible agglutinating effects of the immunoglobulin, univalent antibody fragments of goat anti-mouse IgG² were used in the assay mixture as explained previously. In some experiments, indicated in the text, univalent fragments from the monoclonal antibodies were prepared and assayed by procedures essentially like those described previously (7).

GAMFab Binding—GAMFab (Cappel Laboratories, Inc., Cochranville, PA) was iodinated using Iodobeads (Pierce Chemical Co.) by the method described in the accompanying literature. Labeled

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GAMFab was separated from unreacted iodine on a Sephadex G-25 column (Pharmacia, Piscataway, NJ) and was used to measure the binding of monoclonal antibodies to slime mold cells. A 250-μl aliquot of cells that had been incubated with a monoclonal antibody preparation, as in the cell-cell adhesion assay, was washed with 2 ml of cold 0.5% normal goat serum in 17.5 mm sodium potassium phosphate, pH 7.2. The cells were then resuspended in 250 μl of the medium used for washing to which was added 250 μg of 125I GAMFab. The suspension was shaken on ice for 1 h then thoroughly dispersed by vortexing to dissociate any aggregates. Duplicate 100-μl aliquots were layered over 0.5 ml 10% Ficoll (M, 400,000, Sigma), centrifuged, and the pellet counted in a γ scintillation counter. Control cells were treated identically except that they were not reacted with monoclonal antibody.

The small number of counts they bound was subtracted from the results.

Detection of Relevant Antigens—Whole or aggregated or vegetative D. discoideum cells were solubilized and separated by polyacrylamide slab gel electrophoresis in sodium dodecyl sulfate (14) using a 10% polyacrylamide sample gel. Proteins were transferred to nitrocellulose sheets (14) in an electrophoretic blotting chamber (CBS Scientific, Del Mar, CA) at 10 V/cm, 200 mA overnight at 4°C. Binding of antibodies was determined by incubating solutions containing 0.1 mg/ml in 2.5% normal goat serum, 0.05% Tween 20, 50 mM Tris hydrochloride, pH 7.2, 7.5% NaCl with nitrocellulose strips, and visualizing antibody binding by incubation with a 1:50 dilution of peroxidase-labeled goat anti-mouse IgG (Tago, Inc., Burlingame, CA) in the above solution without antibody and development using 4-chloro-1-naphthol (Polyscience Inc., Warrington, PA).

RESULTS

Thirty-nine hybridomas were identified that secreted antibodies that bound to the surface of aggregating D. discoideum cells. All were grown as peritoneal tumors in mice and the antibodies were isolated by ammonium sulfate precipitation from ascites fluid. The antibodies can be divided into groups based on the number of available surface antigens estimated by preincubating with high antibody concentrations followed by incubation with 125I GAMFab (Table I). The results are expressed as number of 125I GAMFab molecules bound per cell which, based on comparative studies using 125I-labeled antibody d-41, binds at a ratio of about three 125I GAMFab molecules/immunoglobulin molecule. Although this ratio varies with the individual immunoglobulin (15), 125I GAMFab binding provides a convenient estimate, obviating the need to label each antibody directly.

Most of the antibodies bound antigens displayed at less than 10 10 copies/cell surface (Table I) as estimated with 125I GAMFab, but five bound to more abundant antigens (Tables I and II). Four of these blocked-cell-cell adhesion (Tables I and II). Since all bound so extensively, it seemed possible that they acted by nonspecifically coating the cell surface. How-
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Having identified adhesion-blocking monoclonal antibodies by assaying them as intact immunoglobulins, we determined the effect of univalent fragments of antibodies d-41 and d-48 on adhesion in 10 mM EDTA. Both inhibited, but only under special conditions. First, it was necessary to use much higher concentrations and to maintain them in the assay mixture. Second, to get a maximal inhibitory effect, it was necessary to extend the duration of the assay from the usual 10 to 30 min, since inhibition increased up to this time. Under these conditions, univalent d-48 totally inhibited adhesion at a concentration of 12 mg/ml in the preincubation and assay mixtures. Univalent d-41 inhibited 40% at preincubation and assay concentrations of 5 mg/ml, the highest we used. As a control, d-44, which was inactive as intact immunoglobulin, was also inactive as univalent fragments, having no effect at 15 mg/ml in both the preincubation and assay mixtures.

Identification of Relevant Antigens—As a first approach to identifying the antigens that react with the monoclonal antibodies, we fractionated vegetative and aggregating cells by gel electrophoresis and reacted the antibodies with the indicated concentrations of antibodies, and the amount of antibody bound to aliquots of the cells was estimated using 125I GAMFab. Other aliquots were used in adhesion assays in an incubation mixture containing 10 mM EDTA.

These studies show that it is possible to raise monoclonal antibodies that block cell-cell adhesion of D. discoideum. Screening in a cell adhesion assay may be done with the intact immunoglobulins rather than univalent fragments, as suggested previously (7, 16). Intact monoclonal antibodies have also been used recently to block adhesion of cells to substrata (17, 18).

The positive antibodies may be grouped into two classes.

**DISCUSSION**

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**Table III**

Properties of two classes of monoclonal antibodies that block cell-cell adhesion in D. discoideum

<table>
<thead>
<tr>
<th></th>
<th>Class I</th>
<th>Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibodies</td>
<td>d-39, d-41</td>
<td>d-47, d-48</td>
</tr>
<tr>
<td>Block adhesion in 10 mM EDTA</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Block adhesion without EDTA</td>
<td>No*</td>
<td>Yes</td>
</tr>
<tr>
<td>React with developmentally regulated molecules</td>
<td>Yes</td>
<td>No*</td>
</tr>
<tr>
<td>Inhibition of attachment and spreading on plastic</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Inhibition of cell-cell adhesion at partial saturation</td>
<td>Marked</td>
<td>Minimal</td>
</tr>
</tbody>
</table>

* Partial effect at very high concentrations.
* None consistently observed.

(Table III). Each has two members that are not identical, at least by the criterion of immunoglobulin subtype. Class I is specific for a developmentally regulated adhesion process like that described by Gerisch (1). Whether or not Class I reacts with the glycoprotein with $M_r \sim 80,000$ that it implicated in cell adhesion is uncertain. The band with $M_r \sim 90,000$ that reacts with Class I could be identical with the $M_r \sim 80,000$ since it shows variable migration in polyacrylamide gels (19).

Our results should encourage further attempts to evaluate monoclonal antibodies specific for that glycoprotein despite previous negative results (3). Although Class I reacts with a prominent protein band with $M_r$ similar to discoidin I, an endogenous lectin implicated in cell adhesion in D. discoideum (2), the monoclonal antibodies do not bind this protein.

The monoclonal antibodies we have raised can be used to purify all D. discoideum molecules with the antigenic determinants with which they react. We should then be in a position to determine which of these molecules are actually implicated in the cell adhesion process.

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REFERENCES

Monoclonal antibodies block cell-cell adhesion in Dictyostelium discoideum.
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