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The Role of Membrane Lectins in Dictyostelium discoideum Aggregation as Ascertained by Specific Univalent Antibodies Against Discoidin I

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Antibodies against pure discoidin I have been used as a tool to ascertain the role of this lectin in aggregation of Dictyostelium discoideum. Discoidin I is widely expressed over the cell surface of aggregation-competent AX-2 cells, as ascertained by indirect immunofluorescence with specific (antidiscoidin I) antibodies. Univalent antidiscoidin I antibodies (Fab fragments) inhibit the aggregation-specific intercellular adhesion of D discoideum AX-2 cells in an in vitro assay. This inhibition depends on antibody concentration and cell density; a 50% inhibition of cell aggregation was obtained at antidiscoidin I Fab concentration of 4.5 mg/ml and 1×10^6 cells/ml. Aggregation and morphogenesis on solid support is also effectively inhibited when AX-2 cells are starved in the presence of antidiscoidin I Fab fragments. The inhibition of morphogenesis is also dose dependent and more effective than in the in vitro assay. No inhibition of aggregation either in the in vitro assay or on morphogenesis on solid support was observed with preimmune Fab fragments at any of the concentrations tested (up to 9.6 mg/ml).

Key words: intercellular adhesion, Dictyostelium discoideum, discoidin I, antibodies, antidiscoidin I Fab fragments, in vitro reaggregation, morphogenesis

The use of complex antisera [1–3] and more recently monoclonal antibodies [4,5] is one of the few approaches that has provided direct evidence for the involvement of specific molecules in the cell-adhesion system of D discoideum. This approach has been particularly useful in the study of "contact sites A," which have been identified with a membrane glycoprotein of 80 kilodaltons (Kd) [1–4]. In addition to contact sites A, two carbohydrate-binding proteins, discoidin I and II, also accumulate during aggregation [6] and have long since been implicated in the cell-adhesion processes of D discoideum [7]. Both of these lectins have been detected on the surface of aggregation-competent cells [8,9] by radioiodination of the cell surface followed by immunoprecipitation [8]. Up to 92% of discoidin I and 72% of discoidin II can be

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easily eluted from the cell surface with 0.5 M galactose [10], suggesting that most of the endogenous lectins are bound to cell surface receptors. A direct involvement of discoidin I in the aggregation-specific cell adhesion processes is supported by the isolation of an aggregation-defective mutant whose morphogenetic defect can be traced back to a biologically inactive discoidin I [11]. On the other hand, more recently, an 80-Kd glycoprotein with the properties expected for a discoidin I receptor has been shown to interfere with intercellular adhesion in D discoideum [12].

This evidence notwithstanding, doubts about a direct involvement of discoidins in intercellular adhesion still persist, since attempts to inhibit the aggregation processes by antidiscoidin antibodies have so far failed [13], and low concentrations of added discoidin did not affect cell cohesion [7]. Nevertheless, galactose delays the expression of contact sites A-mediated cell adhesion [14], suggesting that discoidins may play a regulatory role rather than a direct role in cell cohesion. In both cases, discoidin is presumed to function by binding to cell surface receptor(s) bearing galactose or a structurally related sugar residue as the interacting ligand. In fact, direct evidence for cell surface receptors for discoidin I have been provided [15–17]. In this report, we present evidence for a direct role of discoidin I in aggregation-specific cell cohesion processes in D discoideum, strain AX-2, showing that univalent antibodies against pure discoidin I can effectively inhibit the intercellular adhesion of aggregation-competent cells in an in vitro assay as well as aggregation and further development when cells are starved on agar in the presence of antidiscoidin I univalent antibodies.

METHODS

Strain, Growth, and Developmental Conditions

Dictyostelium discoideum, axenic strain AX-2, was used in all experiments. Cells were grown in axenic medium HL-5 [18] up to a density of 2–4 $\times$ 10^6 cells/ml. Cells were collected by centrifugation, and after washing three times in distilled water they were resuspended in the appropriate buffer for development. Cells to be developed in suspension were resuspended in 17 mM phosphate buffer, pH 6.0, at a density of 3 $\times$ 10^7 cells/ml and agitated at 22°C in a gyratory New Brunswick bath at 140 rpm. Cells to be developed on filters were resuspended in MES-PDF buffer [19] (per liter: 1.5 g MES, 1.5 g KCl, 0.6 g $MgSO_4$, pH 6.5 with KOH, containing 0.5 mg streptomycin) at a density of 1.5 $\times$ 10^8 cells/ml. Aliquots (0.33 ml) of the cell suspension were spread on Millipore filters (2 $\times$ 10^6 cells/cm^2) supported on two Whatman pads embedded in the same buffer and allowed to develop in a humid chamber at 22°C. Under these conditions, streaming was clearly seen after 7 hr of development and the whole culmination process took about 24 hr. No differences in the timing of streaming or fruiting body construction were observed between AX-2 cells starved in the presence of either MES-PDF or PDF, as reported for AX-3 strain by other authors [19].

Purification of Discoidin I

Discoidin I was purified by affinity chromatography on Sepharose 6-B [16] from whole cell extracts of 2 $\times$ 10^{10} AX-2 cells developed during 11 hr in agitated suspension. After extensive washing of the column (200 ml packed volume) with ECT buffer (15 mM Tris-HCl, 75 mM KCl, 75 mM NaCl, 1 mM EDTA, final pH

7.3) discoidins were specifically eluted with 0.3 M D-galactose in ECT buffer. Discoidin I was obtained free of discoidin II by careful pooling of the chromatographic fractions corresponding to the first leading half of the galactose-specific eluate (pool I in Fig. 1). The rest of the fractions of the protein eluate was also collected (pool II, Fig. 1). After extensive dialysis against water, the samples were lyophilized and stored at −20°C.

Obtainment of the Antiserum Against Discoidin I

Antiserum to the purified discoidin I was obtained by immunization of New Zealand white rabbits with an initial subcutaneous and intradermal injection of 0.5 mg of discoidin I protein in complete Freund's adjuvant, followed by two intradermal injections of the same dose in incomplete adjuvant at weekly intervals. Three weeks after the last injection a booster intradermal injection of 1 mg of discoidin I was given and the animals were bled 4 days later. The sera were stored at −20°C.

Obtainment of the Fab Fragments

IgG from the antiserum and preimmune serum were purified by affinity chromatography on protein A-Sepharose. Univalent antibodies (Fab fragments) from immune (anti-DI) and preimmune purified IgG were obtained by papain digestion [20] and further purified by negative adsorption on protein A-Sepharose. The wash-off fraction (Fab fragments) and 1 M acetic acid eluate (Fc fragments and undigested

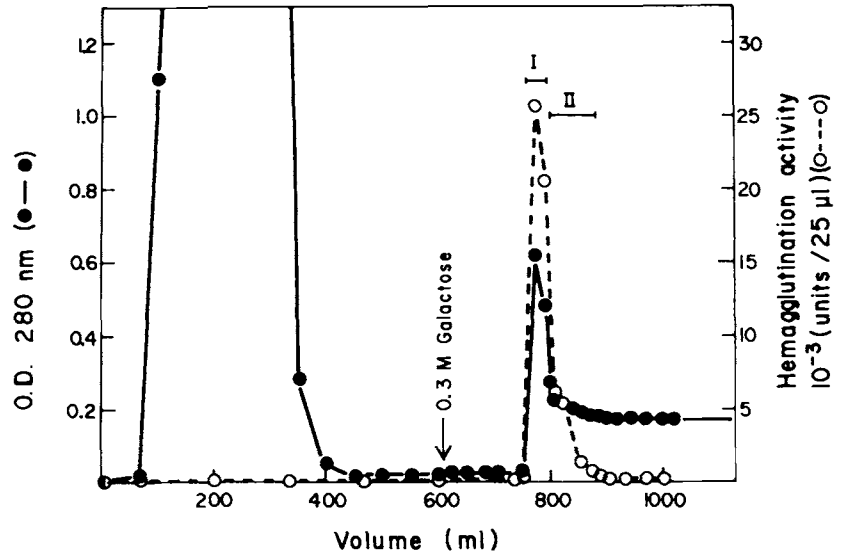

Fig. 1. Affinity chromatography purification of discoidin I. Discoidin I from whole soluble extract of 11-hr-developed cells (2×10^{10} cells) was purified by chromatography on Sepharose-6B, as described in Methods. Fractions of 10 ml were collected and analyzed by optical density at 280 mm (●) and their hemagglutination activity was determined with formalinized rabbit erythrocytes (○) as described [6]. Fractions from the D-galactose eluate with agglutination activity were collected in two pools as indicated.

IgG) were dialyzed against 10 mM $(NH_4)_2CO_3$, lyophilized, and stored at −20°C. Fab fragments were shown to be free of Fc and undigested IgG by electrophoretic analysis (not shown).

Immunoautoradiographic Detection of Protein Antigens

Purified discoidin I and proteins from whole soluble extracts (100,000g) were electrophoresed on 10% polyacrylamide slab gel in the presence of sodium dodecyl sulfate (SDS) and transferred to activated aminophenylthioeter paper (APT) as described [21], except that the pH of the electrophoretic transfer was lowered to 8.5. All the subsequent steps for deactivation of the paper and incubations with the antiserum and ^{125}I-protein A were carried out as described [22]. Following the incubations the paper was exposed to preflashed Kodak X-Omat film and Dupont intensifying screen at −70°C.

Cell Aggregation Assay in the Presence of Fab Fragments

AX-2 cells were developed on Millipore filters for 7 hr, when streaming is clearly visible under microscopic observation (×40 magnification). Cells were then detached from the filters and suspended at 2×10^6 cells/ml in 17 mM phosphate buffer, pH 6.0, containing 10 mM EDTA (PE buffer). Cells were dispersed into single cells by repeated pipetting and vortexing, and their aggregation competence was ascertained by measuring the cohesivity after 20-min incubation in a gyratory bath at 120 rpm and 22°C. Cohesiveness was measured as the loss of single cells and doublets during gyration. The percent of aggregation was calculated as $1 - N_t/N_0 \times 100$, where N_t is the number of single cells and doublets after the incubation period and N_0 is the number of the initially dispersed cells. Cell samples were directly counted in a hemocytometer.

In order to study the effect of Fab fragments on the ability of aggregation-competent cells to reform specific contacts, the cell suspension was dispersed again as above. Aliquots of 100 μl were mixed with an equal volume of PE buffer containing variable amounts of immune (antidiscoidin I) or preimmune Fab fragments in small vials with round plain bottoms, 1 cm in diameter. After a preincubation at 0°C for 30 min, the cells were vortexed for 10 seconds and incubated in the gyratory bath at 22°C and 120 rpm. After 30-min incubation the number of cells remaining as single or doublets was directly counted with a hemocytometer, and the degree of aggregation was calculated as described above.

Development in the Presence of Fab Fragments

To examine the effects of antidiscoidin I Fab fragments on the development of D discoideum, washed vegetative AX-2 cells were resuspended in 17 mM phosphate buffer, pH 6.0. 2×10^5 cells were mixed with different amounts of anti-DI Fab fragments in phosphate buffer in a final volume of 50 μl and plated onto individual wells of a Nunc tissue culture plate (1.5 cm diameter/well) containing 1% agar in 17 mM phosphate buffer, and allowed to develop at 22°C. The effects on cell morphology were determined 9 and 24 hr after plating in a Nikon inverted microscope using phase optics.

Indirect Immunofluorescence

Cells that had developed 4 and 7 hr were dissociated from the filters and, after washing two times with PE buffer, were resuspended in 50 μl of a 1/50 dilution of

anti-D I serum made in PE buffer. After 30-min incubation at room temperature cells were extensively washed with cold PE. Cells were then treated with 50 μl of 1/20 dilution of rhodamine-conjugated goat antirabbit IgG (Sigma Chemical Co) in PE. Following 30-min incubation at room temperature, cells were washed several times with cold PE and finally resuspended in 50 μl of PE. Cells were then viewed with fluorescence optics in a Zeiss microscope.

Other Analytical Methods

Protein concentration of IgG, Fab fragments, and discoidin I were determined by the Lowry method [23]. Purified discoidins and Fab fragments were analyzed on 10% polyacrylamide slab gels containing SDS in the Laemmli system [24].

RESULTS

Preparation and Characterization of Discoidin I and Anti-D I Serum

In order to study the possible effects of anti-D I univalent fragments on cell aggregation and development of D discoideum, we have purified discoidin I from aggregation-competent cells as described in Methods. Care was taken to isolate the first leading half of the specific D-galactose eluate as to obtain a clear-cut separation of discoidin I and II (Fig. 1). The purity of discoidin I preparation was greater than 99% as ascertained by polyacrylamide gel analysis (Fig. 2).

Antiserum to purified discoidin I was raised as described in Methods. The immune serum gave a titer of 0.95 mg of specific IgG per ml as determined by quantitative immunoprecipitation. The specificity of the antiserum was tested by double diffusion in 1% agarose and by immunoelectrophoresis. As can be seen in Figure 3, the immunoelectrophoretic analysis shows a main precipitation arc when the antiserum is tested against whole soluble extracts from vegetative cells and 9-hr developed cells, and a very faint second arc is barely detected in extracts from vegetative cells. This second arc might correspond to discoidin II present in such extracts as it is shown when the antiserum is tested against a mixture of both discoidins, enriched in discoidin II (pool II from Fig. 1). This result indicates a cross-reactivity of the antiserum for both discoidins similar to that recently reported [25].

We have also studied the specificity of the antiserum by immunoradiographic detection of antigen-antibodies complexes on protein extracts transferred to activated APT paper. As shown in Figure 4, discoidin I is the major protein recognized by the immune serum when challenged with total proteins in whole soluble extracts from vegetative cells (lane 1) or aggregation-competent cells (lane 2). This study also showed that the discoidin I concentration in whole extracts increases from vegetative to aggregation-competent AX-2 cells (compare lane 1 and 2) as reported for NC-4 cells [8]. More detailed studies (not shown) indicte that the highest rate of discoidin I synthesis in AX-2 cells is reached between 5–7 hr of development, whereas the period of maximal accumulation (as ascertained by electrophoretic and erythrocyte agglutination analysis) was observed between 7–9 hr of development. Besides discoidin I, two other proteins were recognized by the antiserum (see arrows in Fig. 4). Discoidin II was detected in extracts from vegetative cells (lane 1), confirming the cross-reactivity observed in the immunoelectrophoretic analysis (see above). Another protein, migrating with an apparent molecular weight of 57 Kd (lane 1), could also be detected as a minor component in the discoidin I preparation (lane 4) on longer

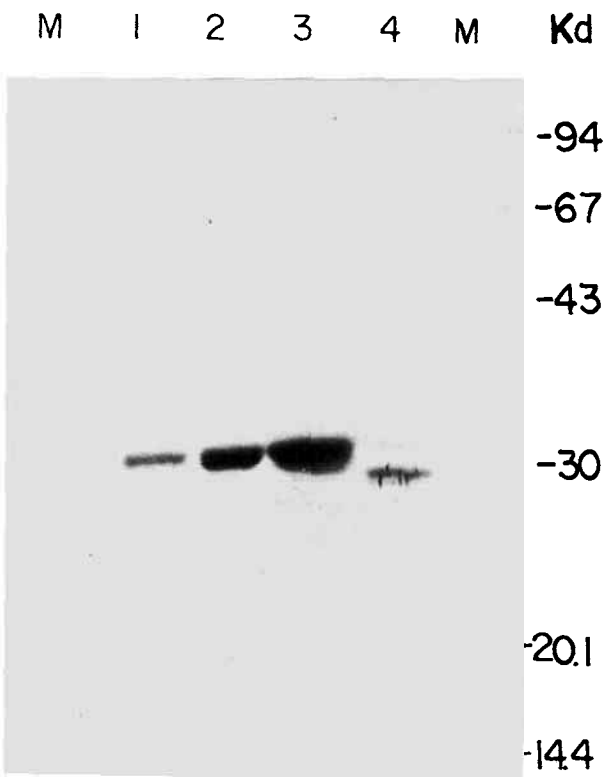

Fig. 2. Electrophoretic analysis of discoidin I preparation. Samples from pool I and pool II from Figure 1 were analyzed on 10% polyacrylamide slab gel in the presence of SDS. The figure shows the Coomassie blue staining of proteins in the gel. Lanes 1, 2, and 3 correspond to aliquots of pool I (3, 6, and 12 μg of protein, respectively) showing one single band of 31 Kd, corresponding to discoidin I. Lane 4 is an aliquot (5 μg of protein) from pool II showing the two bands (31 and 29 Kd) corresponding to the mixture of discoidins I and II. Lane M corresponds to the molecular weight markers.

autoradiographic exposures (4 hr, not shown). The presence of the 57-Kd protein in the "electrophoretically pure" discoidin I preparation suggests that it can represent a high-affinity binding protein for discoidin I copurifying with it. In this context, it is worth mentioning that Siu et al have reported the presence of a 56-Kd protein in their immunoprecipitates of D discoideum membrane proteins with antidiscoidin serum [8].

Localization of Discoidin I on the Cell Surface of Aggregation-Competent Cells

Indirect immunofluorescence studies were carried out in order to demonstrate the binding of anti-D I antibodies to the cell surface. Cells starved on filters were detached after 4 and 7 hr of development and treated with anti-D I serum followed by rhodamine-conjugated goat antirabbit IgG as described in Methods. As shown in Figure 5B, 7-hr-developed cells treated with anti-D I serum display intense fluorescence over the entire cell surface, and some apparent capping can be observed. In

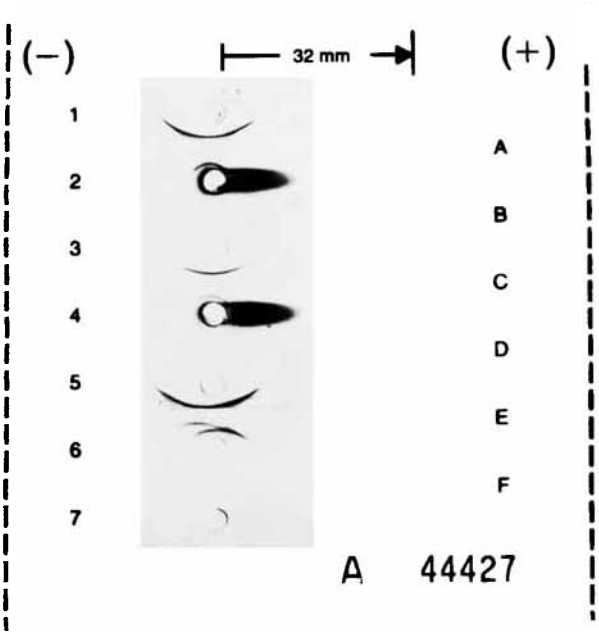

Fig. 3. Immunoelectrophoretic analysis of antidiscoidin I serum. The specificity of the antiserum was analyzed by immunoelectrophoresis against whole soluble extracts and purified discoidins. Wells 1, 3, and 5—22.5 μg of purified discoidin I (pool I from Figs. 1,2): 2 and 4—75 μg of whole soluble extract from vegetative and 9-hr-developed cells, respectively; 6 and 7—18 μg of a mixture of discoidin I and II, enriched in discoidin II (pool II from Fig. 1 and lane 4 from Fig. 2). Slots A, C, E—anti-D I serum; B, D, F—preimmune serum.

contrast, 4-hr-developed cells treated in the same way (Fig. 5A) display less intense fluorescence and it appears more disperse over the whole cell. Treatment with preimmune serum as the first antibody does not induce any fluorescence at all.

Effect of Anti-D I Fab Fragments on Cell Aggregation

The effect of anti-D I Fab fragments on cell aggregation was tested in an in vitro reaggregation assay using cells developed for 7 hr on solid support. At this time streaming is clearly visible on the filters under microscopic observation, and discoidin I is widely expressed over the cell surface, as demonstrated by the immunofluorescence studies. Three independent experiments carried out with the specific immune Fab fragments consistently showed a dose-dependent inhibition of cell aggregation (Fig. 6) with an approximate 50% inhibition at Fab concentration of 4.5 mg/ml (1×10^6 cells/ml). No inhibition of cell aggregation was observed in two independent experiments with control preimmune Fab, at any of the concentrations assayed. The inhibition of cell aggregation by the antidiscoidin I Fab fragments was also evident from a qualitative point of view in the microscopic observation: the size of the aggregates decreased with increasing concentrations of the immune univalent antibodies, while it remained unchanged by the preimmune Fab. The inhibitory effect lasted about 90 min at 22°C. After that, cells started to form aggregates of increasing size, and subsequent disappearance of individual cells was observed. The mechanism of this reversal is unknown, although it could be related to the externalization of

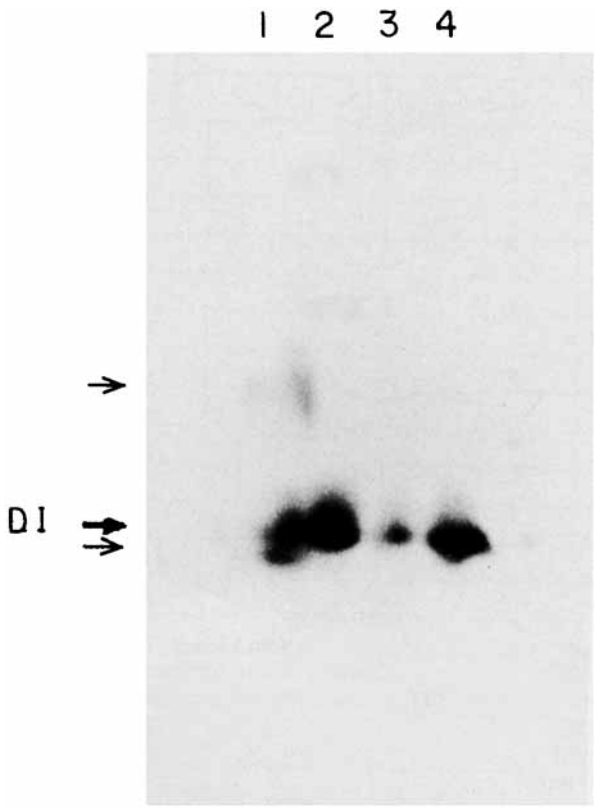

Fig. 4. Specificity of antidiscoidin I serum as ascertained by immunoautoradiography of transferred proteins to APT paper. Purified discoidin I and soluble extracts from vegetative cells and cells developed for 9 hr in agitated suspension were electrophoresed on 10% polyacrylamide slab gel in the presence of SDS and transferred to activated APT paper as described under Materials and Methods. The quality of the protein transfer was checked by staining of the gel with Coomassie blue after the transference; more than 90% of the protein in the gel was transferred to the APT paper by this criterion. The figure shows the autoradiography of the paper after 45-min exposure. Lane 1, soluble extract from vegetative cells (100 μg of protein in the gel); lane 2, extract from 9-hr-developed cells (100 μg of protein); lanes 3 and 4, purified discoidin I (1.5 and 3 μg of protein).

endogenous lectins by divalent antibodies shown in two related Dictyostelium species [26,27]. Whatever its mechanism, the time-dependent reversal of the specific Fab inhibition on cell reaggregation rules out any artifactual alteration of the cells upon its exposure to the immune Fab fragments.

We have also studied the effect of cell density on reaggregation in the presence of anti-D I Fab fragments. As shown in Table I, cell density has a crucial effect on the reaggregation assay: inhibition of reaggregation in the presence of 4.5 mg/ml anti-D I Fab fragments decreases as cell density increases. With 4×10^6 cells/ml, the degree of inhibition observed is only about 12% of the control. The cell density effect is specific, since treatment of the cells at any of the densities with the same concentration of preimmune Fab fragment does not influence cell reaggregation.

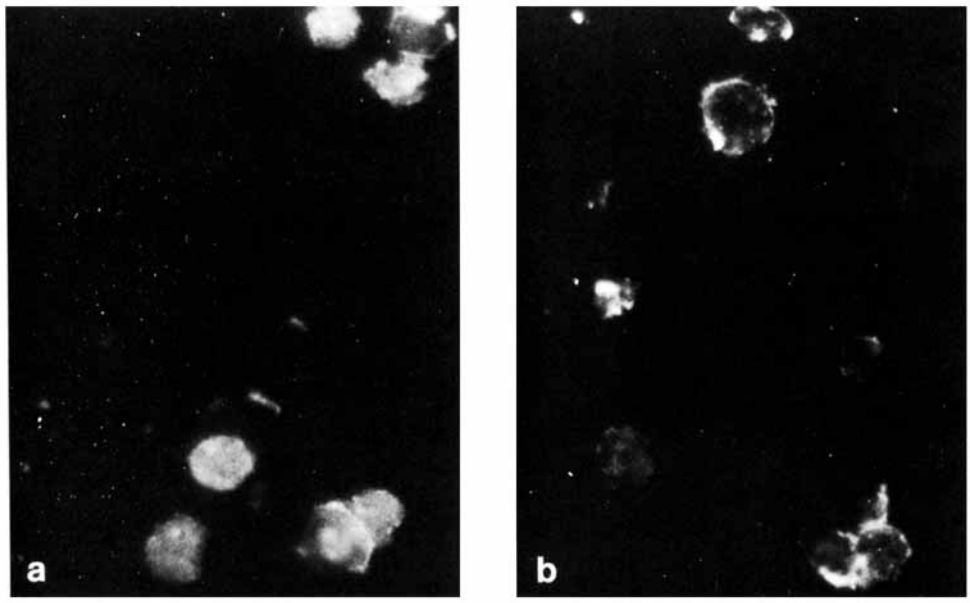

Fig. 5. Indirect immunofluorescence demonstration of cell surface binding of antidiscoidin I serum. 2×10^6 cells were dissociated from filters after 4 hr a) and 7 hr b) of development and treated with anti-D I serum, followed by goat antirabbit rhodamine-conjugated IgG as described in Methods. 10-μl aliquots from each sample were spread on glass slides and viewed with fluorescence optics. ×400.

Fab Inhibition of Development

We have also studied the effects of anti-D I Fab fragments on aggregation and development of D discoideum AX-2 cells on agar. Cells were starved in the presence of different amounts of anti-D I Fab fragments and allowed to develop on 1% agar as described in Methods. Inhibition of aggregation by anti-D I Fab fragments was apparent after 8 hr of development. When 2×10^5 cells were developed in the presence of 900 μg of anti-D I Fab fragments (4.5 mg/10^6 cells) most of the cells remained dissociated on the agar without entering into large streams after 9 hr of development as compared with control cells (compare Fig. 7A, 1 and 4). Further development was also blocked in anti-D I Fab-treated cells, since only a few fruiting bodies are observed after 24 hr of development, while most of the cells remained dissociated on the agar well (Fig. 7B,4). Inhibition of aggregation and late morphogenesis depends strongly on Fab concentration: a gradual decrease in the degree of inhibition is observed with decreasing concentrations of anti-D I Fab fragments. The concentration dependence is clearly evident when morphogenesis was observed after 24 hr of development (compare Fig. 7B, with 4, 5, and 6). Concentrations lower than 50 μg/2×10^5 cells do not affect aggregation or development of cells.

As in the in vitro assay, inhibition of aggregation and development by anti-D I univalent fragments is specific. No effects have been observed at any developmental stage when cells are starved in the presence of different amounts of preimmune Fab fragments (compare Fig. 7A and 7B,1 with 2 and 3).

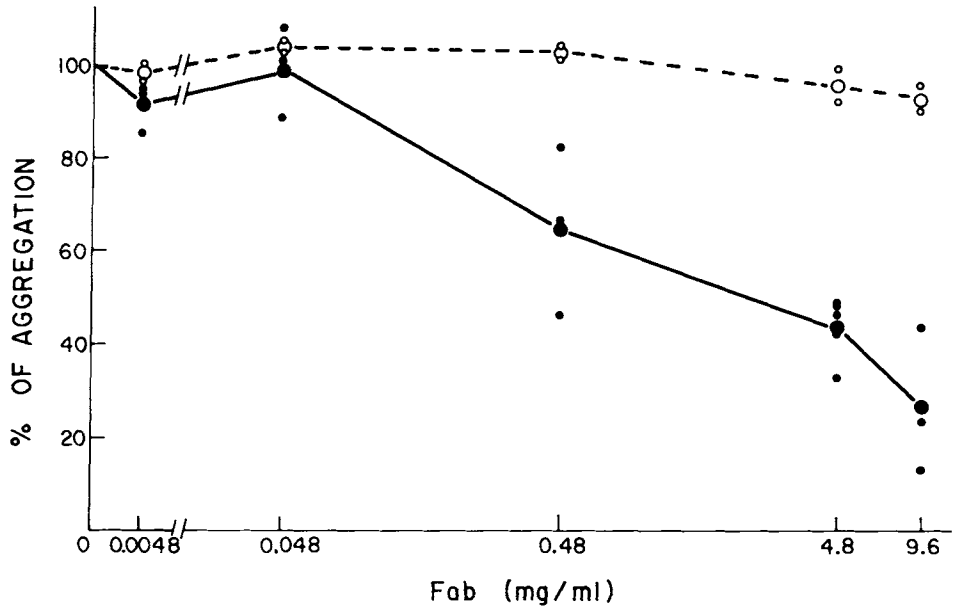

Fig. 6. In vitro reaggregation assay in the presence of antidiscoidin I and preimmune Fab fragments. Aggregation-competent cells were incubated with the indicated amounts of antidiscoidin I and preimmune Fab fragments in the in vitro aggregation assay described in Methods. The values obtained for each individual experiment were normalized to the percent of actual aggregation observed in controls incubated with phosphate-EDTA buffer (between 70% and 85%). Each point in the curve represents the average value of at least three separate experiments with antidiscoidin I Fab (●) and two independent experiments for the preimmune Fab (○). The figure also shows the individual values obtained on each independent experiment.

TABLE I. Inhibition of Cell Reaggregation by Anti-D I Univalent Antibodies in Function of Cell Density

Cell density in assay (cells/ml)	%Aggregation[a]	
	Immune Fab[b]	Preimmune Fab[b]
9.7×10^5	47.5	95.6
2.2×10^6	67.4	101.0
3.2×10^6	87.3	95.3
3.7×10^6	88.1	94.9
4.8×10^6	83.7	92.9

[a]Values were normalized to the reaggregation observed in control cells in PE buffer without Fab additions (normal values in the range of 82–89% of aggregation).
[b]7-hr-developed cells were detached from filters and suspended at double density of indicated. 100-μl aliquots of each sample were mixed with 100 μl of PE buffer containing 9 mg/ml of anti-D I or preimmune Fab fragments, and the reaggregation assay performed as described in Methods. Each sample was counted twice in the hemocytometer and the average value is shown.

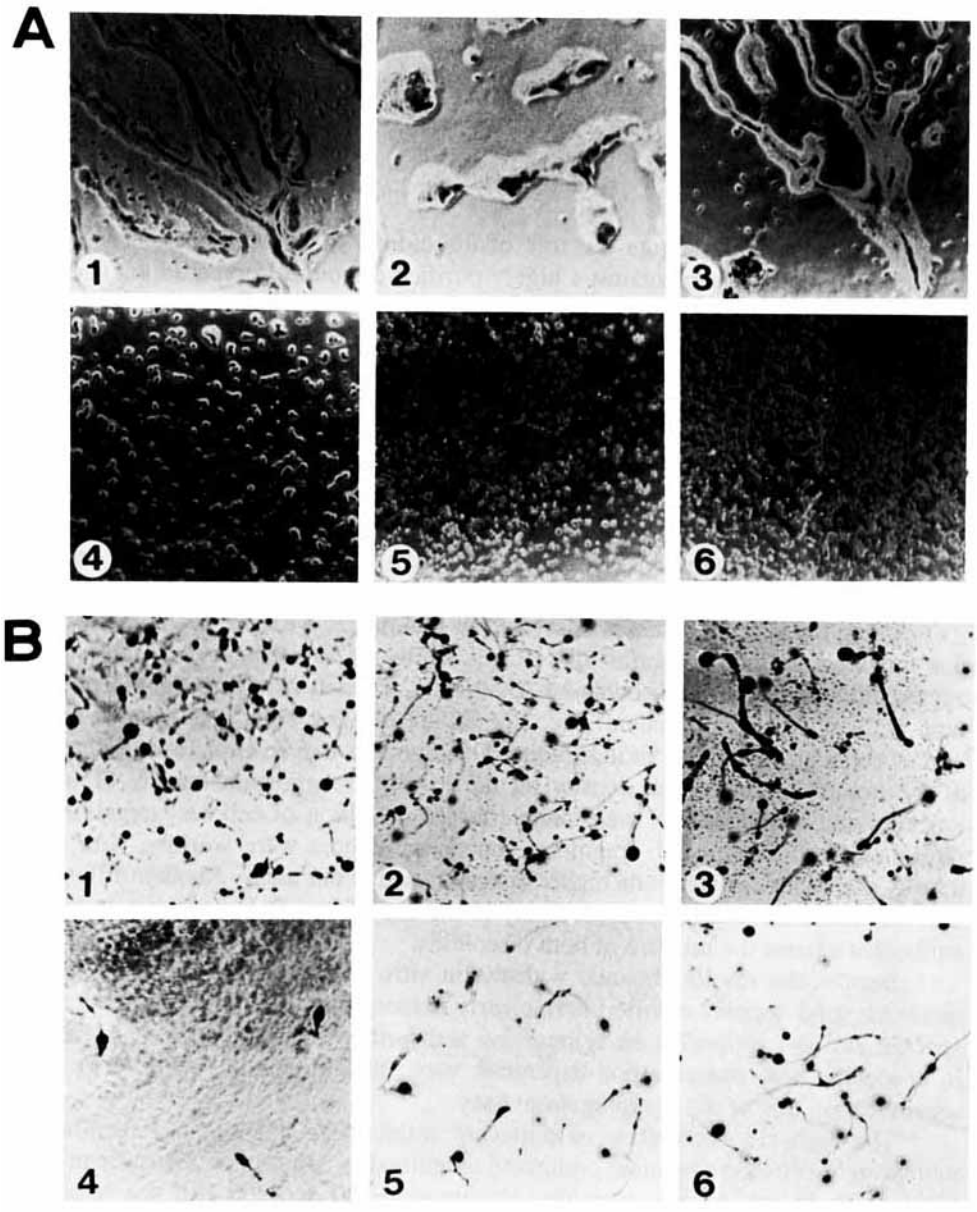

Fig. 7. Aggregation and morphogenesis in the presence of antidiscoidin I Fab fragments. Cells were developed on agar in the absence or presence of different amounts of anti-D I or preimmune sera as described in Methods. Pictures were taken after 9 hr (A) and 24 hr (B) of development. 1) control cells developed in phosphate buffer; 2 and 3) cells developed in the presence of 475 and 100 μg of preimmune Fab fragments, respectively; 4–6) cells developed in the presence of 900, 480, and 100 μg of anti-D I Fab fragments, respectively. A) $\times 100$; B) $\times 40$.

Aggregation and morphogenesis on agar seem to be more sensitive to inhibition by antidiscoidin I antibodies than reaggregation in suspension. While 4.5 mg/ml of anti-D I Fab almost completely inhibited the development on solid support, the same concentration of the antibody only elicited a 50% inhibition of cell reaggregation in the in vitro assay.

DISCUSSION

In order to reinvestigate the role of discoidin I in intercellular adhesion, we have raised rabbit antisera against a highly purified discoidin I preparation. The anti-D I serum obtained shows a high specificity for discoidin I (Figs. 2, 3) and also displays cross-reactivity to discoidin II. These results are in agreement with those of Berger and Armant [25], who provided strong evidence for the presence of a common region in both discoidins. Other minor components are detected by the anti-D I serum in whole soluble extracts, mainly a protein migrating with an apparent molecular weight of 57 Kd. However, it is worth mentioning that anti-D I serum does not react with any protein migrating in the 80-Kd region, either in the antigen preparation or in the soluble extracts. This result argues against the possibility of any contamination in our antigen preparation by some residual 80-Kd glycoprotein associated to "contact sites A."

Using the specific antisera described in this paper, we have been able to show that univalent antibodies against discoidin I inhibit EDTA-resistant cell adhesion of aggregation-competent D discoideum AX-2 cells, in an in vitro assay in a concentration- and cell-density-dependent way. The failure of other authors to observe inhibition of cell aggregation by antidiscoidin Fab fragments can be explained on the basis of the crucial effect of cell density on the in vitro reaggregation assay (Table I). Springer and Barondes [13] were unable to see inhibition of cell reaggregation at 5 mg/ml of antidiscoidin Fab fragments, but these authors were working with a cell density one order of magnitude higher than that used in our assay. Another differential fact that could have improved our assay is the use of anti-D I antibodies instead of antibodies against the mixture of both discoidins.

Besides the results obtained with the in vitro reaggregation assay, the experiments on solid support reported here clearly demonstrate the inhibitory effect of the specific anti-D I antibodies on aggregation and further morphogenesis of AX-2 cells in a specific and concentration-dependent way. This approach seems to be more sensitive than that of the reaggregation assay.

The higher concentration of univalent antidiscoidin I antibodies required for inhibition of cell aggregation as compared to antibodies against other specific proteins involved in D discoideum adhesion (glycoproteins 80 and 150 Kd; see references [3,28]) might be the result of a higher level of expression of discoidin I molecules over the cell surface and/or a lower affinity of antidiscoidin I antibodies for their cell membrane antigens. While the number of cell surface discoidins has not been definitively established [10,13,26], the available binding data suggest a lower affinity of antidiscoidin antibodies for their antigens in intact cells, as compared to antibodies directed to other cell adhesion molecules [13].

Together with other evidence summarized here and reviewed elsewhere [29], the results reported in this paper provide strong support for the direct involvement of

the cell surface lectin, discoidin I, in the aggregation specific intercellular adhesion processes in D discoideum, strain AX-2. More precise and detailed studies should be needed in order to clarify the presumable complex interactions between the different molecules involved in aggregation processes before we can get a comprehensive view of the intercellular adhesion mechanisms operating in D discoideum.

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