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An Integral Membrane Protein Antigen Associated with the Membrane Attachment Sites of Actin Microfilaments Is Identified as an Integrin β-Chain

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A monoclonal antibody (MAb 30B6) was recently described by Rogalski and Singer (J. Cell Biol. 101:785–801, 1985) which identified an integral membrane glycoprotein of chicken cells that was associated with a wide variety of sites of actin microfilament attachments to membranes. In this report, we present a further characterization of this integral protein. An immunochemical comparison was made of MAb 30B6 binding properties with those of two other MAbs, JG9 and JG22, which identify a component of a membrane protein complex that interacts with extracellular matrix proteins including fibronectin. We showed that the 110-kilodalton protein recognized by MAb 30B6 in extracts of chicken gizzard smooth muscle is identical, or closely related, to the protein that reacts with MAbs JG9 and JG22. These 110-kilodalton proteins are also structurally closely similar, if not identical, to one another as demonstrated by ^{125}I-tryptic peptide maps. However, competition experiments showed that MAb 30B6 recognizes a different epitope from those recognized by MAbs JG9 and JG22. In addition, the 30B6 antigen is part of a complex that can be isolated on fibronectin columns. These results together establish that the 30B6 antigen is the same as, or closely similar to, the β-chain of the protein complex named integrin, which is the complex on chicken fibroblast membranes that binds fibronectin. Although the 30B6 antigen is present in a wide range of tissues, its apparent molecular weight on gels varies in different tissues. These differences in apparent molecular weight are due, in large part, to differences in glycosylation.

We have been interested in detecting and characterizing integral membrane proteins that are involved in the attachment of actin microfilaments to membranes. One case in which such attachment arises is the dividing mitotic cell, in which a belt of actin microfilaments is concentrated under the membrane in the cleavage furrow (18). Rogalski and Singer (16) recently described a monoclonal antibody (MAb 30B6) that was elicited by immunization with mitotic chicken embryo fibroblast (CEF) cells and was selected for its unique capacity to label preferentially the cleavage furrow of fixed but impermeable dividing chick cells. In addition, MAb 30B6 immunolabeled a wide variety of other chicken cell surface sites where actin microfilaments are associated with membranes. The protein that was immunoadsorbed from detergent extracts of chicken gizzard on MAb 30B6 columns consisted of two Coomassie blue-staining bands of about 160 and 110 kilodaltons (kDa) on nonreducing sodium dodecyl sulfate (SDS)-polyacrylamide gels, and only the lower-molecular-weight band was immunoblotted by MAb 30B6 (16).

In a different context, several laboratories over the last few years have produced MAbs that were selected for their capacity to inhibit or alter the adhesion of chicken cells to their substrata. These MAbs include JG9 and JG22, derived by Greve and Gottlieb (7), and CSAT, produced by Neff et al. (14). MAbs JG22 and JG9 were shown to react with the same antigen (3, 7). MAb JG22 was found to immunoprecipitate or immunoadsorb a set of three proteins of about 160, 140, and 120 kDa from extracts of whole chicken embryos (5). A closely similar, if not identical, set of three proteins was also isolated by MAb CSAT (10, 12). These proteins were shown to bind to fibronectin (1, 10), suggesting that they were related to the human fibronectin receptor (15). Although the MAbs JG22 and CSAT were not shown to immunoblot any components in extracts of chicken embryos, it was recently indirectly demonstrated that both antibodies were directed only to the lowest-molecular-weight (120,000) protein of the set of three (2). In addition, it was reported that the CSAT antigen bound the cytoskeletal protein talin weakly in vitro (9).

These results raised the possibility that the antigen recognized by MAb 30B6, associated with cytoplasmic actin microfilaments, and the antigen recognized by MAbs JG9, JG22, and CSAT, associated with components of the extracellular matrix, were the same or closely similar proteins, involved in transmembrane linkages between the extracellular matrix and the actin cytoskeleton. Because initial immunolabeling results obtained with MAbs 30B6 and JG9 appeared to be distinguishable, it was tentatively suggested that the antigens recognized by the two antibodies were not identical (16). We examined this problem in greater detail and provide evidence that MAbs 30B6 and JG9-JG22 recognize two different epitopes on the same protein. The antigen specific to the MAbs JG9-JG22 and CSAT was identified as a β-chain of the protein complex integrin (2, 11).

MATERIALS AND METHODS

Antibody reagents. The JG9 hybridoma line was originally obtained from J. Greve and D. Gottlieb. MAb JG9 was isolated and purified as described previously (7). The 30B6 hybridoma line was obtained from A. Rogalski, and MAb 30B6 was isolated and purified as described previously (16). MAb JG22 was obtained from the subclone JG22E of the original JG22 hybridoma and was the gift of W.-T. Chen (Georgetown University).

Cell culture. Primary cultures of 8-day-old CEFs were prepared by trypsinization of decapitated, minced embryos.

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The cells were cultured in Dulbecco modified Eagle-Coon F-12 (1:1, vol/vol) medium containing 10% fetal calf serum and were used after one to two passages. Rous sarcoma virus (RSV)-transformed CEFs were obtained from B. Sefton (Salk Institute).

Antigen isolation. Fresh or frozen adult chicken gizzard or heart was homogenized in 8 volumes of 10 mM Tris chloride (pH 7.4) containing 3 mM $CaCl_2$, 0.25 M sucrose, and 1 mM phenylmethylsulfonyl fluoride for 2 min at high speed in a Waring blender. The extract was centrifuged at 1,700 × *g* for 10 min, and the supernatant was filtered through cheesecloth and then centrifuged at 200,000 × *g* for 60 min. The pellets were extracted overnight at 4°C in 0.5 volumes of phosphate-buffered saline (PBS) containing 0.5% Nonidet P-40 (PBS–NP-40) and 1 mM phenylmethylsulfonyl fluoride, and insoluble material was then centrifuged out at 100,000 × *g* for 60 min. Samples (4 ml) of the supernatants were applied to 2-ml affinity columns of either 30B6 immunoglobulin G (IgG; 3 mg/ml) or JG9 IgG (2 mg/ml) coupled to glutaraldehyde-activated Ultrogel ACA 22 (LKB Instruments, Inc., Rockville, Md.) by a standard technique (19). The extracts were incubated with the columns for 1 h at room temperature and washed with 50 volumes of PBS–NP-40, and specifically bound material was eluted with 0.2 M HCl brought to pH 2.8 with 2 M glycine and containing 0.5% NP-40. Tissue culture cells were rinsed with PBS, extracted directly with PBS–NP-40, and clarified by centrifugation at 100,000 × *g* for 30 min, and the extracts were run on the affinity columns as described above.

Peptide mapping. Two-dimensional mapping of ^{125}I-labeled tryptic peptides from proteins isolated after separation by nonreducing SDS-polyacrylamide gel electrophoresis (PAGE) was done as described previously (20).

Antibody iodination and cell labeling. MAb 30B6 (43 μg) was iodinated with chloramine-T (25 μg) and Na^{125}I (250 μCi) in 0.5 M sodium phosphate (pH 7.5) for 1 min. The reaction was stopped with sodium metabisulfite (300 μg), and the iodinated antibody was separated from free iodine on a 1-ml Dowex AG 1 × 8 (Bio-Rad Laboratories, Richmond, Calif.) column equilibrated with 0.15 M NaCl. The specific activity of the iodinated antibody was 1.5 × 10^7 cpm/μg. CEFs were grown to confluence in 24-well culture dishes. They were rinsed several times with PBS containing 0.1% bovine serum albumin (PBS-BSA) and incubated for 30 min at room temperature with either 0.5 ml of PBS-BSA alone or PBS-BSA containing 126 μg of JG9 per ml or 86 μg of 30B6 per ml. The cells were rinsed twice with PBS-BSA and then labeled for 1 h with ^{125}I-MAb 30B6 (0 to 40 μg/ml) in PBS-BSA. Excess label was rinsed out, the cells were solubilized for 30 min with 1 ml of 0.1 M NaOH–2% Na_2CO_3–1% SDS per well, and samples were taken for scintillation counting and protein determination by the method of Hartree (8). The results from the protein assay were compared with a standard curve in which the number of CEFs was plotted against cellular protein determined in the same way. Thus, counts per minute per microgram of protein could be converted to counts per minute per 10^6 cells.

SDS-PAGE and immunoblotting. SDS-PAGE was done as described previously (13) on 7.5% gels except that the final SDS concentration in the samples was 1%, the samples were not boiled, and if immunoblotting was to be done, no β-mercaptoethanol was used. SDS-PAGE gels were either stained with Coomassie blue or transferred to nitrocellulose as described previously (21) except SDS was omitted from the transfer buffer. This omission was found to enhance immunoblotting greatly. Blots were blocked for 2 h at room temperature with 3% BSA in Tris-buffered saline (TBS), rinsed, and incubated with MAb 30B6 or MAb JG9 (10 μg of IgG per ml in TBS-BSA) overnight. Excess antibody was washed out, and the blots were incubated for 2 h with rabbit anti-mouse IgG (1 μg/ml in TBS-BSA), washed, treated with peroxidase-conjugated goat anti-rabbit IgG (1/1,000 dilution; Organon Teknika, Malvern, Pa.) for 30 min, washed again, and developed with diaminobenzidine (Sigma Chemical Co., St. Louis, Mo.) (0.5 mg/ml in 50 mM Tris [pH 7.5]–0.15% H_2O_2).

Lectin blotting. Samples were separated by 7.5% SDS-PAGE, transferred to nitrocellulose, and blocked as described above. They were then treated for 4 h at room temperature with concanavalin A (ConA; 0.5 mg/ml; Vector Laboratories, Burlingame, Calif.), peroxidase-conjugated wheat germ agglutinin (WGA; 1/1,000 dilution; E-Y Laboratories, San Mateo, Calif.), or peroxidase-conjugated *Ricinus Communis* agglutinin I (RCA; 1/5,000 dilution; E-Y Laboratories) in TBS-BSA. Excess reagent was washed out, and the ConA blot was treated with horseradish peroxidase (10 μg/ml in TBS-BSA; Sigma) for 30 min. All blots were developed with diaminobenzidine as described above.

Fibronectin affinity chromatography. Fibronectin was prepared from chicken plasma (17) and coupled to Affi-gel 10 (Bio-Rad) as described in the Bio-Rad catalog. Gizzard extract (4 ml) prepared as described above was mixed with 2 ml of coupled gel for 1 h at room temperature with constant rotation of the column. Unbound material was washed out with 50 volumes of PBS–NP-40, and specifically bound material was eluted with 2 ml of GRGDSP peptide (the gift of M. Pierschbacher; used at 1 mg/ml in PBS–NP-40) for 1 h at room temperature with constant rotation. Fractions (1 ml) were collected, and 100-μl samples were analyzed by SDS-PAGE and immunoblotting with MAb 30B6.

Glycosidase treatments. The *N*-glycanase reaction mixture containing 10 μl of affinity-purified antigen in PBS–NP-40–0.20 M sodium phosphate (pH 8.6)–10 mM 1,10-phenanthroline hydrate–1.5% NP-40–1.2 ml of *N*-glycanase (2.50 U/ml; Genzyme Corp., Boston, Mass.) was incubated overnight at 37°C, and the samples were analyzed by SDS-PAGE and immunoblotting with MAb 30B6. Controls were run omitting the enzyme.

The procedure for *O*-glycanase treatment was somewhat different since the reaction was inhibited in PBS–NP-40. Gizzard or cardiac extracts (100 μl) were mixed with 50 μl of MAb 30B6 conjugated to Ultrogel ACA 22 for 2 h at room temperature. The beads were rinsed twice with 0.1 M sodium acetate (pH 5.0)–0.2 mM EDTA–2 mM calcium acetate and were incubated overnight at 37°C in 125 μl of the same buffer containing 2.5 U of *Vibrio cholerae* neuraminidase (Calbiochem-Behring La Jolla, Calif.). A 2-μl sample of *O*-glycanase (1,000 U/ml; Genzyme) was then added, and the reaction was continued for 6 h at 37°C. The antigen was then eluted from the beads with 0.2 M HCl brought to pH 2.8 with 2 M glycine and containing 0.5% NP-40, for 1 h, and the supernatants were analyzed by SDS-PAGE followed by immunoblotting with MAb 30B6. Controls were run with no enzyme, no neuraminidase, or no cell extract.

Tissue homogenate preparation. Fresh chicken tissues were rinsed in 1 mM $NaHCO_3$ (pH 8.2) and homogenized in 10 volumes of the same buffer containing 1 mM phenylmethylsulfonyl fluoride, 5 mM EGTA, aprotinin (0.15 to 0.30 trypsin inhibitor units per ml), and 5 μg of leupeptin per ml for 30 s at high speed in a Waring blender. The protein concentration of each homogenate was determined by the

method of Hartree (8), using BSA as the standard, and aliquots were stored frozen at −70°C.

RESULTS

MAb 30B6 recognizes the JG9 antigen and MAb JG9 recognizes the 30B6 antigen. A first series of experiments involved immunoadsorbing chicken gizzard extracts on columns of either MAb 30B6 or JG9 and then immunoblotting the column eluates with one or the other MAb. Affinity columns were prepared by coupling MAb 30B6 or JG9 to glutaraldehyde-activated Ultrogel ACA 22. NP-40 extracts of chicken gizzard were adsorbed, and the adsorbed protein was then eluted from both columns. In SDS-PAGE under nonreducing conditions, each eluate contained two Coomassie blue-staining bands of about 170 and 110 kDa (Fig. 1, lanes A and B) as described previously (16). The apparent molecular size of the latter band increased to 140 kDa after reduction, while that of the upper band did not change markedly (Fig. 1, lanes C and D). Transfers to nitrocellulose made from the nonreducing gels in the absence of SDS allowed more efficient immunoblotting with the MAbs than earlier procedures. Using the same MAb for immunoblotting as was employed in the immunoadsorption step, only the lower-molecular-weight band was found to react (Fig. 1, lanes E and F). The results of particular interest, however, were that the heterologous MAb also blotted the lower-molecular-weight band in each eluate (Fig. 1, lanes G and H). The specificity of the immunoblotting was demonstrated by the failure of an unrelated MAb to react with either eluate under the same conditions (Fig. 1, lanes I and J). Thus, the MAbs 30B6 and JG9 recognize the same cross-reactive antigen. MAb 30B6 also reacted with the 110-kDa band in the eluate from a gizzard extract adsorbed on a JG22 column (data not shown). However, MAb JG22 did not react in immunoblotting experiments under the same conditions.

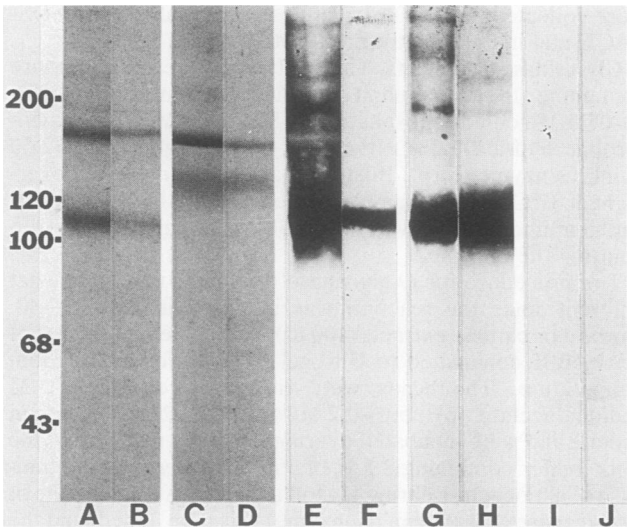

FIG. 1. Analysis of the immunoaffinity-purified 30B6 and JG9 antigen complexes from adult chicken gizzard. Coomassie blue staining is shown of the nonreduced (lanes A and B) and reduced (lanes C and D) 30B6 antigen complex (lanes A and C) and JG9 antigen complex (lanes B and D) eluted from their respective immunoadsorption columns and separated on a 7.5% SDS-polyacrylamide gel. Immunoblotting results are given for the unreduced 30B6 antigen complex (lanes E, G, and I) and JG9 antigen complex (lanes F, H, and J) with MAb 30B6 (lanes E and H), MAb JG9 (lanes F and G), and an unrelated MAb (lanes I and J). Only the lower-molecular-weight bands of the antigen complexes are immunoblotted, and either MAb blots the band eluted from either immunoaffinity column. Molecular sizes are shown on the left in kilodaltons.

Similar two-dimensional peptide maps of the 30B6 and JG9 antigens. To investigate further the relationship between the 30B6 antigen and the JG22-JG9 antigen, the 170- and 110-kDa proteins isolated from the two MAb affinity columns were separated on nonreducing gels and analyzed by two-dimensional ^{125}I-tryptic peptide mapping. The peptide maps for the 110-kDa proteins eluted from the two columns were nearly superimposable (Fig. 2A and B); the maps of the two 170-kDa proteins were also very similar to one another (Fig. 2C and D). These results indicate a close structural homology, if not identity, between the two 110-kDa proteins on the one hand and the two 170-kDa proteins on the other.

Recognition of different epitopes by MAbs 30B6 and JG9. Once the close structural relationship or identity between the proteins recognized by MAbs 30B6 and JG9 had been established, it was then of interest to determine whether these two antibodies recognized the same epitope in the 110-kDa protein. MAb 30B6 was iodinated with chloramine-T and used to label unfixed CEFs either alone or after pretreatment with excess MAb JG9 or 30B6. The results are shown in Fig. 3. Although MAb 30B6 substantially reduced the subsequent binding of ^{125}I-MAb 30B6 to the cells, 1.5 times as much MAb JG9 had no effect on the subsequent binding of ^{125}I-MAb 30B6. These results showed that MAb JG9 did not block the binding of MAb 30B6. A similar result was obtained in a double-immunofluorescence study with biotin-conjugated MAb 30B6 and unlabeled MAbs 30B6, JG9, and JG22. The conclusion, therefore, is that the epitopes recognized by MAb 30B6 and MAbs JG9 and JG22 on the 110-kDa antigen are different.

Binding of the 30B6 antigen complex to fibronectin. Since it had been reported that the set of proteins isolated with MAb CSAT and with MAb JG22 had binding affinity for fibronectin (1, 10), it was of interest to determine whether the 30B6 antigen-containing complex also bound to fibronectin. A gizzard extract enriched in the 30B6 antigen was incubated with chicken fibronectin coupled to glutaraldehyde-activated Ultrogel ACA 22 for 1 h with constant rotation. After extensive washing, specifically fibronectin-bound material was eluted with the peptide GRGDSP as described previously (15). The presence of the 30B6 antigen in the eluant was shown by immunoblotting of the column fractions with MAb 30B6 (Fig. 4).

Variation of the apparent molecular weight of 30B6 antigen from tissue to tissue. Immunoblotting of extracts of a variety of adult chicken tissues with MAb 30B6 demonstrated the presence of the 30B6 antigen in most cell types (Fig. 5) except lens and erythrocytes. However, the apparent molecular size of the antigen varied somewhat. This was most obvious when gizzard and cardiac muscle were compared. The 30B6 antigen in cardiac muscle had an apparent molecular size of 140 kDa in nonreducing gels, whereas the gizzard antigen was 110 kDa. To determine the reason for these molecular size differences, we isolated the 30B6 antigen from cardiac muscle on an MAb 30B6 affinity column. Only a single broad Coomassie blue-staining band of protein was obtained from cardiac muscle in contrast to the two protein bands that were isolated from chicken gizzard (Fig. 6, lanes A and B). The molecular size of the cardiac 140-kDa protein was not altered after reduction (Fig. 6, lane C), in contrast to the 110-kDa band in the gizzard extract (Fig. 6, lane D). One

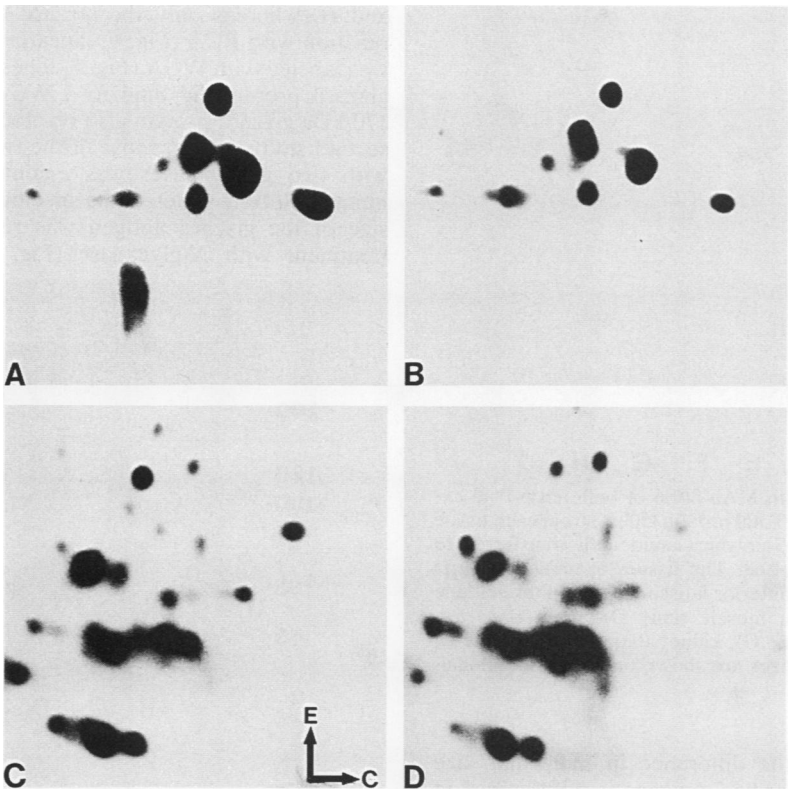

FIG. 2. Two-dimensional ^{125}I-peptide maps of trypsin digests of the 110-kDa (A and B) and 170-kDa (C and D) proteins eluted from MAb 30B6 (A and C) or MAb JG9 (B and D) immunoaffinity columns. The coordinates labeled E and C indicate the directions of electrophoresis and chromatography, respectively.

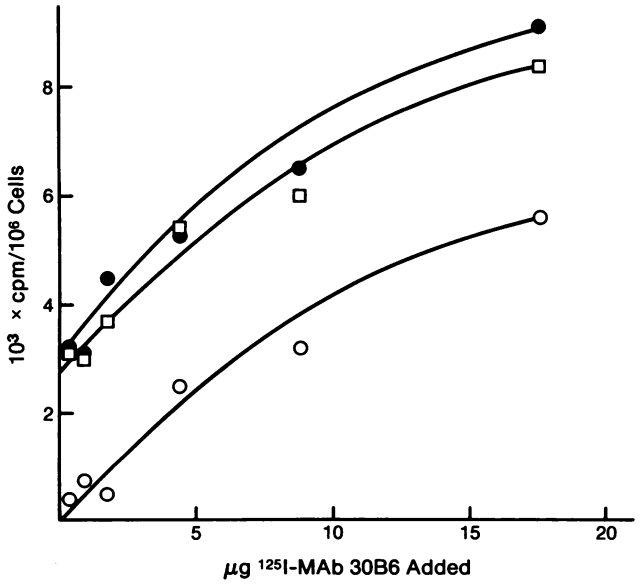

FIG. 3. ^{125}I-labeled MAb 30B6 binding to unfixed CEFs. Labeling was done in the absence (●) of a prior treatment with unlabeled MAb 30B6 or in the presence of either 43 μg of unlabeled MAb 30B6 (○) or 63 μg of unlabeled MAb JG9 (□). The results shown are the averages of three determinations each done in duplicate.

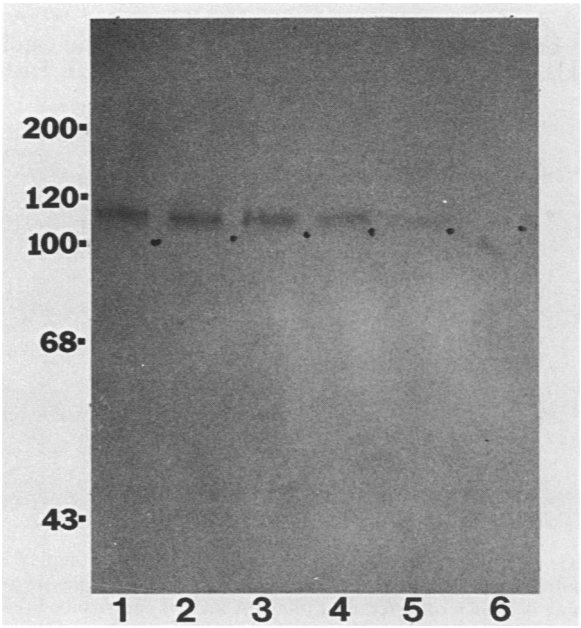

FIG. 4. Immunoblotting with MAb 30B6 of fractions of GRGDSP eluant from a chicken fibronectin-ACA 22 affinity column which had been used to adsorb a gizzard extract enriched in the 30B6 antigen. The column was washed with 50 column volumes of buffer and eluted with the GRGDSP peptide (1 mg/ml) in the same buffer. Samples (100 μg) of each successive 1-ml fraction (1 to 6) of the eluant were separated on a 7.5% SDS-polyacrylamide gel, transferred to nitrocellulose, and immunoblotted positively for MAb 30B6. Molecular sizes are shown on the left in kilodaltons.

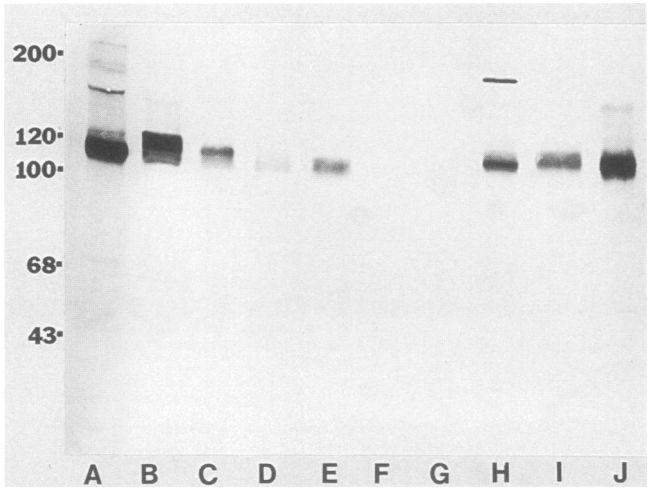

FIG. 5. Immunoblotting with MAb 30B6 of buffered NP-40 extracts of adult chicken tissues. Total protein (50 μg) from each tissue was separated on a 7.5% SDS-polyacrylamide gel, transferred to nitrocellulose, and immunoblotted. The tissues examined are gizzard (lane A), heart (lane B), anterior latissimus dorsi muscle (lane C), posterior latissimus dorsi muscle (lane D), brain (lane E), erythrocytes (lane F), lens (lane G), kidney (lane H), liver (lane I), and lung (lane J). Molecular sizes are shown on the left in kilodaltons.

possible explanation for the difference in molecular size between the gizzard and cardiac antigens is a difference in glycosylation of the two proteins. This was examined in two experiments. Initially, the reaction of the two antigens with several different lectins was compared. The antigens were separated on SDS-polyacrylamide gels, transferred to nitrocellulose, and treated with peroxidase-conjugated WGA or RCA or with ConA. Both the gizzard 110-kDa and cardiac 140-kDa proteins reacted strongly with ConA (Fig. 6, lanes I and J), whereas only the gizzard antigen gave a positive reaction with RCA (Fig. 6, lanes E and F). Neither antigen was labeled with WGA (Fig. 6, lanes G and H), although the gizzard protein did bind to a WGA-agarose column. The 170-kDa gizzard protein also reacted with the lectins. In the second study, the response of the two antigens to treatment with two glycosidases was examined, using MAb 30B6 immunoblotting to detect the specific antigen. The molecular size of the gizzard antigen was reduced to ~85 kDa by treatment with *N*-glycanase (Fig. 7-1, lanes A and B),

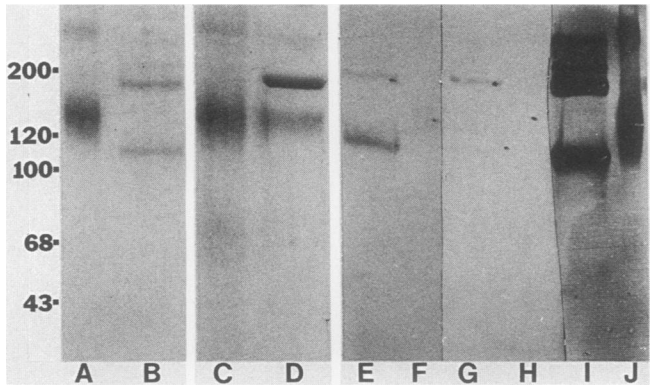

FIG. 6. Comparative analysis of the 30B6 antigen complex isolated from chicken cardiac muscle and gizzard. Coomassie blue staining is shown of unreduced (lanes A and B) and reduced (lanes C and D) forms of the cardiac (lanes A and C) and gizzard (lanes B and D) 30B6 antigen complex. Lectin binding of the gizzard (lanes E, G, and I) and cardiac (lanes F, H, J) 30B6 antigen was examined. The proteins were separated on a 7.5% SDS-polyacrylamide gel, transferred to nitrocellulose, and stained with RCA (lanes E and F), WGA (lanes G and H), or ConA (lanes I and J). Both the lower- and higher-molecular-weight components of the gizzard antigen complex reacted with all three lectins, but the cardiac antigen complex reacted only with ConA. Molecular sizes are shown on the left in kilodaltons.

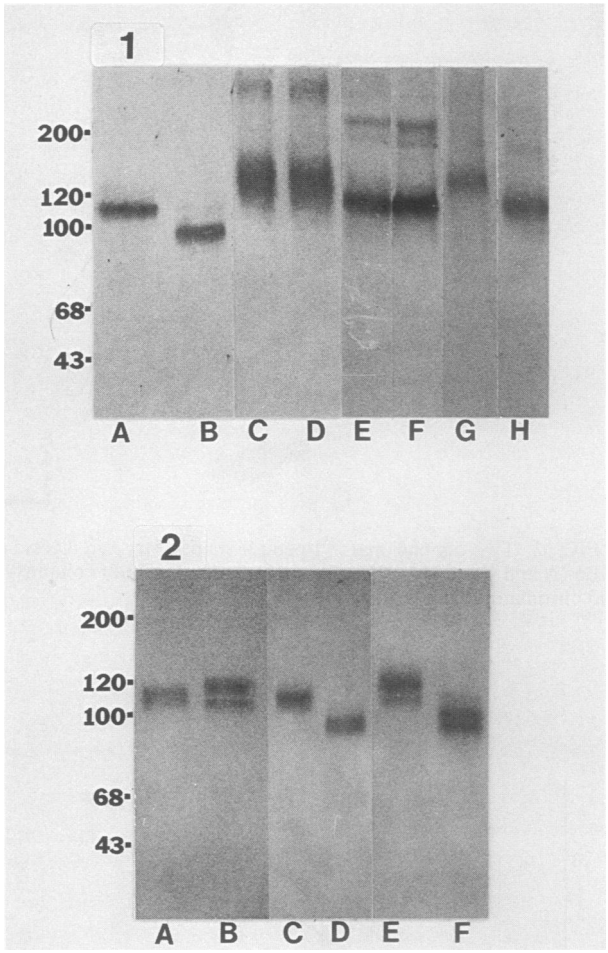

FIG. 7. Analysis of treatment of the 30B6 antigen with *N*-glycanase and *O*-glycosidase. The unreduced 30B6 antigens were separated on 7.5% SDS-polyacrylamide gels, transferred to nitrocellulose, and immunoblotted with MAb 30B6. (1) Gizzard 30B6 antigen that was incubated overnight at 37°C in either the absence (lane A) or presence (lane B) of *N*-glycanase is shown. Similarly, cardiac 30B6 antigen was incubated in the absence (lane C) or presence (lane D) of *N*-glycanase. The gizzard antigen was changed in apparent molecular weight by this treatment, but the cardiac antigen was not. Gizzard 30B6 antigen was incubated in the absence (lane E) or presence (lane F) of *O*-glycanase; similarly, cardiac 30B6 antigen was incubated in the absence (lane G) or presence (lane H) of *O*-glycanase. Only the cardiac antigen was affected by this enzyme treatment. (2) Untreated 30B6 antigen isolated from normal CEFs (lane A) and RSV-transformed CEFs (lane B) is shown. Normal CEF 30B6 antigen that was incubated in either the absence (lane C) or presence (lane D) of *N*-glycanase and RSV-transformed CEF 30B6 antigen that was incubated in the absence (lane E) or presence (lane F) of *N*-glycanase are shown. Both the normal and RSV-transformed antigens have their apparent molecular weights reduced by the enzyme treatment. Molecular sizes are shown on the left in kilodaltons.

whereas the molecular size of the cardiac antigen was unaltered (Fig. 7-1, lanes C and D). On the other hand, the molecular size of the cardiac antigen was reduced to ~100 kDa by treatment with *O*-glycanase (Fig. 7-1, lanes G and H), whereas the molecular size of the gizzard antigen remained constant (Fig. 7-1, lanes E and F). The molecular size of the 30B6 antigen also varied between that found in normal and in RSV-transformed CEFs in a manner similar to that seen with the gizzard and cardiac antigens (Fig. 7-2, lanes A and B). To see whether this difference was also due to different glycosylation patterns, the 30B6 antigen was isolated by affinity chromatography from normal and RSV-transformed CEFs and treated with *N*-glycanase. The molecular sizes of the *N*-glycanase-treated proteins from normal and transformed cells had become essentially the same (Fig. 7-2, lanes D and F).

DISCUSSION

Our results indicate that MAb 30B6, produced in response to immunization with intact mitotic CEFs and selected for its immunofluorescent localization to the actin microfilament-enriched cleavage furrow of the dividing cells (16), and the separately derived MAbs JG9 and JG22, produced in response to immunization with chicken embryo myoblast preparations and selected for their capacity to inhibit or disrupt myoblast attachment to their substrate (7) and which recognize the same antigen (3, 7), are all directed to the same, or a closely similar, cell surface integral membrane protein.

This relationship was demonstrated in two ways, immunochemically and structurally. Extracts of chicken gizzard were passed over columns of either MAb 30B6 or JG9. The immunoadsorbed proteins were then eluted from each column, electrophoresed, transferred by an improved procedure to nitrocellulose, and immunoblotted with either MAb. The proteins eluted from both columns showed specific immunoblotting of the same 110-kDa protein band with either MAb (Fig. 1). Furthermore, the 110-kDa bands eluted from the two MAb immunoadsorbent columns yielded closely similar ^{125}I-tryptic peptide maps (Fig. 2A and B). These maps are simple enough to be consistent with the presence of only a single protein species in the 110-kDa band, but the possibility that more than one closely homologous species of the same molecular size was present cannot be excluded by the peptide maps alone. As discussed below, MAbs 30B6 and JG9-JG22 recognize different epitopes. The simplest conclusion from all our results is that the two MAbs are directed to different epitopes on the same 110-kDa protein. If it were suggested, on the contrary, that the epitopes were on two different but closely homologous 110-kDa protein species, these proteins would have had to be noncovalently associated with one another in the extracts because each would have had to be immunoadsorbed on the heterologous MAb column. This seems unlikely.

Consistent with the conclusion that the 30B6 and JG9-JG22 antigens are the same protein, in double-immunofluorescence labeling experiments with a polyclonal antibody rendered monospecific for the 30B6 110-kDa protein and either MAb JG9 or JG22, the pair of antibodies always exhibited identical localizations on a given cell (data not shown). In competition assays, however, saturation binding with MAb 30B6 did not block further binding of MAb JG9 and vice versa (Fig. 3). The MAbs 30B6 and JG9-JG22 must therefore recognize different and sterically separate epitopes on the 110-kDa protein.

Both MAbs 30B6 and JG9 immunoadsorbed, in addition to the 110-kDa band, a 170-kDa band from the chicken gizzard extracts, which, however, did not immunoblot with either antibody (16) (Fig. 1). Furthermore, the ^{125}I-tryptic peptide maps of the 170-kDa proteins eluted from the two MAb columns were closely similar to one another (Fig. 2C and D), but different from the maps of the 110-kDa proteins. As suggested elsewhere (3, 16), the 170-kDa protein is therefore immunochemically and structurally probably not related to the 110-kDa protein but is very likely complexed to it in the membrane, the complex resisting dissociation upon NP-40 solubilization. The results are consistent with the conclusion that the same complex is immunoadsorbed by both MAbs 30B6 and JG9.

An MAb, CSAT (2, 10, 12, 14), has been extensively studied and has been shown to be related to JG9-JG22 (2). This MAb was independently produced and selected (14) by methods similar to those used to generate MAbs JG9 and JG22 (7). MAb CSAT was found to immunoprecipitate a complex of three integral membrane proteins of 160, 135, and 120 kDa from nonreduced extracts of whole chicken embryos (12). Although MAb CSAT did not immunoblot any of these components, it was recently shown indirectly that both MAbs CSAT and JG22 reacted with only the 120-kDa protein (2). Taken together, these results therefore demonstrate that the MAbs 30B6, JG9-JG22, and CSAT are all directed to the same, or closely similar, protein of 110 to 120 kDa. Following the suggestions and nomenclature of Hynes (11), the protein complexes immunoadsorbed by these several MAbs are thought to consist of heterodimers or mixtures of heterodimers; the 110 to 120-kDa components are called β-chains, and the 160- to 170-kDa components are called α-chains. The 135-kDa component observed in extracts of whole chicken embryos, but not in extracts of adult chicken gizzard, may be a second kind of α-chain. A heterodimer complex is called integrin (11). MAbs 30B6 and JG9-JG22, and probably CSAT as well, are therefore specific for the same chicken integrin β-chain.

Integrin complexes in vitro function as receptors for fibronectin (1, 2, 10, 11) (Fig. 4). In addition, a weak interaction of the integrin complex with the cytoskeletal protein talin has been reported (9). These in vitro interactions complement immunofluorescence labeling experiments which have shown, on the one hand, a close correspondence in the surface distributions of MAbs JG9-JG22 and CSAT (5, 6) with extracellular fibronectin, and on the other hand, of MAbs JG22, CSAT, and 30B6 (4, 6, 16) with actin microfilaments. These results together support the conclusion that the integrin complex can serve as a transmembrane link between the extracellular matrix and cytoskeletal microfilaments (9, 11).

The ability to perform efficient immunoblotting with MAb 30B6 allowed us to survey extracts of chicken tissues other than gizzard for proteins that are immunochemically related to the integrin β-chain. This showed not only the widespread distribution of the β-chain in different cell types (Fig. 5), as was indicated by earlier immunofluorescence microscopic studies (4, 16), but also revealed that the β-chain can have somewhat different apparent molecular weights in different cells and tissues. This is obviously important for SDS-PAGE comparisons of integrins isolated from complex systems (such as whole chicken embryos) or from a variety of tissues. The molecular weight difference was most apparent when comparing the adult gizzard and cardiac antigens (Fig. 5 to 7). We obtained evidence that at least part of this molecular weight difference was due to differences in glyco-

sylation of the protein in the two tissues. The apparent molecular weight of the gizzard protein could be decreased by an enzyme that hydrolyzes asparagine-linked oligosaccharides, whereas the molecular weight of the cardiac antigen was only affected by an enzyme that removes a gal-β-(1,3)galNAc core disaccharide linked to either serine or threonine (Fig. 7). In addition, the gizzard and cardiac proteins showed different reactivities with two different lectins (Fig. 6). It is possible that the gizzard and cardiac integrin β-chains are isoforms that are glycosylated differently. On the other hand, it is possible that alterations in the glycosylation of the same β-chain occur. Such differences may function in regulating the interaction of integrin with fibronectin or other components of the extracellular matrix or with the actin microfilaments. In connection with β-chain variation, the studies of the 30B6 antigen of normal and RSV-transformed CEFs are of interest. It was observed that the 30B6 antigen had an increased apparent molecular weight in RSV-transformed CEFs as compared with that in normal CEFs (Fig. 7) and that this difference was eliminated by treatment of both antigens with *N*-glycanase. Transformed fibroblasts are well known to have a decreased cell-cell adhesivity and show marked changes in microfilament-membrane interactions, as compared with normal cells, and the altered glycosylation of their integrin β-chains may play a role in these changes.

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