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Immunochemical and Biochemical Characterization of a Glioma-Associated Extracellular Matrix Glycoprotein

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A novel human glioma-associated extracellular matrix (ECM) glycoprotein has been identified by murine monoclonal antibody 81C6. The glycoprotein, designated GMEM, is expressed in the ECM of glioma and mesenchymal cell cultures, in the perivascular matrix of endothelial proliferations of human gliomas, and in the stroma of human glioma xenografts in athymic mice, where it has been used as a target antigen for monoclonal antibody tumor localization and radioimaging. We report here on the immunochemical and biochemical characterization of GMEM. Polyacrylamide gel analysis of immunoprecipitated [^{3}H]-leucine- and [^{3}H]-glucosamine-labeled ECM from the human glioma cell line U-251MG has shown that GMEM is a high-molecular-weight macromolecule (M_r ~ 1,000,000) composed of M_r ~ 230,000 disulfide-bonded glycoprotein subunits. Immunoprecipitation, immunoblot, and one-dimensional peptide map analysis have shown that GMEM is distinct from human fibroblast and plasma fibronectin. These results support previous immunohistology and absorption analysis findings, indicating that GMEM is distinct from fibronectin, laminin, and glycosaminoglycans secreted by U-251MG.

Key words: extracellular matrix, glioma-associated glycoprotein, fibronectin, GMEM

The human glioma cell line U-251MG, which expresses the intracellular glial differentiation marker glial fibrillary acid protein (GFAP), is an adherent cell line secreting a variety of extracellular matrix (ECM) products. Studies have demonstrated the secretion by U-251MG of hyaluronic acid and chondroitin proteoglycan [1,2], fibronectin [3], laminin, type IV collagen, and EC collagen [4]. The composition of the extracellular matrix includes glycosaminoglycans [1] and laminin [4]. In addition, the ECM of U-251MG expresses a distinctive glioma-mesenchymal extracellular matrix (GMEM) antigen [5]. Fibronectin, type IV collagen, and EC collagen have not been found in detectable amounts in the ECM of U-251MG [3–5]. Many of these

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ECM macromolecules have important roles in cell adhesion, motility, and differentiation of both normal and tumor cells [6,7]. Within the nervous system, studies have suggested a role for fibronectin and glycosaminoglycans in normal development [8,9]. Neurite extension by dissociated neurons in culture has also been shown to be dependent on the presence of an ECM produced by other cells [10].

The functional role of GMEM is unknown; however, its presence in the ECM and association with sites of cell-substrate adhesion indicate a possible role in either cell adhesion or structural maintenance of the ECM. Its role in vivo appears to be highly specific, given its limited distribution in several organs and its association with tumor vasculature [5]. The association of GMEM with the perivascular matrix of human glioma endothelial proliferations, but not normal brain, may be an indication of its role in tumor neovascularization [11].

We describe here the partial immunochemical and biochemical characterization of GMEM from the ECM of glioma and fibroblast cell lines.

METHODS

Cell Culture

The human glioma cell line U-251MG and human adult skin fibroblasts 199 and IMR 90 (ATCC) were cultured as adherent monolayers in Reichert's zinc option media [12]. Glioma cultures were passaged twice weekly and fibroblast cultures every 5–6 days. Incorportion of 20 μCi/ml [^{3}H]-leucine (specific activity 50 Ci/mM; New England Nuclear) into cellular proteins was performed in leucine-deficient RPMI1640 media supplemented with 5% dialyzed fetal calf serum (FCS) for a period of 72 hr. Cells were labeled with 20 μCi/ml [^{3}H]-glucosamine (specific activity 32 Ci/mM; New England Nuclear) for 72 hr using RPMI1640 supplemented with 10% FCS. Labeling of confluent cultures with 100 μCi/ml [^{35}S]-methionine (specific activity 1,200 Ci/mM; New England Nuclear) was performed for 6 hr in HB/102 methionine-deficient media (Hana Media Inc.) supplemented with 2% dialyzed FCS.

Antibodies

Monoclonal antibodies 81C6 and B2B7 and myeloma immunoglobulin 45.6 all IgG_{2b}, were purified by protein A-Sepharose affinity chromatography from spent culture supernatant as previously described [5]. The myeloma immunoglobulin 45.6 has no known specificity and served as a negative immunoglobulin control, as did the monoclonal antibody B2B7, which binds a cell surface-associated antigen expressed by virtually all human cell lines [unpublished observation]. Monoclonal antibody 3E1 (IgG_1) ascites and rabbit anti-human fibronectin antisera were provided by M. Pierschbacher [13].

Solubilization of U-251MG Extracellular Matrix

Cultures of U-251MG in 96-well microtiter plates were treated with 0.02% EDTA in Dulbecco's phosphate-buffered saline (DPBS) to detach adherent cells and were washed to remove the cells. Triplicate wells were treated with 100 μl DBPS, sodium dodecyl sulfate (SDS), Triton X-100, deoxycholate (DOC), or urea at the concentrations indicated in Figure 1, either alone or in combination with 1% 2-mercaptoethanol for 1 hr at 37°C. Treated wells were then rinsed first with solubilizing agent and finally with DPBS prior to assaying for 81C6 antibody binding.

Binding of a limiting amount of 81C6 antibody (0.1 μg/ml) to the treated wells was determined by an indirect immunoassay using radiolabeled affinity-purified rabbit anti-mouse immunoglobulin antibodies.

Cultures of U-251MG labeled for 72 hr with [^{3}H]-leucine were first treated with 0.2% EDTA-DPBS to detach adherent cells, and culture plates were rinsed with DPBS. The culture plates were then treated with 1 ml 0.2% SDS, 6 M urea, or 0.2 M NaOH for 1 hr at 37°C. The supernatant was removed and plates were treated with 1 ml 0.2 M NaOH to solubilize any remaining material. The total activity in the first supernatant and NaOH supernatant was determined by liquid scintillation counting of 20-μl aliquots in 2 ml Aquasol-2 (New England Nuclear).

Immunoprecipitation and Immunoblots

Metabolically labeled extracellular matrix material was prepared for immunoprecipitation by first detaching adherent cells with 0.02% EDTA in DPBS and then removing cells by rinsing culture dishes with 0.02% EDTA-DPBS, DPBS alone, and finally distilled water. Substrate-attached matrix material was next solubilized by treatment with 0.2% SDS containing 1 mM phenylmethylsulfonyl fluoride (PMSF) in distilled water for 1 hr at 37°C [14]. This procedure removed greater than 90% of both [^{3}H]-leucine and [^{3}H]-glucosamine matrix incorporated counts. Immunoprecipitation of solubilized matrix material was carried out by overnight incubation at 4°C with equal volumes of solubilized matrix and antibody preadsorbed to formalin-fixed Staphylococcus aureus [15]. Secondary rabbit anti-mouse immunoglobulin (Cedar Lane) was used as a bridging antibody to bind the antibody 3E1 to Staphylococcus aureus. Immunoprecipitates were washed with 0.01 M Tris, pH 7.2, 0.14 M NaCl, 1% NP 40, 0.1% SDS. Radiolabeled material was eluted from immunoprecipitates by addition of 200 μl of sample buffer (0.1 M Tris, 0.1% SDS) with or without 0.1% 2-mercaptoethanol and heating to 100°C for 2 min. Treated samples were stored frozen (−20°C) until used.

Proteins were blotted on nitrocellulose paper by the procedure of Towbin et al [16]. Matrix material from 8–15 confluent cultures in 100-mm-diameter plastic petri dishes was prepared as above, pooled, and lyophilized. Matrix material was solubilized in sample buffer (final SDS concentration 2%), heated to 100°C for 2 min, and electrophoresed on 5% SDS-polyacrylamide gels as described below. Gels were placed between two nitrocellulose filters and blotted 24 hr. Transfer of high-molecular-weight peptides from gels required addition of 0.01% 2-mercaptoenthanol to the transfer buffer.

The nitrocellulose blots were soaked in 0.1% bovine serum albumin-DPBS (BSA-DPBS). Immunoblots were prepared by incubating nitrocellulose filters with 100 μg purified antibody, 50 μl ascites fluid, or antisera for 4 hr at 4 °C with constant mixing, followed by washing and incubation with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse immunoglobulin antibodies. Bands were visualized as described by Bell and Engvall [17].

Extracellular Matrix Enzyme Digests

The sensitivity of matrix antigen to various proteases and glycosaminoglycan glycosidases was examined by immunoprecipitation and SDS-PAGE electrophoresis of U-251MG matrix digests. Cultures of U-251MG were plated in 60-mm plastic petri dishes and labeled with 20 μCi/ml [^{3}H]-leucine as described above. Following

incubation for 72 hr cells were removed with EDTA-DPBS and substrate-attached material was washed with DPBS. Dishes were incubated with 1-ml solutions of either 100 μg/ml trypsin in DPBS, 100 μg/ml pepsin in 0.5 M sodium acetate, pH 3.0, 50 μg/ml bacterial collagenase (Sigma type VII) in 0.1 M Tris, pH 7.2, 0.02 M $CaCl_2$, 1 mm PMSF, 10 mM N-ethylmaleimide [18], 20 units hyaluronidase (testis) (Sigma) [19], or 0.1 unit bacterial chondroitinase AC or ABC (Sigma) [20]. Incubations were for 1 hr at 37°C, except the pepsin digest, which was for 24 hr at 4°C. Following incubation, digests were further incubated in 0.2% SDS, 0.02% EDTA, and 1 mM PMSF for 30 min at 37°C to solubilize undigested matrix. Digests were dialyzed against 0.01 M Tris, 7.2, 0.15 M Nacl, 0.2% SDS, concentrated against polyethylene glycol, and immunoprecipitated as described above.

Isolation of Human Plasma and Fibroblast Fibronectin

Fibronectin from freshly drawn human plasma and [^{35}S]-fibronectin from [^{35}S]-methionine-labeled human fibroblast culture supernatant were isolated by gelatin-agarose (Sigma) affinity chromatography according to the procedure of Engvall and Ruoslahti [21].

SDS-Polyacrylamide Gels

Immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis on 5% and 7.5% polyacrylamide slab gels with 4% stacking gel and discontinuous buffer system [22]. Nonreduced samples were also electrophoresed on 4% polyacrylamide gels without a stacking gel. In those samples in which sulfhydryl bonds were reduced, the sample buffer also contained 1% 2-mercaptoethanol. Following electophoresis, gels were stained with Coomassie R250, impregnated with DMSO-PPO for fluorography, and dried. Kodak AR-5 X-ray film was placed over gels and exposed for various lengths of time at −70°C. Molecular weight standards included unlabeled proteins (thyroglobulin, 330,000; ferritin, 220,000; phosphorylase b, 94,000; albumin, 67,000; ovalbumin, 43,000 [Pharmacia]), and [^{14}C]-labeled proteins (myosin, 200,000; phosphorylase b, 94,000; albumin, 67,000; ovalbumin, 43,000; and carboxylase, 30,000) (New England Nuclear).

Peptide Maps

Peptide maps of purified [^{35}S]-methionine-labeled human fibroblast fibronectin and [^{35}S]-methionine-labeled antigen immunoprecipitated with 81C6 from U-251MG extracellular matrix were performed by the Cleveland-Laemmli procedure using staphylococcal V8 protease [23]. Labeled proteins for peptide maps were first electrophoresed on 12% polyacrylamide gels with 20 μg human plasma fibronectin. Gels were stained with Coomassie R250 and the high-molecular-weight bands cut from the preparative gel were utilized for peptide maps. Analytical 7% polyacrylamide gels were employed to demonstrate that the samples contained a single major high-molecular-weight band and only trace levels of small-molecular-weight peptides. Preparative gel samples were next placed over a 30-cm-long 15% polyacrylamide gel with 4% polyacrylamide stacking gel, and overlayed with 40-μl electrophoresis buffer containing 5 μg of staphylococcal V8 protease. Peptide maps were prepared for fluorography as described above.

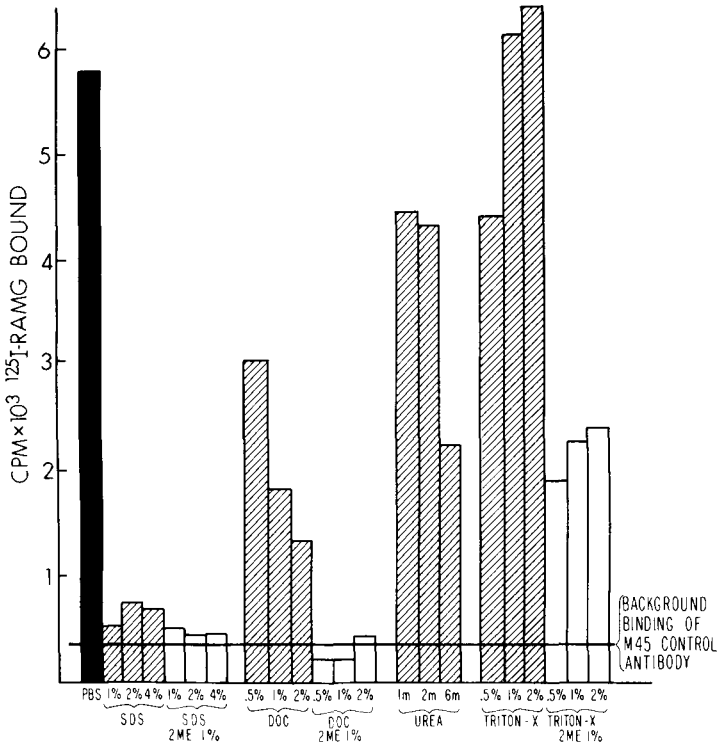

Fig. 1. Binding of 81C6 to U-251MG ECM following solubilization of ECM with SDS, DOC, urea, or Triton X-100. Confluent cultures of U-251MG in 96-well plates were treated with 0.2% EDTA-PBS to detach and remove cells. Wells in triplicate were treated with 100 μl of solubilizing agent and incubated for 1 hr at 37°C. Binding of 81C6 was determined by indirect assay using ^{125}I-rabbit anti-mouse immunoglobulin antibody [5].

RESULTS

Solubilization of Extracellular Matrix Antigen

Previous immunofluorescence and absorption studies have demonstrated that the GMEM antigen defined by the monoclonal antibody 81C6 is expressed primarily as a substrate-attached extracellular matrix antigen [5]. Solubilization of antigen was demonstrated in two ways: first, by direct binding of 81C6 to matrix previously treated with solubilizing agents; and second, by observing the amounts of [^{3}H]-leucine-labeled matrix products liberated by solubilizing agents. The results of solubilization and subsequent 81C6 binding to matrix are shown in Figure 1. Binding of 81C6 to U-251MG matrix was not significantly affected by prior treatment of matrix material with Triton X-100. Pretreatment of matrix with 6M urea reduced 81C6 binding 61%. Approximately 70% reduction in binding of 81C6 was observed following matrix solubilization with 2% deoxycholate. DOC concentrations below 0.5% did not reduce 81C6 binding. Binding by 81C6 was reduced 90–100% in wells treated with 1% SDS or 1% 2-mercaptoethanol in combination with urea or DOC. The lack of 81C6 binding to SDS-treated matrix is not the result of denaturation of

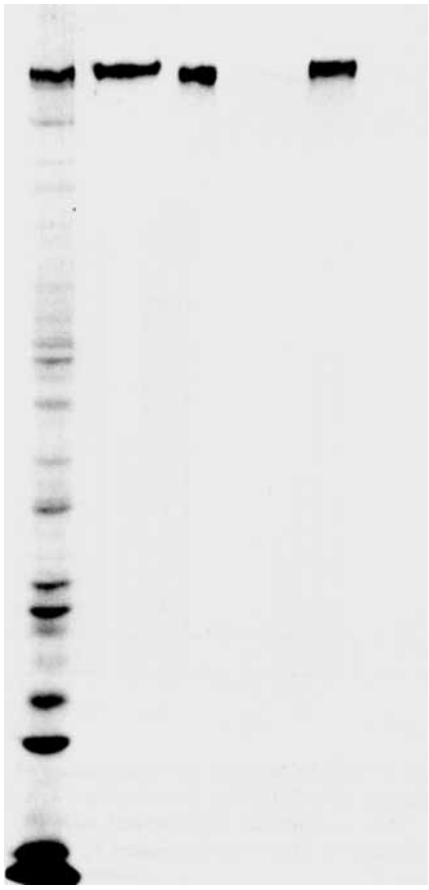

Fig. 2. SDS-polyacrylamide gel electrophoresis of [^{3}H]-leucine- and [^{3}H]-glucosamine-labeled U-251MG ECM immunoprecipitated with 81C6. Samples were electrophoresed under reducing conditions on 7.5% gels. Lanes 1,3–5 are [^{3}H]-leucine-labeled U-251MG alone (lane 1) and immunoprecipitated with 81C6 (lane 3), 45.6 (lane 4), and B2B7 (lane 5). Lanes 2,6–8, are [^{3}H]-glucosamine-labeled U-251MG ECM alone (lane 2) and immunoprecipitated with 81C6 (lane 6), 45.6 (lane 7), and B2B7 (lane 8).

antigen, since 81C6 binds antigen in immunoblots following treatment of antigen with 2% SDS and heat denaturation (Fig. 5).

Treatment of [^{3}H]-leucine-labeled extracellular matrix with 6 M urea did not solubilize more than 5–10% of matrix-bound counts. However, treatment of matrix with 0.2% SDS solubilized greater than 93% of [^{3}H]-leucine counts. As a result of these studies, solubilization of matrix material was routinely performed for 1 hr at 37°C in the presence of 0.2% SDS and 1 mM PMSF as described [14].

Immunoprecipitation of [^{3}H]-Leucine- and [^{3}H]-Glucosamine-Labeled Extracellular Matrix

Fluorographs of [^{3}H]-leucine and [^{3}H]-glucosamine-labeled extracellular matrix and immunoprecipitates are shown in Figure 2. The extracellular matrix is composed

of approximately 22 separate peptides under reducing conditions. The major high-molecular-weight [3H]-leucine-labeled peptide (M_r ~ 230,000) corresponds to the major band from [^{3}H]-glucosamine-labeled extracullar matrix.

Immunoprecipitation of U-251MG extracellular matrix with 81C6 identified a M_r ~ 230,000 molecule in both [^{3}H]-leucine- and [^{3}H]-glucosamine-labeled extracellular matrix, indicating that the antigen is a glycoprotein (Fig. 2). The M_r ~ 230,000 glycoprotein appears to be the major extracellular matrix glycoprotein in U-251MG ECM as judged by the relative amounts of [^{3}H]-leucine and [^{3}H]-glucosamine incorporated.

Under nonreducing conditions the antigen immunoprecipitated by 81C6 migrated only a few millimeters into a 4% polyacrylamide gel, indicating a multimer molecular weight in the range of M_r ~ 1,000,000 (Fig. 3). Intermediate-molecular-weight bands between M_r ~ 230,000 and 1,000,000 were not detected.

Immunoprecipitation of Extracellular Matrix: Enzyme Digests

The extracellular matrices of [^{3}H]-leucine-labeled U-251MG cultures were subjected to enzyme digestion and immunoprecipitated with 81C6 in order to further define the matrix glycoprotein. Labeled matrix was digested with the proteases trypsin, pepsin, and bacterial collagenase, and the glycosidases neuraminidase, hyaluronidase (testis), chondroitinase AC, and chondroitinase ABC (Fig. 4). Under the conditions employed for digestion, only trypsin and pepsin digestion resulted in a change in the mobility of the immunoprecipitated glycoprotein on 7.5% polyacrylamide gels. The extracellular matrix treated with these enzymes gave minimal immunoprecipitation of [^{3}H]-leucine-labeled peptides and these migrated with the dye front.

Immunologic Comparison of GMEM and Fibronectin

The similar molecular weights of GMEM and fibronectin, and the known secretion of fibronectin by U-251MG cells [3], prompted a comparison of immunoprecipitates of [^{35}S]-methionine-labeled matrix proteins with 81C6 and antifibronectin monoclonal antibody 3E1 [13]. Fibronectin was not detected in immunoprecipitates of U-251MG matrix utilizing 3E1, although GMEM was immunoprecipitated with 81C6. Fibronectin labeled with [^{35}S]-methionine and isolated from fibroblast culture supernatant by gelatin-agarose affinity chromatography was immunoprecipitated by 3E1 but not by 81C6 (results not shown).

Immunoblotting

The GMEM glycoprotein was further analyzed on immunoblots of U-251MG and fibroblast ECM electrophoresed on 5% polyacrylamide gels under reducing conditions (Fig. 5). A single band of approximately M_r 230,000 was identified on immunoblots of reduced U-251MG (lane 2) and fibroblast ECM (lane 1) using monoclonal antibody 81C6. Monoclonal antibody 81C6 did not react with plasma fibronectin (lane 3).

Rabbit polyclonal anti-human fibronectin antisera identified a single fibronectin band on immunoblots of reduced fibroblast ECM and human plasma fibronectin, The fibronectin band had a slightly faster mobility than GMEM (Fig. 5). Fibronectin was not detected by antifibronectin antisera on U-251MG immunoblots.

Fig. 3. SDS-polyacrylamide gel electrophoresis of reduced and unreduced U-251MG ECM immunoprecipitates run on 7.5% and 4% gels, respectively. Lane 1–4 are samples reduced with 2-mercaptoethanol and run on 7.5% gels; lane 1) [^{3}H]-leucine-labeled U-251MG ECM; lane 2) 81C6 immunoprecipitate; lane 3) 45.6 immunoprecipitate; lane 4) B2B7 immunoprecipitate. Lanes 5–8 are samples not reduced and run on 4% gel; lane 5) [^{3}H]-leucine-labeled U-251MG ECM; lane 6) 81C6 immunoprecipitate; lane 7) 45.6 immunoprecipitate; lane 8) B2B7 immunoprecipitate.

Peptide Maps

Peptide maps of 81C6-immunoprecipitated GMEM from U-251MG ECM and fibroblast fibronectin were compared. Fluorographs of staphylococcal V8 Cleveland-Laemmli peptide digests showed major differences in peptide fragments between GMEM and fibronectin (Fig. 6). There was no homology in major digest fragments,

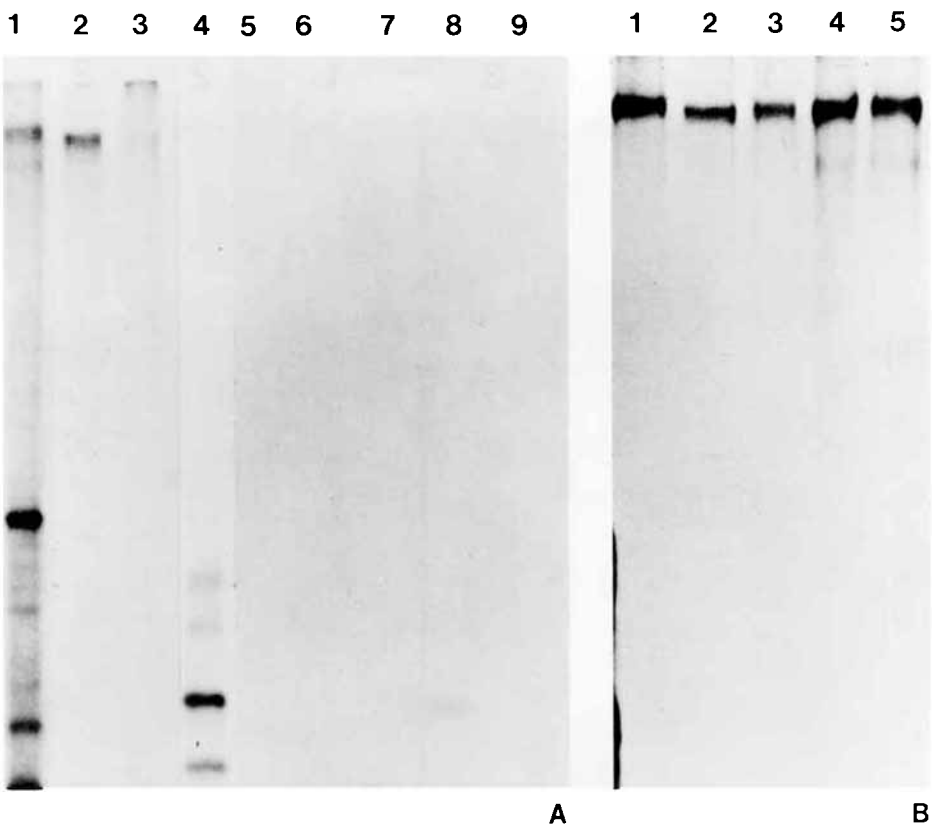

Fig. 4. SDS-polyacrylamide gel electrophoresis on 7.5% gels of [^{3}H]-leucine-labeled U-251MG ECM enzyme digests immunoprecipitated with 81C6. A) [^{3}H]-leucine-labeled U-251MG ECM treated with collagenase (lanes 1–3), trypsin (lanes 4–6), or pepsin (lanes 7–9) and immunoprecipitated with 81C6 (lanes 2,5,8) or 45.6 (lanes 3,6,9). B) Samples are [^{3}H]-leucine-labeled U-251MG ECM treated with DPBS (lane 1), neuraminidase (lane 2), hyaluronidase (testis) (lane 3), bacterial chondroitinase AC (lane 4), or chondroitinase ABC (lane 5) and immunoprecipitated with 81C6.

although several minor peptide fragments had the same molecular weights in both GMEM and fibronectin peptide maps (Fig. 6).

DISCUSSION

In this study we have described the immunochemical isolation and partial biochemical characterization of a novel M_r ~ 230,000 extracellular matrix glycoprotein from human glioma and fibroblast cell cultures. The glycoprotein, designated GMEM, is defined by the murine monoclonal antibody 81C6 and is expressed in extracellular matrix of human glioma and fibroblast cell lines, as well as in the perivascular matrix of human glioma endothelial proliferations [5].

SDS-PAGE analysis of immunoprecipitates of [^{3}H]-leucine-labeled U-251MG ECM demonstrates that GMEM immunoprecipitated by 81C6 exists within the ECM as a high-molecular-weight (M_r ~ 1,000,000) macromolecule composed of M_r ~ 230,000 disulfide-bonded subunits.GMEM appears to be a glycoprotein because it

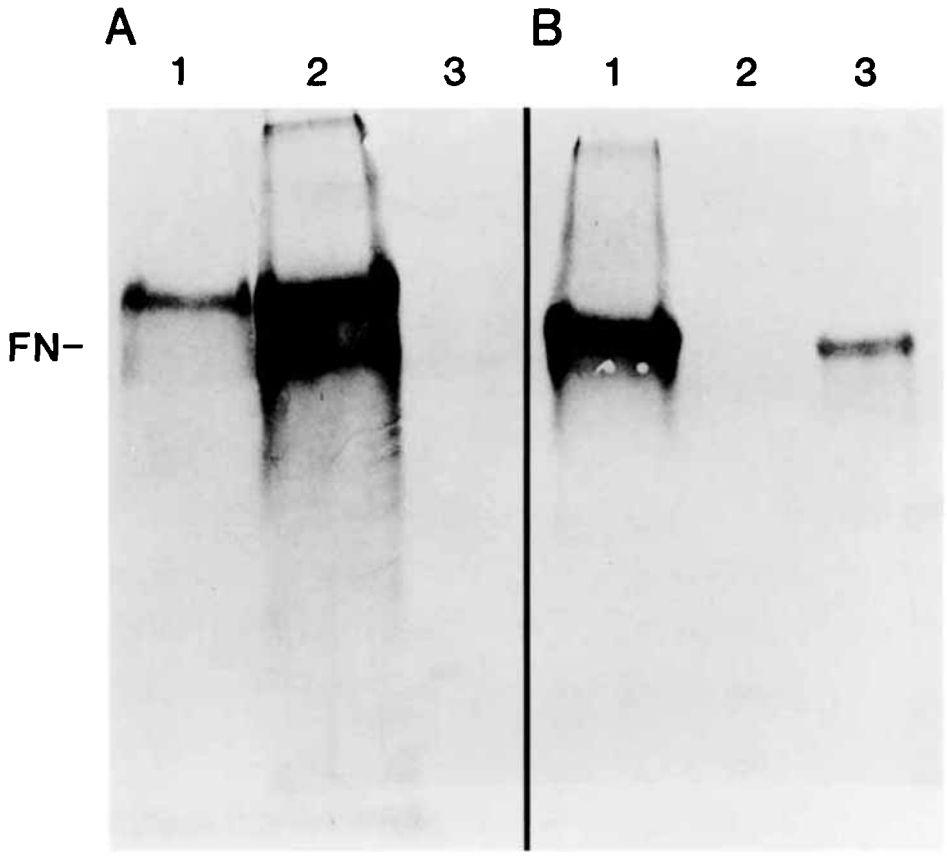

Fig. 5. Immunoblots on nitrocellulose paper of U-251MG ECM, fibroblast ECM, and plasma fibronectin reacted with 81C6 (A), or rabbit anti-human fibronectin (RAHFN) antisera (B). A) Lane 1, fibroblast ECM; lane 2, U-251MG ECM; lane 3, human plasma fibronectin. B) Lane 1, fibroblast ECM; lane 2, U-251MG ECM; lane 3, human plasma fibronectin. Position of plasma fibronectin (FN) indicated.

also incorporates [^{3}H]-glucosamine. Interestingly, GMEM was the only major ECM protein labeled with glucosamine in the total ECM of the U-251MG cells, whereas leucine-labeling revealed numerous other peptides. This suggests that GMEM may be the major extracellular protein in the matrix preparation.

We have previously reported that GMEM was serologically and immunohistologically distinct from fibronectin, laminin, collagen types I–V, a melanoma-associated proteoglycan, and hyaluronic acid, chondroitin sulfate, and heparin [5]. In this study we have shown that GMEM is susceptible to enzyme digestion by trypsin and pepsin, but not bacterial collagenase, hyaluronidase, or chondroitinases AC and ABC (Fig. 4A,B), suggesting that GMEM is neither a collagen protein nor a glycosaminoglycan.

Although results presented here and elsewhere [5] demonstrate that 81C6 antibody does not bind soluble fibroblast or plasma fibronectin, the possibility remained that the glycoprotein detected by 81C6 could be a fibronectin-related molecule given its prominence in the glioma cell line ECM and a molecular weight similar to that of

Fig. 6. One-dimensional Cleveland-Laemmli peptide map of [^{35}S]-methionine GMEM from U-251MG ECM and [^{35}S]-methionine fibroblast fibronectin digests with staphylococcal V8 protease. Lane 1) GMEM 81C6 immunoprecipitate; lane 2) fibroblast fibronectin. Lanes 3 and 4) untreated GMEM and fibroblast fibronectin.

fibronectin. Fibronectin has been shown to exist in at least a cellular fibronectin and a plasma form, and there may be differences in the molecular weights of cellular fibronectin from normal and transformed cells [6,24–26]. In addition, while there appears to be only one gene for fibronectin, the coding regions of fibronectin mRNA are heterogeneous, allowing for the potential synthesis of many forms of fibronectin that differ from one another with regard to the presence of short internal stretches of amino acids [27,28].

We have considered the possibility that the 81C6 antibody could be detecting an antigenic determinant that is present in fibronectins from some sources but not from others. Our evidence indicates, however, that GMEM differs from fibronectin more than could be expected on the basis of short difference regions in a fundamentally identical polypeptide. The peptide maps of GMEM and fibroblast fibronectin were quite different. Moreover, no reaction was obtained with polyclonal antifibronectin antibodies in immunoblotting of samples where GMEM could be readily detected with the 81C6 antibody, indicating that GMEM lacks the antigenic determinants detected by such antisera. However, more distant relatedness between GMEM and fibronectin seems possible.

The presence of GMEM in the ECM of both normal and transformed cell lines indicates that it is an important component of the ECM, perhaps with a role in cell adhesion as is the case with fibronectin [6,7]. The high-molecular-weight disulfide-bonded nature of the GMEM macromolecule in the ECM and its limited solubility characteristics may be an indication that the antigen is cross-linked in a lattice. The punctate pattern of the distribution of GMEM in the substrate of glioma and fibroblast cultures as seen by immunofluorescence indicates a localization at sites of substrate-cell adhesion [5]. The in vivo distribution of GMEM in the perivascular matrix of glioma endothelial cells, but not normal brain endothelium [5], is particularly interesting. It is quite different from the localization of fibronectin and may be an indication that GMEM is associated with tumor cell–endothelial interactions involved in the vascular proliferation that is prominent in human anaplastic gliomas.

In summary, in this study we have partially characterized the GMEM antigen in terms of molecular and immunochemical properties and compared these to human fibronectin, which has a similar molecular weight and distribution in extracellular matrices. Our findings demonstrate that GMEM is a novel glycoprotein that is structurally different from fibronectin in both monomer and multimer molecular weight, in immunoreactivity, and in peptide maps. Their distribution in extracellular matrix of glioma cells and fibroblasts suggests the possibility that similarities in the functional properties may exist.

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