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Monoclonal antibodies that recognize the trisaccharide epitope Galα1–3Galβ1–4GlcNAc present on Ehrlich tumor cell membrane glycoproteins

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Abstract. Monoclonal antibodies were prepared against the trisaccharide Galα1–3Galβ1–4GlcNAc, a sequence which occurs on the surface of Ehrlich ascites tumor cells as well as in thyroglobulin, laminin and a variety of other proteins. This was accomplished by immunizing BALB/c mice with the fraction of Ehrlich cell membrane glycoproteins obtained by affinity chromatography on a *Griffonia simplicifolia* I (GS I) column which selectively binds α-D-galactosyl-terminated structures. Detection of Galα1–3Galβ1–4GlcNAc-specific antibodies was accomplished by employing glycoproteins containing the trisaccharide sequence; fusion with spleen cells from an immunized mouse was accomplished in the presence of polyethylene glycol (PEG1500). An enzyme-linked immunosorbent assay (ELISA) system was used to identify two clones (2.10G and 6.8E), which recognized the desired trisaccharide conjugate. These clones also recognized a thyroglobulin fraction isolated by GS I affinity chromatography and murine laminin, both of which possess the Galα1–3Galβ1–4GlcNAc sequence. Inhibition of antibody-trisaccharide reactivity, examined employing an ELISA assay, revealed that two trisaccharides, Galα1–3Galβ1–4GlcNAc/Glc, were the best inhibitory haptens; Galβ1–4GlcNAc (LacNAc), Galα1–3Gal and Galβ1–4Glc (lactose) were poor inhibitors. Indirect immunofluorescence staining of unfixed Ehrlich cells using the monoclonal antibody at 4° C revealed fluorescence over the entire cell surface. Indirect immunogold labeling of semithin and ultrathin sections of aldehyde fixed and Lowicryl K4M-embedded Ehrlich cells resulted in specific labeling of the cell surface and internal structure. Immunoblot analysis revealed that removal of the α-galactosyl residues of laminin by α-galactosidase abolished reactivity with the monoclonal antibodies. The availability of this antibody, which belongs to the IgM family of immunoglobulins, now makes possible the detection of this sugar sequence on cells and tissue sections, as well as on glycoproteins in solution.

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Introduction

The galactose α1–3 galactose β1–4 *N*-acetylglucosamine (Galα1–3Galβ1–4GlcNAc) oligosaccharide structure has been reported to be present on cell-membrane proteins of bovine thyroid cells (Edge and Spiro 1985), murine Ehrlich ascites tumor cells (Eckhardt and Goldstein 1983b), murine lymphoma cells (Cummings and Kornfeld 1984), murine NIH 3T3 fibroblasts (Santer et al. 1989) and bovine thyroglobulin (Cummings and Kornfeld 1982; Dorland et al. 1984; Spiro and Bhoyroo 1984). This structure does not normally occur on human cells and tissues nor on human thyroglobulin (Peters and Goldstein 1979; Galili et al. 1988). Galili and coworkers (1988) have reported that humans produce large amounts of a natural IgG antibody that interacts with α-galactosyl-containing oligosaccharide structures on mammalian glycoconjugates, and that this antibody (anti-Gal) interacts specifically with synthetic Galα1–3Galβ1–4GlcNAc (Galili et al. 1984, 1985a, b; Davin et al. 1987; Avila et al. 1989). It was argued that abnormal expression of Galα1–3Galβ1–4GlcNAc residues on human glycoproteins may result in anti-Gal-mediated autoimmune processes (Thall and Galili 1990). Mice were immunized with rabbit erythrocytes and a monoclonal antibody (Gal–13) was raised which reacted with the neoglycoprotein Galα1–3Galβ1–4GlcNAc-BSA and Galα1–3Galβ1–4GlcNAcβ1–3Gaβ1–4Glcβ1–1 Ceramide (Galili et al. 1987).

Gil and coworkers (1990) prepared monoclonal IgM antibody (A10) with specificity to Ehrlich ascites tumor cell surface carbohydrates but did not characterize its specificity. Further occurrence of the Galα1–3Galβ1–4GlcNAc carbohydrate structure was reported by Randle (1982, 1983). The monoclonal antibody 2C5, which had been raised to P13 embryonic carcinoma, was shown to react with embryonic and extraembryonic endoderm, thymus and the female reproductive tract. Varani and coworkers (Varani et al. 1983a, b) have shown that the expression of α-D-galactosyl residues on the cell surface is strongly correlated with the metastatic potential of

murine fibrosarcoma cells, using the *Griffonia simplicifolia* I–B4 isolectin. When these cells were treated with anti-blood type B antisera, the selected cells, lacking terminal α-linked galactosyl groups were also poorly metastatic. Addition of the glycoconjugate, laminin, restored the metastatic property. In this study, we have immunized BALB/c mice with plasma membrane glycoprotein isolated from Ehrlich ascites tumor cell and prepared and characterized a monoclonal antibody, which recognized the Galα1–3Galβ1–4GlcNAc carbohydrate trisaccharide epitope. The monoclonal antibody was employed to detect this structure on Ehrlich cells and on several glycoproteins.

Materials and methods

Materials

The following materials were obtained commercially: Freund's complete adjuvant and Freund's incomplete adjuvant (Difco Laboratories, Detroit, Mich., USA); coffee bean α-galactosidase (Boehringer Mannheim, Indianapolis, Ind., USA) glutaraldehyde, vacuum distilled (Fluka, Buchs, Switzerland); paraformaldehyde and tetrachloroauric acid (Merck, Darmstadt, Germany). Affinity-purified rabbit anti-mouse IgG (L&H) (Jackson ImmunoResearch Laboratories, West Chester, Pa., USA) was complexed with 8 nm gold particles (Lucocq and Baschong 1986). Galβ1–4GlcNAc (LacNAc) and Galβ1–4GlcNAc-BSA conjugate were available from previous studies. Galα1–3Galβ1–4GlcNAc-β-*O*-$(CH_2)_9COOH$ and Galα1–3Galβ1–4GlcNAc-Synsorb were the gift of Murray Ratcliffe of Chem Biomed (Edmonton, Canada). The Galα1–3Galβ1–4GlcNAc-bovine serum albumin (BSA) conjugate was prepared by Nike Plessaas in our laboratory; a second sample was a gift of Murray Ratcliffe.

Isolation of Ehrlich cell plasma membrane glycoprotein

Ehrlich ascites tumor cells were maintained by weekly intraperitoneal injection of outbred CD–1 mice (Charles River, Portage, Mich., USA) with 0.6 ml of ascites fluid. For experimentation, cells were harvested 8 or 9 days after transfer and washed. Plasma membrane glycoproteins were prepared as previously described (Eckhardt and Goldstein 1983a).

Preparation of Galα1–3Galβ1–4GlcNAc-enriched bovine thyroglobulin

Bovine thyroglobulin, enriched for Galα1–3Galβ1–4GlcNAc residues, was isolated from a commercial preparation of bovine thyroglobulin (Sigma Chemical Co., St Louis, Mo., USA) on a GS I-Sepharose column (1.5 × 21 cm). Bovine thyroglobulin in solution was passed through a GS–I column. After extensive washing with phosphate-buffered saline (PBS), pH 7.5, the bound material was eluted with 10 m*M* methyl α-galactoside (Shibata et al. 1982; Spiro and Bhoyroo 1984; Thall and Galili 1990).

Immunization of animals

Female BALB/c mice (5 to 8 weeks old) were immunized by intraperitoneal injection with isolated α-D-galactopyranosyl (α-D-Gal*p*)-containing Ehrlich cell plasma membrane glycoproteins.

Cell fusion

Cell fusion was carried out with spleen cells from an immunized mouse (3 days after the final antigenic boost), following the method of Galfre et al. (1977) and McKearm (1980). Briefly, murine spleen cells (1×10^2) were fused with murine myeloma cells (2×10^7) in polyethlene glycol (PEG1500, Boehringer Mannheim, Indianapolis, Ind., USA). The fused cells were plated into 96-well plates and cultured in HAT-Iscove's Modified Dulbecco's Medium (Gibco BRL, Grand Island, N.Y., USA) at 37° C in a 5% CO_2 incubator.

Screening

Screening of hybrid cells was conducted initially by enzyme-linked immunosorbent assay (ELISA) with a Galα1–3Galβ1–4GlcNAc-BSA conjugate and Galα1–3Galβ1–4GlcNAc-enriched bovine thyroglobulin adsorbed onto 96-well plates (Nunc, Naperville, Ill., USA). Antigen glycoproteins (10 mg/ml in 0.5 *M* carbonate buffer, pH 9.5) were bound to microtiter plates by overnight incubation at 4° C. The wells were washed with PBS and subsequently incubated with PBS–1% BSA for 1 h at room temperature in order to block nonspecific binding. After washing with PBS–0.05% BSA, the hybridoma cell culture supernatant solution was added and incubated for 1 h at room temperature. Plates were washed with PBS–0.05% Tween and incubated with a dilution (1:500) of an anti-mouse Ig-antibody Fab fragment from sheep conjugated with alkaline phosphatase (Boehringer Mannheim, Indianapolis, Ind., USA) Following washing, a *p*-nitrophenyl phosphate substrate solution (1 mg/ml, Sigma 104 phosphatase substrate) was added and the color allowed to develop for 30 min. The enzymatic reaction was quenched with 3 *N* NaOH and the absorbance read at 405 nm. Colonies showing reactivity towards the above carbohydrates were cloned three times by limiting dilution using macrophages as feeder layers, identifying wells where a single clone could be assured microscopically. Stable cultures of antibody-producing hybridomas were expanded and monoclonal antibodies produced in pristane-treated BALB/c mice.

Purification of monoclonal antibodies

Cell-free ascites fluid was applied to a column of Galα1–3Galβ1–4GlcNAc-Synsorb (0.6 × 4.0 cm). Before applying the sample, the column was eluted by PBS–1% BSA followed by PBS. Elution of antibodies was achieved by passing 0.1 *M* glycine-hydrochloric acid buffer, pH 2.5, as described previously (Lapresle and Webb 1965; Lapresle and Goldstein 1969; Hudson and Hay 1976), through the column at 4° C. Eluted fractions were collected and neutralized immediately with solid *Tris*.

Chromatography of monoclonal antibodies on laminin-Sepharose

The hybridoma culture supernatant was applied to a column of laminin-Sepharose (0.6 × 8.5 cm) and incubated for 1 h. The column was eluted with PBS and bound fractions were eluted with 0.5 *M* NaCl containing 10 m*M Tris* buffer, pH 7.5.

Chromatography of monoclonal antibodies on Galanthus nivalis *agglutinin (GNA)-Sepharose*

The hybridoma culture supernatant fluid was applied to a column of GNA-Sepharose (0.6 × 8.5 cm) and incubated for 1 h. The column was eluted with PBS, followed by 0.5 *M* methyl α-D-mannoside in PBS at a flow rate of 1 ml/h and fractions were monitored by absorbance at 280 nm. All experiments were conducted at 4° C. (Shibuya et al. 1988).

Characterization of monoclonal antibodies

The isotype of monoclonal antibodies was determined by using a Mouse-Typer Isotyping Kit (Bio-Rad Laboratories, Richmond, Calif., USA). To determine the specificity of monoclonal antibodies, we used an inhibition test in microtiter wells. Antigen glycoproteins (10 mg/ml in 0.5 *M* carbonate buffer, pH 9.5) were bound to microtiter plates by incubating overnight at 4° C. The GS-I-bound thyroglobulin was used as antigen with which to coat the plates, inasmuch as the Galα1–3Galβ1–4GlcNAc structure occurs on bovine thyroglobulin (Cummings and Kornfeld 1982; Dorland et al. 1984; Spiro and Bhoyroo 1984). The wells were washed with PBS and subsequently incubated with PBS–1% BSA for 1 h at room temperature in order to block nonspecific binding. After washing with PBS–0.05% Tween, the monoclonal antibody solution (5 μg/ml in PBS) was incubated with inhibitors (monosaccharide, disaccharides, trisaccharides, and glycoproteins). Plates were washed with PBS–0.05% Tween and incubated with a dilution (1:500) of an anti-mouse Ig-antibody Fab fragment from sheep conjugated with alkaline phosphatase (Boehringer Mannheim). Following washing, a *p*-nitrophenyl phosphate substrate solution (1 mg/ml, Sigma 104 phosphatase substrate) was added and the color allowed to develop for 30 min. The enzymatic reaction was quenched with 3 *N* NaOH and the absorbance read at 405 nm.

Indirect immunofluorescence of Ehrlich cells

To 50 μl of a suspension of washed Ehrlich cells in PBS–5% fetal calf serum (FCS; 5×10^7 cells/ml) was added 50 μl of the monoclonal antibody solution (5 μg/ml in PBS), and the suspension was stirred gently for 30 min at 4° C. In other experiments, non-specific mouse IgM was added to the cell suspension as a negative control. The cells were washed twice with 200 μl of PBS–5% FCS and 50 μl of fluorescein isothiocyanate (FITC)-coupled anti mouse Ig(G + M) antibodies (Boehringer Mannheim) was added and the mixture stirred gently for 30 min at 4° C. The cells were washed four times with 200 μl of PBS–5% FCS, resuspended in 100 μl PBS–5% FCS, and examined.

Indirect immunogold labeling of Ehrlich cells

A suspension of washed Ehrlich cells in Hanks balanced salt solution was fixed with 2% formaldehyde (freshly prepared from paraformaldehyde) and 0.1% glutaraldehyde at 37° C for 30 min. The suspension was subsequently washed with PBS twice for 5 min each, treated with 50 m*M* NH_4Cl in PBS for 30 min to amidate free aldehyde groups and stored in PBS at 4° C until embedding. For embedding, cells were pelleted and enclosed in 2% agar. Small pieces of agar containing the enclosed cells were dehydrated in graded ethanol at progressively lowered temperature (down to −40° C), infiltrated with Lowicryl K4M at −40° C and polymerized at −40° C by indirect UV-light irradiation as described (Roth 1989; Roth et al. 1981). Semithin (1 μm thick) and ultrathin sections were prepared and incubated with the monoclonal antibody (5–25 μg/ml) diluted with PBS containing 1% BSA and 0.05% Tween 20 for 2 h at room temperature. This was followed by two washes with PBS (5 min each) and incubation with gold-labeled rabbit anti-mouse IgG (diluted with PBS containing 1% BSA, 0.01% Triton X–100 and 0.01% Tween 20 to an $OD_{525\ nm}$ of 0.05–0.1) for 1 h at room temperature. Ultrathin sections were contrasted with uranyl acetate (5 min) and lead acetate (45 s) and examined with a Zeiss electron microscope 910 at 80 kV. Semithin sections were fixed with 1% glutaraldehyde for 20 min, subsequently thoroughly washed with distilled water, air dried and subjected to silver intensification (Roth 1989). Specificity controls included treatment of sections with coffee bean α-galactosidase, or preabsorption of the monoclonal antibody with Galα1–3Galβ1–4GlcNAc trisaccharide.

Purification of laminin

The Engelbreth-Holm-Swarm (EHS) murine sarcoma was grown as a solid tumor in C57B16 female mice (Orkin et al. 1977). Laminin was isolated from EHS tumor on a GS I-Sepharose affinity column as previously described by Shibata and co-workers (1982).

Dot immunoblot analysis

An Immobilon-P transfer membrane (Millipore Co., Bedford, Mass., USA) wetted with 100% methanol was placed in deionized water and 10 m*M Tris* buffer. Laminin (200 μg in 0.2 ml) was incubated for 14 h with 0.4 Units of coffee bean α-galactosidase in 10 m*M Tris* buffer, pH 6.0. The solutions of laminin (5 μl) and α-galactosidase-treated laminin were blotted onto a membrane and incubated for 1 h at room temperature. Following incubation, blots were washed for 5 min in 10 m*M Tris*-buffered saline (TBS), pH 7.4, the wash cycle being repeated three times. The blots were then incubated with 1% BSA in *Tris*-buffered saline (TBS) for 1 h at room temperature. After washing with 0.05% Tween in TBS, monoclonal antibody solution (5 μg/ml) was added and incubated for 1 h at room temperature. Blots were washed with 0.05% Tween in TBS and incubated with a (1:500) dilution of an anti-mouse Ig-antibody Fab fragment from sheep conjugated with alkaline phosphatase (Boehringer Mannheim). Following washing, a substrate solution containing 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine and nitroblue tetrazolium (Gibco BRL) was added.

Results

Purification of Ehrlich tumor cell plasma membrane

Plasma membranes solubilized in dimethyldecylphosphine oxide (DC10PO) were subjected to affinity chromatography on a GS I-Sepharose column. α-D-Galactosyl-containing glycoproteins were eluted as a single peak from the column with 0.1 *M* methyl α-D-Gal*p* (Fig. 1).

Table 1. Inhibition by oligosaccharides and glycoconjugates of interaction between monoclonal antibody and bovine thyroglobulin

Inhibitor	Concentration (μM) required for 50% inhibition
Gal	$>5 \times 10^3$
Galβ1-4Glc (Lactose)	$>5 \times 10^3$
Galα1-3Gal	>50
Galα1-6Glc (Melibiose)	$>5 \times 10^3$
Galβ1-4GlcNAc (LacNAc)	$>5 \times 10^3$
Galα1-3Galβ1-4Glc	5
Galα1-3Galβ1-4GlcNAc	5
Galα1-3Galβ1-4GlcNAc-BSA	0.04 μg/ml
Galβ1-4GlcNAc-BSA	>1.5 μg/ml
BSA	>1.5 μg/ml
Galα1-3Galβ1-4GlcNAc-R[a]	0.003 μg/ml
Galβ1-4GlcNAc-R[b]	>1.5 μg/ml

[a] GS I, *Griffonia simplicifolia* I, GS I-bound fraction of bovine thyroglobulin

[b] α-Galactosidase-treated, GS I-bound bovine thyroglobulin

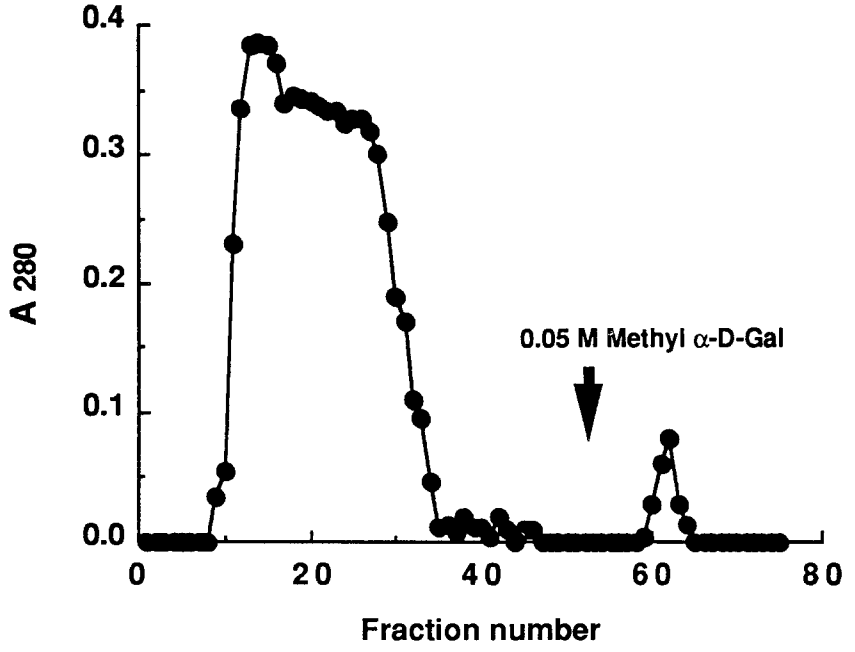

Fig. 1. Isolation of Ehrlich cell plasma membrane glycoproteins. A preparation of plasma membrane glycoproteins from Ehrlich ascites tumor cells were applied to a GS I-Sepharose column (1.5 × 21 cm) and eluted with 50 m*M* methyl-α-D-galactopyranoside. Fractions of 5.5 ml were collected

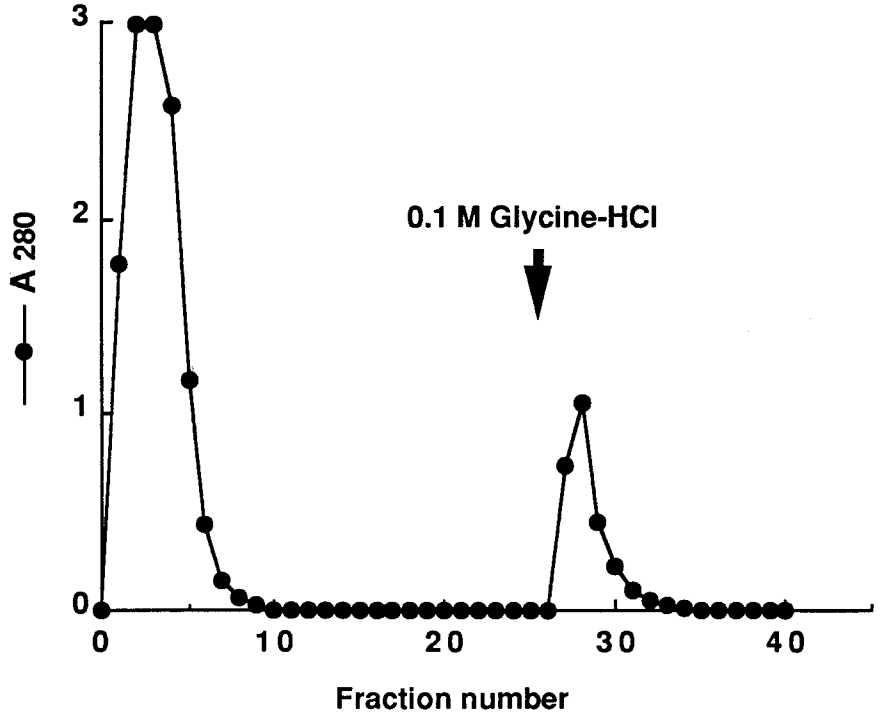

Fig. 2. Purification of monoclonal antibody from hybridoma clone 6.8E. Cell-free ascites fluid was applied to a column of Galα1–3Galβ1–4GlcNAc-Synsorb (0.6 × 4.0 cm). Antibodies were eluted by addition of 0.1 *M* glycine-HCl buffer, pH 2.5

Characterization of monoclonal antibodies

The hybridoma clone 6.8E, isotype IgM, was selected for further study (Fig. 2). The capacity of the monoclonal antibody to serve as a reagent for detection of Galα1–3Galβ1–4GlcNAc residues was determined by ELISA. The assay was based on inhibition of monoclonal antibody binding to Galα1–3Galβ1–4GlcNAc-enriched bovine thyroglobulin by incubation of the antibody with various oligosaccharides and carbohydrate conjugates (Table 1). Low concentrations of Galα1–3Galβ1–4GlcNAc and Galα1–3Galβ1–4Glc were excellent inhibitors of the interaction. Inhibition did not occur when α-Gal-terminated disaccharides and β-Gal-terminated carbohydrates were assayed, i.e. Galα1–3Gal, Galβ1–4Glc and Galβ1–4GlcNAc were extremely poor inhibitors. The binding of monoclonal antibody to Galα1–3Galβ1–4GlcNAc-enriched bovine thyroglobulin was inhibited by Galα1–3Galβ1–4GlcNAc-containing glycoproteins; this inhibition activity was abolished by treatment with

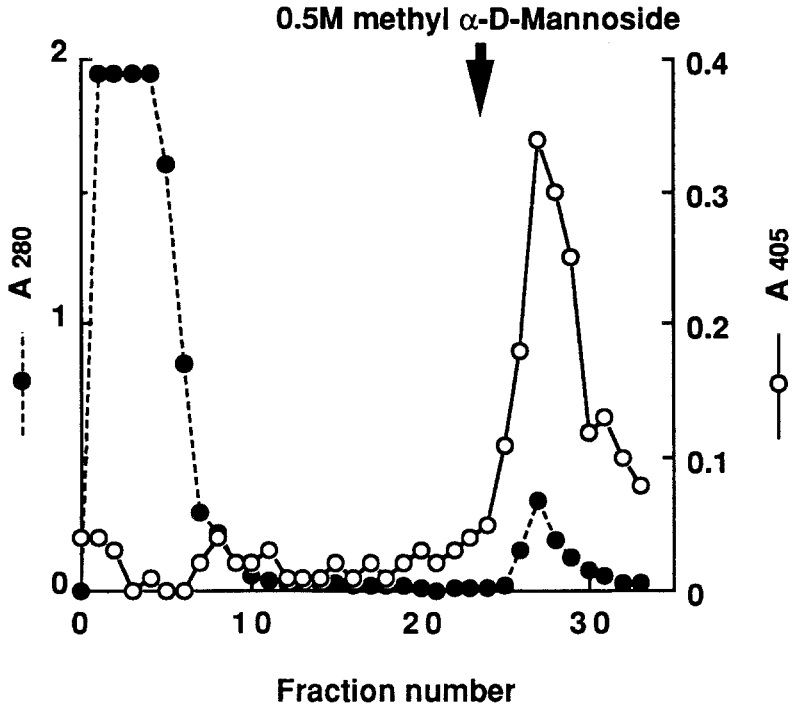

Fig. 3. Purification of monoclonal antibody using *Galanthus nivalis* agglutinin (GNA)-Sepharose. Supernatant solutions of hybridoma cultures were applied to a column of GNA-Sepharose (0.6 × 8.7 cm). The column was washed with 20 column volumes of phosphate-buffered saline (PBS) and bound antibody was eluted with PBS containing 0.5 *M* methyl α-D-mannoside. Monoclonal antibody as detected by using anti-mouse Ig(G + M) conjugated to alkaline phosphatase. Fractions of 2.2 ml were collected

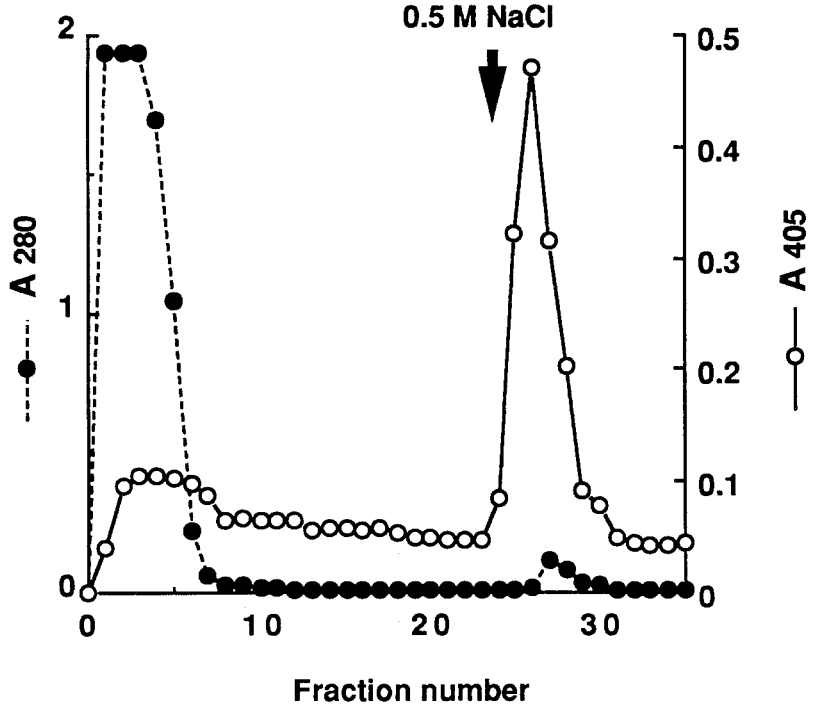

Fig. 4. Binding of monoclonal antibody (MoAb) to laminin-Sepharose. A supernatant solution of hybridoma cultures was applied to a column of laminin-Sepharose (0.6 × 8.5 cm) in 10 m*M Tris* buffer, pH 7.5. The column was washed with this buffer and the bound fraction eluted with 0.5 *M* NaCl containing 10 m*M Tris* buffer, pH 7.5. Monoclonal antibody was detected by using anti-mouse Ig(G + M) conjugated to alkaline phosphatase

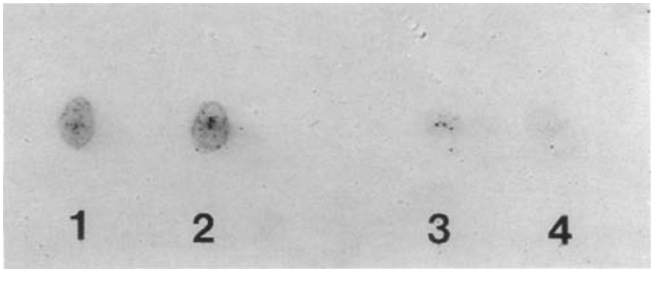

Fig. 5. Dot immunoblot analysis MoAb 6.8E binding to native and α-galactosidase treated laminin: 5 µl of the laminin solution (1 mg/ml) and α-galactosidase-treated laminin solution (1 mg/ml) were blotted onto an Immobilon-P transfer membrane and reacted with MoAb 6.8E. Samples 1 and 2 are native laminin; samples 3 and 4 are laminin treated with coffee bean α-galactosidase

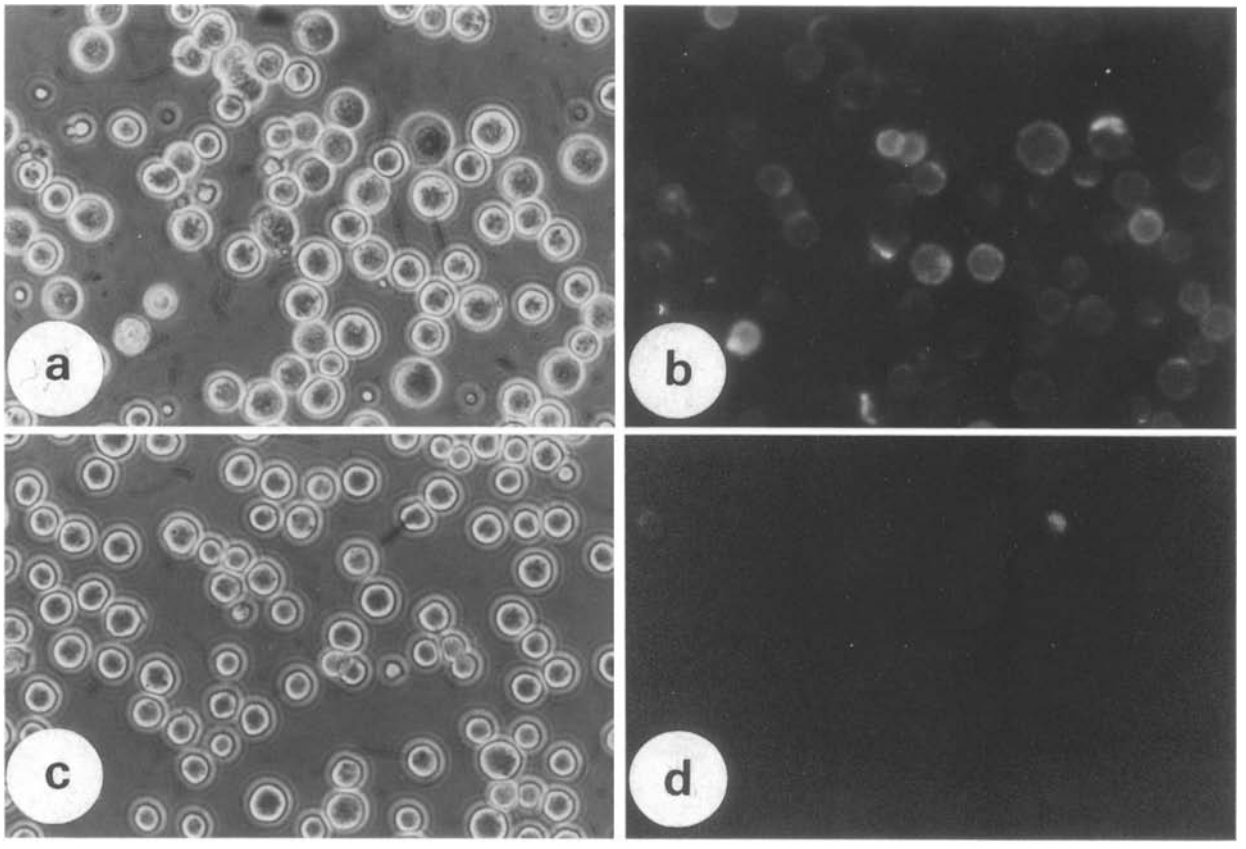

Fig. 6a, c. Indirect immunofluorescence labeling of Ehrlich ascites tumor cells with MoAb 6.8E. **b, d** Non-specific mouse IgM was added to the cell suspension as a control **a–d** × 300

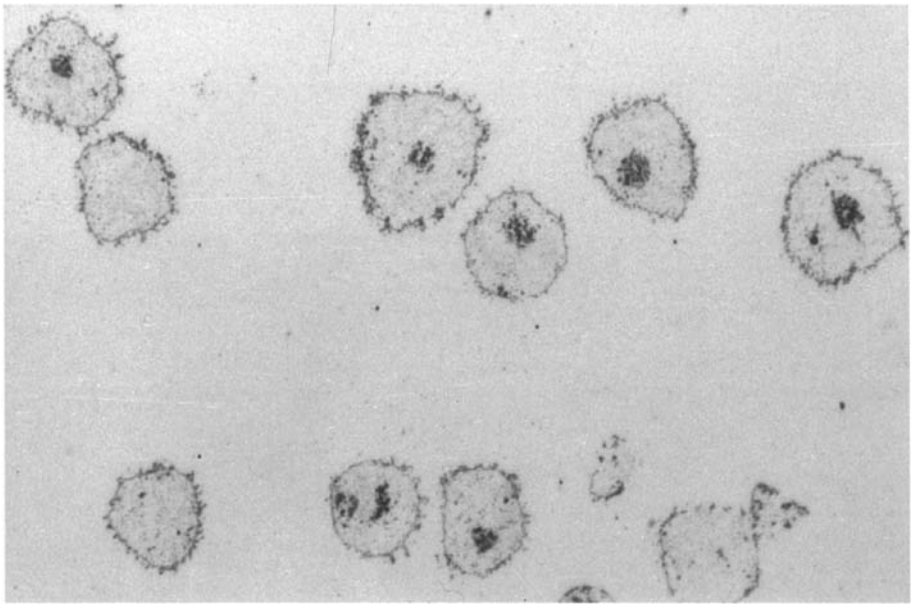

Fig. 7. Semithin section of Lowicryl K4M embedded Ehrlich cells treated by indirect immunogold labeling followed by silver intensification. Positive staining is seen at the cell surface and in perinuclear locations. × 950

α-galactosidase. These results indicate that monoclonal antibody 6.8E interacts in a very specific manner with Galα1–3Galβ1–4GlcNAc epitopes. When the hybridoma culture supernatant fluid was applied to a column of GNA-Sepharose, the bound fraction were eluted as a single peak from the column (Fig. 3)

Reactivity of monoclonal antibodies against laminin

The hybridoma culture supernatant fluid was applied to a column of laminin-Sepharose and the bound fraction eluted as a single peak from the column (Fig. 4). Following the report that terminal, non-reducing α-D-galactosyl

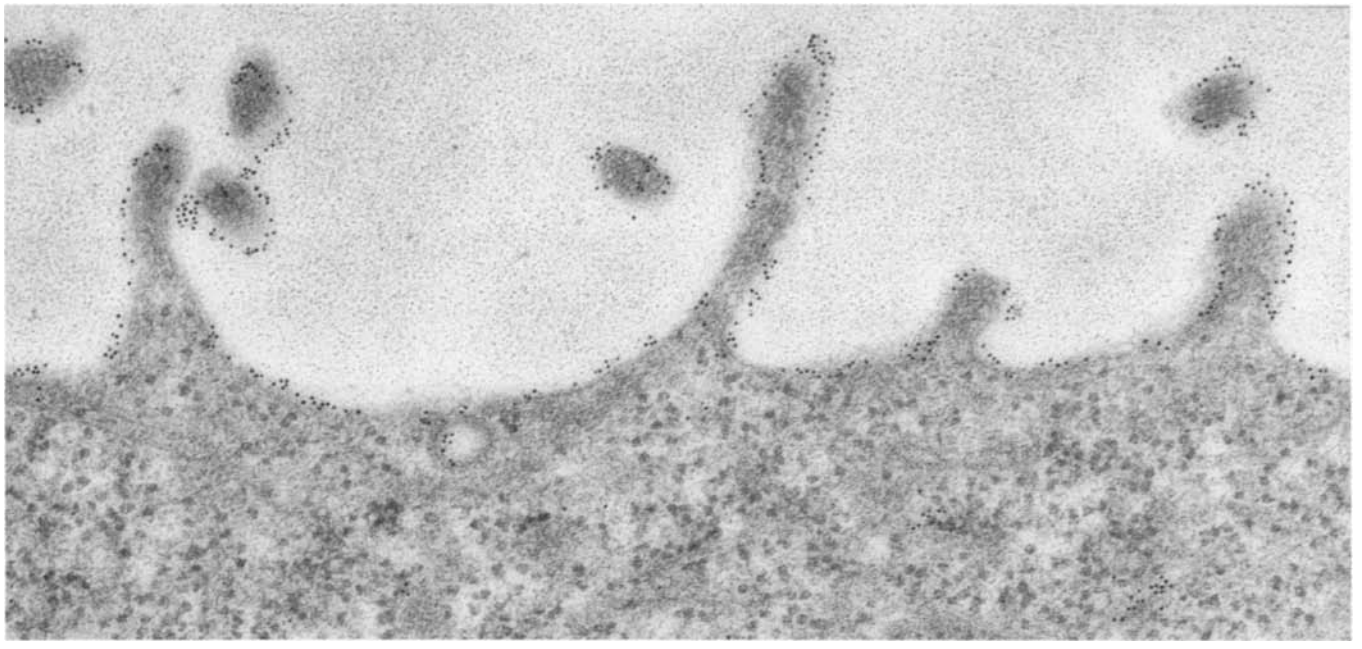

Fig. 8. Ultrathin section of Lowicryl K4M embedded Ehrlich cells treated by indirect immunogold labeling. Numerous gold particles are present at the outer surface of the plasma membrane. ×76000

units comprise 15.5–19.5% of the total galactosyl content of laminin (Knibbs et al. 1989), we assayed the reactivity of monoclonal antibodies with laminin using a dot immunoblot procedure. Antigens employed were laminin and α-galactosidase-treated laminin. The results in Fig. 5 demonstrated that our monoclonal antibody did not react with α-galactosidase-treated laminin. Following dialysis against PBS, we applied the α-galactosidase-treated laminin to a GS I-Sepharose column. The agalactolaminin did not react with GS I, indicating the absence of α-D-galactosyl units.

Detection of Galα1–3Galβ1–4GlcNAc residue of Ehrlich tumor cell surface

Fluorescence microscopy of Ehrlich tumor cells incubated with monoclonal antibody revealed intense fluorescence over the entire cell surface (Fig. 6). Indirect immunogold labeling of semithin and ultrathin Lowicryl K4M embedded cells also resulted in labeling along the cell surface and internal structures (Figs. 7 and 8). The intracellular labeling pattern will be reported elsewhere. Under the different conditions of the specificity controls no labeling was observed (data not shown).

Discussion

Eckhardt and Goldstein (1979, 1983a, b) isolated a series of α-D-Gal*p*-containing glycoproteins from Ehrlich ascites tumor cells. The distribution and quantitation of α-D-Gal*p* groups on the cell surface was determined by using the α-D-galactopyranosyl-specific isolectin *Griffonia simplicifolia* I–B4 labeled with fluorescein isothiocyanate, ferritin, or [^{3}H]propionate. In the present study, we prepared Ehrlich cell plasma membrane glycoproteins in the same manner and immunized BALB/c mice with them. Following cell fusion with spleen cells from an immunized mouse, we obtained hybridomas producing monoclonal antibodies that reacted with glycoproteins containing Galα1–3-Galβ1–4GlcNAc epitopes. These monoclonal antibodies were characterized by hapten inhibition using ELISA. Two preparations recognized the Galα1–3Galβ1–4GlcNAc/Glc residue. The binding of Galα1–3Galβ1–4GlcNAc-containing glycoprotein was inhibited by Galα1–3Galβ1–4GlcNAc and Galα1–3Galβ1–4Glc, but not by Galβ1–4GlcNAc (LacNAc) or Galβ1–4Glc (lactose). Therefore the *N*-acyl group at the C–2 position of glucose is not necessary for recognition by this antibody. This monoclonal antibody reacted with laminin and Galα1–3Galβ1–4GlcNAc-enriched bovine thyroglobulin and also reacted strongly with Ehrlich tumor cells.

Recently, clinical interest has focused on the terminal α-galactosyl residues of glycoproteins including laminin. Galili et al. (1984) reported that human antibodies to the Galα1–3Galβ1–4GlcNAc structure, normally present in the serum, constituted 1% of circulating IgG; the epitope itself is not ordinarily expressed on human cells and glycoproteins (Thall and Galili 1990). However in at least two human disease states, namely metastatic cancer and Chagas' disease, it is thought that these antibodies may participate in defense processes. Human cancer cells often express this epitope (Castronovo et al. 1987) and the antibody will inhibit adhesion of these cells to laminin (Castronovo et al. 1989). The parasitic trypanosome which causes Chagas' disease also carries the same epitope (Milani and Travassos 1988; Avila et al. 1989). It has been suggested that laminin may be one of the major α-D-Gal*p*-containing moieties in many tumor cell lines of

murine origin (Varani et al. 1983a, b; McCoy et al. 1984). Knibbs et al. (1989) reported that the Galα1–3Galβ1–4GlcNAcβ1–6 branching structure is present on the oligosaccharide structures of laminin; these investigators also showed that the Ehrlich cell plasma membrane glycoprotein reacted with anti-laminin antibodies when examined by ELISA (Knibbs et al. unpublished observation). In this study, our monoclonal antibodies reacted with laminin; treatment with α-galactosidase abolished this reaction. Using these monoclonal antibodies, we now plan to investigate the occurrence of the Galα1–3-Galβ1–4GlcNAc epitope in normal and tumor murine and human cells.

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