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Characterization of a 140Kd Cell Surface Glycoprotein Involved in Myoblast Adhesion

Ann E. Chapman

Department of Biology, University of California, San Diego, La Jolla, California 92093

Two monoclonal antibodies that cause changes in the morphology of cultured chick myogenic cells have been described previously [8]. In this paper, these antibodies are shown to interact with the same 140Kd protein. The 140Kd protein has been further characterized as a cell-surface glycoprotein by lactoperoxidase-catalyzed iodinations and lectin affinity chromatography. The protein is resistant to digestion by trypsin and collagenase and has been shown to be unrelated to fibronectin by immunoprecipitation studies and by peptide mapping. A second protein, of approximately 170Kd MW, is also immunoprecipitated by the monoclonal antibodies. This protein is probably unrelated to the 140Kd protein since the peptide maps are quite distinct.

Key words: adhesion, cell surface glycoprotein, monoclonal antibodies

An area of much recent scientific investigation has to do with molecules that are involved in cell-cell or cell-substrate interaction. These molecules probably serve key roles in development and tissue maintainance. Several "matrix" proteins, including fibronectin, collagen, laminin, and entactin [1–4], have been implicated in cell-cell adhesion, but little is known about integral membrane proteins that may be involved in cell-cell or cell-substrate interactions. Part of the problem involved in detecting such cell-surface molecules exists because they occur in much lower amounts than the secreted matrix proteins. One approach that shows great promise in the identification of cell-surface molecules involved in adhesion is the use of antibodies directed against cell-surface moieties that can block cell-cell or cell-substrate interaction. This approach has been used successfully to isolate cell-surface antigens involved in cell-cell or cell-substrate interaction between chick neural cells [5], teratocarcinoma cells [6], and fibroblasts [7], among others. This study characterizes antigens recognized by two monoclonal antibodies isolated by Greve and Gottlieb [8], which are directed against chicken myoblast and fibroblast cell-surface molecules. These monoclonal antibodies cause myoblasts and myotubes, but not fibroblasts, to round up when

Ann E. Chapman's present address is Department of Cell Biology, University of Texas Health Sciences Center at Dallas, Dallas, TX 75235.

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added to cells grown on collagen-coated plastic. A JG22-Sepharose affinity column specifically bound a 140Kd protein present in chicken embryo extracts but not from a control nonimmune mouse IgG-Sepharose column. This paper characterizes the 140Kd and 170Kd antigens recognized by the JG22 and JG9 antibodies.

METHODS

Cell and Cell and Tissue Extraction Conditions

Primary cultures of chick embryo fibroblasts (CEF) were prepared by trypsin treatment of minced tissue from eight embryos. The cells were grown in monolayer culture at 37°C in Dubecco modified Eagle Medium/Coon modified F12 medium (1:1) supplemented with 10% fetal calf serum (Reheis) and antibiotics. For the antigen identification studies, cells were extracted for 20 min at 40°C with 0.5% NP40/phosphate-buffered saline (PBS) containing 0.5 mM phenylsulfonylfluoride (PMSF). The extracts were then subjected to ultracentrifugation at 200,000g for 45 min to remove insoluble material, and were then subjected to either immunoprecipitation or affinity chromatography on a JG9 or JG22 antibody affinity column. Large-scale embryo extracts were prepared by isolating a crude membrane fraction from homogenized 14 chicken embryos, as described by Greve and Gottlieb [8]. The membranes were extracted in 0.5% NP40/PBS 0.5 mM PMSF by stirring overnight at 4°C, and nonextracted material was removed by centrifugation at 200,000g for 45 min.

Antibody Reagents

JG9 and JG22 antibodies are mouse myeloma antibodies directed against chicken muscle antigens. They were a kind gift from Drs. Jeff Greve and David Gottlieb, and were isolated and purified as described [8]. Rabbit antifibronectin was prepared from serum from rabbits immunized with fibronectin from chicken serum which was purified by affinity chromatography over a gelatin-Sepharose 4B affinity column as desribed [9].

Radiolabeling of Cells or Proteins

Two 100-mM Petri dishes of semiconfluent CEF cells were incubated with 100 μCi of [^{35}S]methionine (1266 Ci/mmol, New England Nuclear) in 4 ml of Dulbecco modified Eagle medium (DME) containing 1/1OX methionine (5 μM) and 10% fetal calf serum plus 50 units/ml of pen strep for 16 hr at 37°C, and then the cells were extracted with 0.5% NP40/PBS at 4°C in the presence of PMSF. For the trypsin resistance experiment, the cells were incubated with nonradioactive medium for a 5-hr "chase" period before extraction.

Cells were metabolically labeled with [^{3}H]glucosamine or [^{3}H]mannose by incubating one 100-mM Petri dish of semiconfluent CEF cells with 100 μCi of D-6-[^{3}H]glucosamine (20 Ci/mmol, New England Nuclear) or 100 μCi of 2-[^{3}H]mannose (8 Ci/mmol, Amersham) in DME/F12 plus 10% fetal calf serum (FCS) and pen strep (50 units/ml) for 16 hr. The cells were then extracted as described above.

Lactoperoxidase iodination was performed on one dish of confluent CEF cells which had been plated 16 hr before the experiment and incubated at 37°C in DME/F12 + 10% fetal calf serum. The cells were washed three times with cold Hanks Bufffered Salt Solution (HBSS) before subjecting the cells to lactoperoxidase iodination as described [11]. Chloramine T iodination of proteins [12] was performed on JG9-Sepharose 4B affinity column eluates by incubating 100 μl of the eluate (contain-

ing the proteins in 0.5% NP40/PBS) with 30 μl of chloramine T (0.5 mg/ml) in 0.5 M NaPhosphate buffer, pH 7.5, and 500 μCi of ^{125}I (13–17 mCi/μg, 100 mCi/ml New England Nuclear) at room temperature for 30 sec for samples to be subjected subsequently to immunoprecipitation, or for 20 min for samples to be subjected to peptide mapping. Then the reaction was stopped with 30 μl of sodium metabisulfite (5 mg/ml in 0.5 M NaPhosphate buffer, pH 7.5), and the samples were immediately desalted by passage over a 5-ml column of Sephadex G-50 equilibrated in 0.5% NP40/PBS which had been previously rinsed with 1 mg/ml bovine serum albumin (BSA) to lower nonspecific binding. The iodinated protein eluate was then collected and was stored frozen at −20°C until use. Chicken fibronectin was iodinated under the same reaction conditions so that the results would be directly comparable to those of the JG9 eluate.

Isolation of the Antigen

Immunoprecipitation of the antigen from 0.5% NP40/PBS extracts of CEF cells was performed by incubating the extracts with the mouse monoclonal antibodies at 4°C for 1 hr. Since the mouse monoclonal did not bind protein A, the immunoprecipitation was performed by adding Pansorbin (Calbiochem) coated with rabbit anti-mouse IgG to the cell extracts. The extracts were then spun in a microfuge for 2 min. The pellets were rinsed three times with 0.5% NP40/PBS, and were then dissolved in Laemmli sample buffer, followed by SDS gel electrophoresis [13].

Affinity chromatography of 14-d-old chick embryo extracts was performed on columns of JG9-Sepharose 4B or JG22-Sepharose 4B as described previously [8].

Peptide Mapping

Peptide mapping was performed as described. Since the samples to be mapped contained very low levels of protein, a problem with high background labeling was encountered. To minimize this problem, the JG9 extracts were iodinated by the chloramine T procedure before subjecting them to SDS gel electrophoresis (the JG22 140Kd and fibronectin samples were iodinated in the usual way—the proteins were subjected to SDS gel electrophoresis, the gels were stained with Coomassie Blue to identify the bands, and then the bands were cut out of the gel and subjected to iodination by the chloramine T procedure). The major peaks of the 140Kd peptide map pattern were identical whether the sample was iodinated before or after gel electrophoresis (data not shown) and background peaks were reduced considerably.

SDS-PAGE

One-dimensional SDS polyacrylamide slab gel electrophoresis (PAGE) was performed as described by Laemmli [13]. Autoradiography was performed after the gels were treated with En^{3}Hance (New England Nuclear) and were dried down. Silver staining of the gels was performed by the method of Morrissy [16].

Collagenase Digestion

Collagenase digestion was performed using a purified bacterial collagenase isolated from Clostridium histolyticum (obtained as a kind gift from Dr. E. Harper); 10^6 cpm of the ^{125}I JG9 affinity column eluate (described above) was mixed with 5 μg of purified chick skin collagen (a kind gift from Dr. Charles Little) in a 250-μl reaction volume containing 5 μl of C histolyticum enzyme in a buffer containing 25 mM TRIS, 10 mM $CaCl^2$, 50 mM NaCl. The mixture was incubated at 22°C, and

50-μl aliquots were taken at the time points indicated. The reaction was stopped by the addition of 20 mM EDTA, and Laemmli sample buffer was then added to the samples which were boiled. To insure that the resistance of the iodinated JG9 eluate was not due to the chloramine T iodination of the proteins, 10 μg of the chick skin collagen was ^{125}I-labeled under the same conditions as the JG9 extract. The iodinated collagen was as susceptible to collagenase digestion as uniodinated collagen, indicating that the resistance of the ^{125}I 140K/170Kd JG9 species is not an artifact.

RESULTS

JG9 and JG22 antibodies interact with a 140Kd protein from [^{35}S]-methionine-Labeled CEF Cells

Out of the series of 22 monoclonal antibodies directed against chick cell-surface molecules which were characterized by Greve and Gotttlieb, only two of these, JG9 and JG22, caused myoblasts and myotubes to round up when antibodies were added to the live cells in culture [8]. In order to determine more information about the nature of the antigens responsible for the rounding effect, chick embryo fibroblasts (CEF) were metabolically labeled with [^{35}S]methionine and extracted with 0.5% NP40/PBS. (Previous studies using a radioimmunoassay to detect cellular JG9 and JG22 antigens showed that the antigens JG9 and JG22 are 90–95% extractable with 0.5% NP40 [J. Greve, personal communication]). The NP40 extracts were then subjected to immunoprecipitation by JG9, JG22, or nonimmune mouse IgG antibodies as shown in Figure 1. Both JG9 and JG22 antibodies immunoprecipitate a 140Kd protein which is not present in the control (lanes 3 and 4). JG22 also appears to immunoprecipitate some high molecular weight [^{35}S]methionine-labeled material which runs at the top of the gel (lane 3). While this high molecular weight material consistently appears in [^{35}S]methionine-labeled immunoprecipitates of cell extracts by JG22 it is never present in JG9 immunoprecipitates, nor is it present in JG22 immunoprecipitates of [^{125}I]lactoperoxidase or [^{3}H]glucosamine-labeled cell extracts (Figs. 5, 6). It is also absent in Coomassie-blue-stained gels of JG22-Sepharose affinity column eluates of 0.5% NP40/PBS extracts of crude membrane preps isolated from d 14 chick embryos (Fig. 1C, lane 6) or chicken cardiac muscle membrane (data not shown). One possible explanation for the presence of the high molecular weight [^{35}S]methionine-labeled material is that it may be intracellular rather than membrane-bound material which may be eluted as a complex with the 140K protein in the NP40/PBS extracts, although the possibility that the 140Kd molecule is a fragment isolated from a larger protein or protein complex cannot be discounted.

In some experiments using identical labeling and extraction conditions to those just described, both JG9 and JG22 antibodies immunoprecipitated a 170Kd molecule in addition to the 140Kd species. Figure 1B shows immunoprecipitation of both 140Kd and 170Kd species by JG22 of [^{35}S]methionine-labeled 0.5% NP40/PBS extract performed under conditions to which are identical to that of the panel A experiment. The 170Kd species is also found in immunoprecipitates of [^{3}H]glucosamine and [^{125}I]lactoperoxidase-labeled cell-surface protein, or by Coomassie-blue-stained affinity column eluates (Fig. 1B,C, lane 6).

In order to investigate the possibility that the 140Kd and 170Kd molecules might exist as a disulfide-linked dimer, samples of the affinity column eluate shown in Figure 1C were boiled in Laemmli sample buffer in the presence or absence of

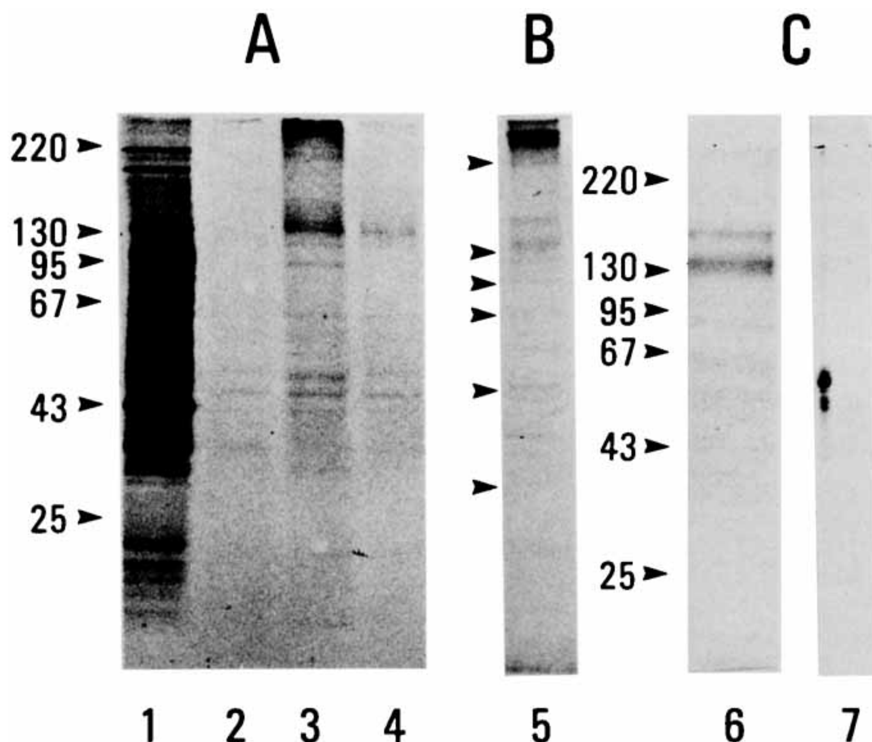

Fig. 1. SDS-PAGE (6–15%) of the antigen isolated from [^{35}S]methionine-labeled CEF cells and from a large-scale preparation of 14 chicken embryo extracts. A shows an autoradiogram of 0.5% NP-40/PBS extracted [^{35}S]methionine-labeled CEF cells. Lane 1 represents the total [^{35}S]methionine-labeled proteins present in the NP-40/PBS extract. Lane 2 shows [^{35}S]methionine-labeled material which was immunoprecipitated by control (nonimmune) mouse IgG. Lane 3 shows proteins which were immunoprecipitated by the mouse monoclonal antibody JG22, and lane 4 shows the labeled material which was immunoprecipitated by the JG9 antibody. Lane 5 (B) shows a JG22 immunoprecipitation of another [^{35}S]methionine-labeled CEF cell extract, which was performed under identical conditions to those in panel A. C, lane 6, shows Coomassie-blue-stained protein isolated by affinity chromatography of 0.5% NP40/PBS extract of a crude membrane preparation of d 14 chicken embryos on a JG22-Sepharose 4B affinity column. Lane 7 shows extracted material which stuck to a column of Sepharose 4B with no antibody bound to it. The standard marker compounds indicated are fibronectin (220,000), vinculin (130,000), α-actinin (95,000), bovine serum albumin (67,000), human IgG heavy chain (50,000), and actin (43,000).

β-mercaptoethanol. Some samples were also dissolved in Laemmli buffer without β-mercaptoethanol, and without being boiled. When subjected to SDS gel electrophoresis, there was no shift in the molecular weights of the proteins to that of a higher molecular weight (data not shown), indicating that the two species are not normally a disulfide linked dimer.

When crude chick embryo membrane 0.5% NP40/PBS extracts were passed over a JG22 affinity column once, as shown in Figures 1C and 2, lane 1, both 140Kd and 170Kd species were specifically eluted from the column with 0.05 M diethylamine, pH 11.5, in 0.5% NP40/PBS. When a portion of this eluate was neutralized and passed again over the JG22 column, the 140Kd protein was enriched, and most of the 170Kd protein did not bind to the column a second time (Fig. 2, lane 3). This result

may indicate that the 170Kd species is not recognized by the JG22 antibody, but instead exists in a noncovalent interation with the 140Kd species which is destroyed by diethylamine treatment. Alternative explanations include the possibilities that diethylamine treatment changes the conformation of the 170Kd species in such a way that it can no longer be recognized by the antibody, or that the 170Kd species binds either too tightly or too weakly to the affinity column, so that it does not elute to the same extent as the 140Kd species. Additional evidence that the 140Kd and 170Kd

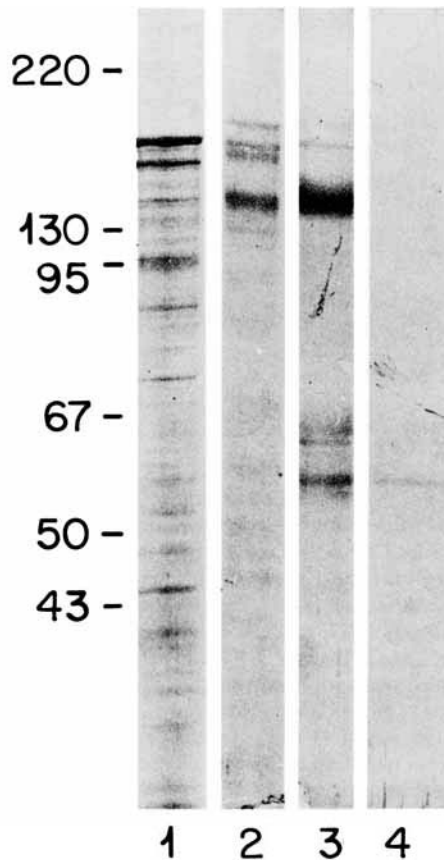

Fig. 2. JG22-Sepharose 4B affinity chromatography of 0.5% NP40/PBS extract of a crude membrane fraction of d 14 chick embryos. Isolation of the antigen from a 0.5% NP40/PBS extract of a crude membrane fraction of d 14 chick embryos after two passages over a JG22-Sepharose 4B affinity column. Lane 1 shows proteins present in the initial 0.5% NP40/PBWS extract before passage over the affinity column. Lane 2 shows proteins which bound to the JG22-Sepharose column, and which were eluted from the column with .05 M diethylamine in 0.5% NP40/PBS, pH 11.5. The eluate was neutralized with 1 M TRIS buffer, pH 7.0, and a portion of the neutralized eluate was passed a second time over the JG22-Sepharose column. Lane 3 shows the material which stuck specifically to the JG22 column the second time, and lane 4 shows material from the initial extract which stuck to a column of Sepharose 4B (no antibody). The standard marker compounds are the same as those listed in the legend to Figure 1. The initial extract and the affinity column eluates were fractionated by SDS gel electrophoresis on a 6–15% slab gel and were then stained with the silver stain [9]. Standard compounds described in the legend to Figure 1.

species are not related proteins comes from peptide mapping studies (Fig. 4A,B) which show that the peptide fragments formed from ^{125}I-140Kd and 170Kd are totally different.

Both JG9 and JG22 Antibodies Recognize the Same 140Kd Molecule

In a previous study by Greve and Gottlieb, both JG22 and JG9 antibodies were shown to cause myoblasts and myotubes in culture to round up, although the morphology of the rounded cells was somewhat different in the two cases [8]. These authors also demonstrated that addition of cold JG22 antibody could inhibit binding of ^{125}I-JG9 antibodies to cultured chick myoblasts, which suggested that the antigens were spatially or structurally related. The studies presented here have demonstrated that both antibodies recognize a 140Kd species. In order to confirm that both antigens recognize the same 140Kd species, two types of experiments were performed. First, a 0.5% NP40/PBS extract from a crude membrane fraction from d 14 chick embryos was passed over a JG9-Sepharose 4B affinity column. The material which bound specifically was eluted with diethylamine, and was then iodinated by the chloramine T iodination procedure [12]. Both 140Kd and 170Kd species were specifically eluted from the JG9 column (the 50,000 MW band is JG9 heavy chain which leached off the column, and the 43,000 MW peak which is probably actin is also frequently seen in immunoprecipitates of control IgG). The iodinated material was then subjected to immunoprecipitation by JG9 and JG22 monoclonal antibodies and by control mouse IgG. Figure 3 (lanes 3 and 4) shows that both JG9 and JG22 can immunoprecipitate the 140Kd species from the JG9 affinity column eluate, suggesting that the 140Kd band is identical in each case. Less of the 170Kd species is immunoprecipitated by the JG9 and 22 antibodies, suggesting that it is either antigenically distinct from the 170Kd species, or that its antigenicity is largely destroyed by the iodination procedure.

These results strongly suggest that the JG9 and JG22 antibodies recognize the same 140Kd antigen. These results also suggest that the 170Kd species may not be recognized by the JG9 and JG22 antibodies, since (1) the 170Kd species which elutes from the JG9 affinity column then is not immunoprecipitated by JG9 and JG22 antibodies to any great degree (Fig. 3), and (2) the peptide maps of the JG9 170Kd and 140Kd species are quite different (Fig. 4).

The 140Kd Molecule Is a Cell-Surface Glycoprotein

In order to determine if the 140Kd molecule is present on the cell surface, intact CEF cells were iodinated by the lactoperoxidase iodination procedure, which labels cell-surface proteins [11]. The cells were extracted with 0.5% NP40/PBS, and were then immunoprecipitated with JG22 or control IgG. Figure 5 shows that the 140Kd species recognized by the JG22 antibody is iodinated by the lactoperoxidase iodination procedure, suggesting that it is indeed a surface antigen (the 170Kd species is also iodinated in some experiments; data not shown). The absence of ^{125}I-labeled actin indicates that the cells were intact during the labeling procedure.

The glycoprotein nature of the 140Kd (and 170Kd species) was determined by lectin affinity column chromatography of the solubilized antigen, and by immunoprecipitation of antigen from 0.5% NP40/PBS extracts of cells metabolically labeled with 2-[^{3}H]mannose or [^{3}H]glucosamine (Fig. 6). JG9-Sepharose affinity column eluates were iodinated by the chloramine T method, and were then passed over a variety of lectin columns which recognize different sugar moieties. The 140Kd and

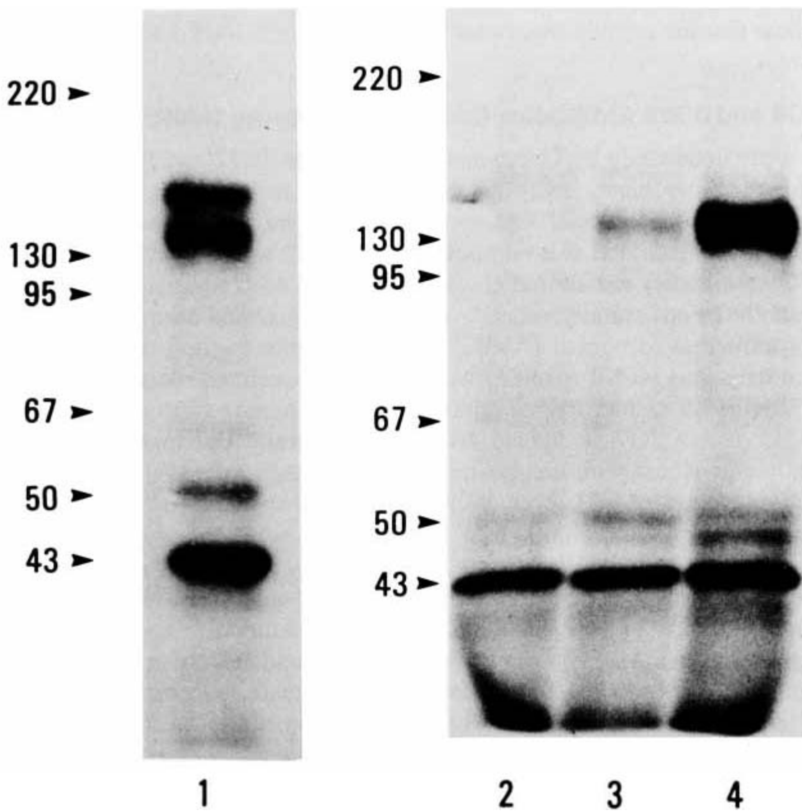

Fig. 3. SDS-PAGE (8%) of immunoprecipitation by JG9, JG22, and control mouse IgG of material which bound to an affinity column of JG-Sepharose 4B. Lane 1 shows an autoradiogram of the proteins present in a 0.5% NP40/PBS extract of a crude membrane prep of d 14 chick embryos which were eluted specifically from a JG9-Sepharose 4B affinity column. The eluted material was labeled with ^{125}I by the chloramine T iodination procedure, as described in Materials and Methods. Lanes 2–4 show materials from the ^{125}I eluate which could be immunoprecipitated by control mouse IgG, JG9 antibody, and JG22 antibody, respectively. Standard compounds are described in the legend to Figure 1.

170Kd species bound specifically to several lectin columns, including ConA, lentil, ricin, and wheat germ agglutinin columns (data not shown).

The 140Kd/170Kd Molecules Are Not Related to Fibronectin or Collagen

Since myoblast cell rounding might be caused by antibodies to fibronectin or other known matrix proteins, it was important to show that the JG22 antibodies were not merely interacting with a 140Kd fragment of fibronectin. While there is no "matrix"-type immunofluorescent staining of the cells (data not shown), it could be possible that the antigenic site recognized by the monoclonals was covered in the matrix cables, and so only nonfibrillar antigens could interact with the antibody. Our evidence that the 140Kd molecule is not fibronectin is as follows:

(1) Immunofluorescent staining of CEF cells by JG22 and JG9 antibodies cannot be blocked by the addition of exogenous fibronectin under conditions that would

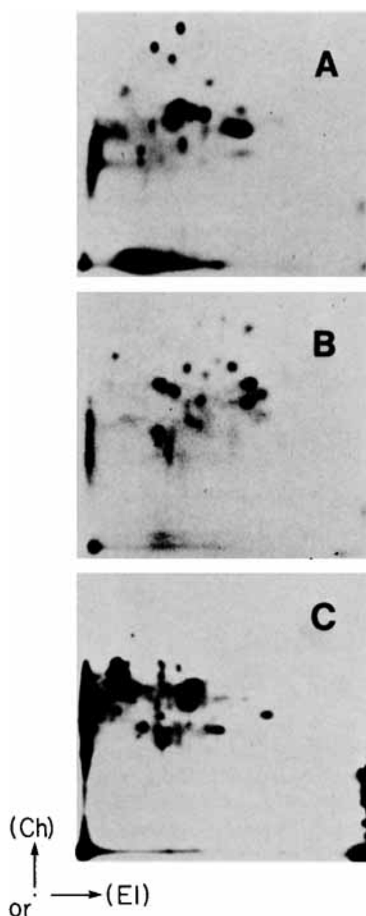

Fig. 4. Peptide maps of chloramine-T-iodinated samples: A shows an autoradiogram of the mapping pattern of the 140Kd protein isolated from a JG9-Sepharose 4B affinity column eluate. B shows the map of the 170Kd protein isolated from the JG9 affinity column eluate. C shows the mapping pattern of iodinated chick serum fibronectin. "Or" indicates the origin, "El" indicates the direction of electrophoresis, and "Ch" indicates the direction of chromatography.

completely block staining of the cells by antifibronectin antibodies (data not shown).

(2) The 140/170Kd species could not be immunoprecipitated by polyclonal rabbit antibodies directed against chicken fibronectin (Fig. 7), although it could be demonstrated that antifibronectin was able to quantitatively precipitate intact iodinated chicken fibronectin and 43–140Kd fibronectin fragments generated by thermolysin digestion (data not shown).

(3) The peptide map of intact fibronectin (Fig. 4C) did not resemble maps of the 140Kd/170Kd material (Fig. 4A–C).

Since collagen also has monomers in the same general molecular weight range as the 140Kd and 170Kd species, aliquots of iodinated affinity purified 140Kd/170Kd species were subjected to colllaginase treatment with a purified collagenase from Clostridium histolyticum. Under conditions which completely digested chick skin collagen, the 140Kd/170Kd species remained intact (data not shown).

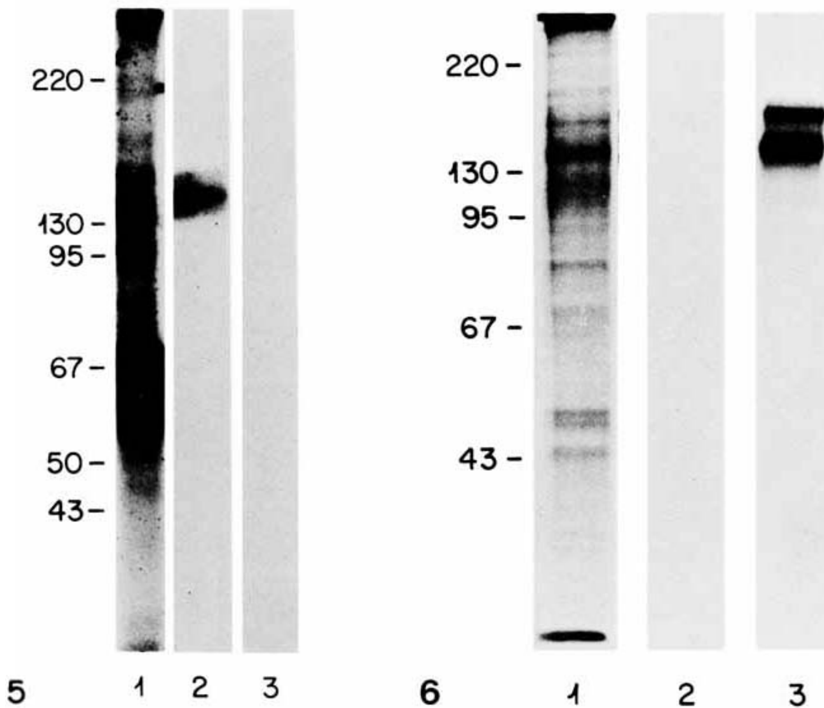

Fig. 5. One 100-mM dish of semiconfluent CEF cells (plated 12 hr before the experiment) was iodinated by the lactoperoxidase iodination procedure [4]. Cells were then extracted with 0.5% NP40/PBS containing 1 mM PMSF for 20 min at 4°C, centrifuged at 200,000 for 45 min, removing insoluble material, and subjected to immunoprecipitation, as described in Materials and Methods, followed by SDS gel electrophoresis on an 8% gel. Lane 1 shows the initial iodinated extract; lane 2 shows JG22 immunoprecipitate; and lane 3 shows the mouse IgG control immunoprecipitate. Standard compounds are described in the legend to Figure 1.

Fig. 6. SDS-PAGE of 0.5% NP40/PBS extracts of [^{3}H]glucosamine-labeled cells (lane 1) subjected to immunoprecipitation by JG22 (lane 3) and control IgG (lane 2). Identical results were obtained by JG22 immunoprecipitation of [^{3}H]mannose-labeled CEF extracts. Standard compounds are described in the legend to Figure 1.

The 140Kd and 170Kd Species on Intact Cells Are Resistant to Trypsin Treatment

When [^{35}S]methionine-labeled CEF cells in monolayer culture were detached from the dish by trypsin treatment, the 140Kd/170Kd species were resistant to trypsin treatment (data not shown). The immunofluorescent staining intensity was also undiminished by trypsin treatment under conditions which totally abolished fibronectin staining of the cells (data not shown). 140Kd and 170Kd species are, however, readily digested by trypsin, after they have been extracted in NP40/PBS.

DISCUSSION

The experiments in this paper have demonstrated that the 140Kd protein recognized by the JG9 and JG22 mouse monoclonal antibodies is a cell-surface glycoprotein

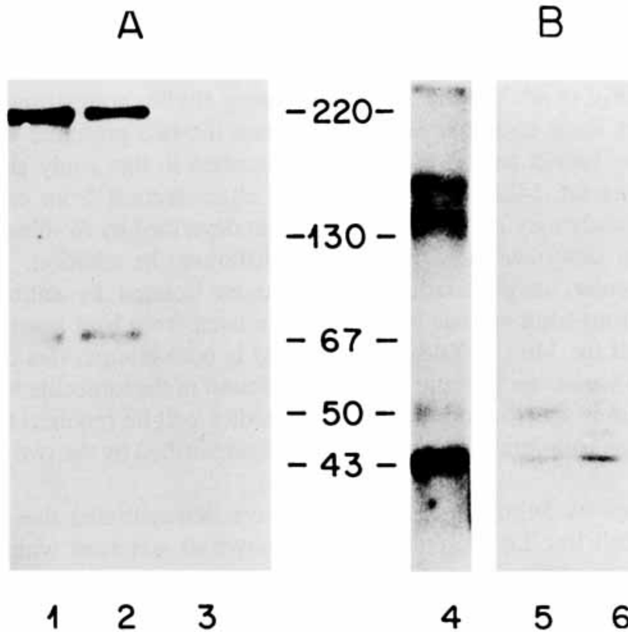

Fig. 7. The 140Kd/170Kd proteins are not immunoprecipitable by a rabbit antifibronectin antibody. A shows 180,000 intact ^{125}I-fibronectin (lane 1) subjected to immunoprecipitation by rabbit antifibronectin (lane 2) or control rabbit IgG (lane 3). B shows the immunoiprecipitation of 100,000 cpm of the ^{125}I affinity column eluate from a JG9 column (lane 1) which was subjected to immunoprecipitation by the rabbit antifibronectin antibody (lane 2) or control IgG (lane 3). The immunoprecipitates were dissolved in Laemmli sample buffer and subjected to SDS gel electrophoresis on an 8% acrylamide gel, followed by autoradiography. Standard compounds are described in the legend to Figure 1.

that is trypsin-resistant on intact cells. This molecule is unrelated to fibronectin as determined by lack of immunoreactivity and by differences in the peptide. It is probably also unrelated to collagen, as determined by its resistance to collagenase digestion. A 170Kd cell-surface glycoprotein, which sometimes appears in JG22 or JG9 immunoprecipitates, may not be related to the 140Kd antigenic glycoprotein, based on peptide mapping studies. It may represent a glycoprotein that exists in a noncovalent interaction with the 140Kd protein.

Antibodies which block cell-cell or cell-substrate interaction have been used as a tool in a number of laboratories to isolate cell-surface molecules implicated in cell adhesion. The approach was first used for studies of Dictyosteleum aggregation [21] and was next used to characterize cell-surface molecules involved in neural cell aggregation [5,22], and in a number of other vertebrate cell types [6–8,19, 23–25]; 120–140Kd molecules which interact with adhesion-blocking antibodies have been identified in neural cells [26,20], fibroblasts [27], epithelial cells [23], and myogenic cells [8,19]. The 140Kd protein described in this study may be similar or identical to those described by Neff et al [19]. These authors characterized the antigen recognized by a monoclonal antibody which causes chick skeletal muscle myoblasts and myotubes plated on a gelatin-coated substrate to round and detach. The antigens, isolated by antibody affinity chromatography, had a molecular weight of 120 to 160Kd.

It is not known if the 140Kd antigen described here is the same as that described in a study by Rothbard et al [20]. These investigators identified a glycoprotein involved in neural cell adhesion in the chicken brain shown to have a molecular weight of 150–180Kd in adult tissue. Peptide mapping studies comparing their 150Kd and 180Kd species show strong similarities between the two proteins. Peptide mapping studies of the 140Kd and 170Kd species described in this study show no such homology, so while the 140Kd protein we have characterized from chick embryo fibroblasts in this study may well be identical to that described by Rothbard et al [20], the 170Kd protein described here is probably different. In addition, no apparent variation in molecular weight existed in the antigen isolated by antibody affinity chromatography from adult cardiac membranes (isolated from beef heart) embryonic chick fibroblasts. If the 140–150Kd species isolated in both laboratories is identical it is possible that the variations in sialic acid content found in the molecule in embryonic vs adult brain tissue is specific to brain. Further studies will be required to determine whether the antigens from brain and from fibroblasts described by the two laboratories are identical.

Recent studies by Schubert et al [28,29] have demonstrated that the skeletal muscle myoblast cell line L6 secretes particles known as adherons which appear to be high molecular weight complexes composed of glycosaminoglycns and "matrix"-type glycoproteins, including fibronectin and collagen. It has also been shown by Damsky et al [23] that epithelial cells, which contain a 120Kd cell-surface molecule implicated in cell-cell adhesion, also shed an 80Kd fragment of this protein into the medium. It may be that the 140Kd protein described in this study also sheds fragments into the medium, or exists as part of an adheron-type complex of molecules. Future studies to test this possibility should prove interesting.

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