| **Title** | A monoclonal antibody directed to N-acetylneuraminosyl-alpha 2 leads to 6-galactosyl residue in gangliosides and glycoproteins |
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| **Note** | |
A hybridoma cell line producing a monoclonal antibody directed to N-acetylneuraminosyl-α2→6-galactosyl residue has been established. The antibody is IgG2b and reacts only with lacto-series gangliosides as well as with glycoproteins having an N-acetylneuraminosyl-α2→6-galactosyl residue, but does not react with gangliosides or glycoproteins having an N-acetylneuraminosyl-α2→3- or α2→4-galactosyl residue. The antibody is useful for detecting the specific carbohydrate chain having this terminal structure by immunostaining of glycolipids separated on thin layer chromatography or glycoproteins separated on gel electrophoresis after blotting on nitrocellulose sheet. A remarkable accumulation of a few gangliosides having this terminal structure has been detected by this monoclonal antibody in some human cancer.

Monoclonal antibodies directed to defined carbohydrate structures have been isolated and have proven to be useful probes in determination of the carbohydrate profile at the cell surface and in the secreted reagents in determination of carbohydrate structures (1-9). This approach has been conveniently applied for analysis of antigen profiles, in combination with the immunostaining of glycolipids separated on thin layer chromatography (10) and glycoproteins separated on gel electrophoresis followed by blotting on nitrocellulose sheet (11). The specificity of these glycoproteins with the antibody was determined by solid-phase carbohydrate assay (see for a review Refs. 15-17). The present paper describes establishment of a hybridoma secreting the IgG2b antibody, which reacts specifically with the sialosyl-α2→6-lacto-neotetraosylceramide, and shows no reactivity to sialosyl-α2→3-lacto-neotetraosylceramide. They were derived from the same well (lIIIC8) of the original fusion plate and were derivatives of the same clone.

**RESULTS**

Production of the Hybridoma—Fusion of the NS/1 myeloma with spleen cells of mice immunized against sialosyl-α2→6-lacto-neotetraosylceramide yielded about 3% positive clones by the first assay after the fusion. Two clones, IB2 and IB9, were isolated which appeared to have the same reactivity, specifically to sialosyl-α2→6-lacto-neotetraosylceramide, and showed no reactivity to sialosyl-α2→3-lacto-neotetraosylceramide. They were derived from the same well (IIIIC8) of the original fusion plate and were derived from the same clone. Both hybridomas, IB2 and IB9, can be propagated as ascites form in BALB/c mice, producing high titer antibody. Antibodies from these hybridomas were identified as IgG2b class, and were successfully propagated as ascites form in "pris-tane"-treated BALB/c mice.

**Specificity of the Antibody**—Antibodies produced by both hybridomas, IB2 and IB9, showed identical specificity, and the supernatant fluid of the hybridomas showed the same specificity as ascites. The results described in this paper are those with the IB9 antibody. The reactivity of the antibody with various gangliosides and glycoproteins is shown in Figs. 1 and 2, respectively. It is clear that only lacto-series gangliosides containing the sialosyl-α2→6-galactosyl residue showed a specific reactivity. All other gangliosides failed to react with the antibody. 

**MATERIALS AND METHODS**

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Fig. 1. Reactivity of various gangliosides with the monoclonal antibody IB9 determined by solid phase radioimmunoassay. A shows the reactivity of various gangliosides using 100 ng/well with lectin and cholesterol (see text). The activity was determined with various dilutions of hybridoma ascites. C, sialosyl-α2→6-lacto-neotetraosylceramide; Δ, sialosyl-α2→6-lacto-norhexaosylceramide; Δ, sialosyl-α2→3-lacto-neotetraosylceramide; C, GM1 ganglioside; V, GM1 ganglioside. B shows the reactivity of various gangliosides with different concentrations by solid phase radioimmunoassay. The original concentration (100 ng/well) was diluted and coated on the plate with cholesterol and lectin and determined with 100× diluted ascites of IB9 hybridoma. C, sialosyl-α2→6-lacto-neotetraosylceramide; Δ, lacto-neotetraosylceramide; V, GM1 ganglioside; ■, GM1 ganglioside; △, cholesterol and lectin without ganglioside.

1 The abbreviations used are: Ga, Fucol→2Galβ1→4GlcNAcβ1→6[NeuAcα2→3Galβ1→4GlcNAcβ1→3]Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→1Cer (other assignments for ganglio-series gangliosides are according to Svensson (33)); HPTLC, high performance thin layer chromatography.
Monoclonal Antibody to NeuAcα2→6Gal

The various gangliosides were separated on high performance thin layer chromatography plate (J. T. Baker Chemical Co., Phillipsburg, NJ) in a solvent mixture of chloroform/methanol/water (60:35:8, v/v/v). Lanes 1-4 were revealed by orcinol/sulfuric acid reaction. Lanes 5-8 are an autoradiogram of the chromatogram developed simultaneously and immunostained with monoclonal antibody IB9. Lane 1, a mixture of standard brain gangliosides. Bands a-f are, respectively, GM1, GM3, GM2, GD3, GD1b, and GT1b gangliosides. Designation of these brain gangliosides is according to Svennerholm (33). Lane 2 is a mixture of standard lacto-series gangliosides isolated and previously characterized (12). A doublet band g represents sialosyl-α2→3-lacto-neotetraosylceramide (the two bands represent different ceramides); a doublet band h represents sialosyl-α2→6-lacto-neotetraosylceramide (the two bands represent different ceramides); band i, sialosyl-α2→6 lacto-norhexaosylceramide; band j, G9 ganglioside (a branched fucoganglioside with ceramide decasaccharide structure (30). Lane 3, ganglioside fraction of hepatocarcinoma (diagnosed as adenocarcinoma). Lane 4, ganglioside fraction of primary lung carcinoma metastatic to liver.

**FIG. 4 (right). Immunostaining of fibronectins separated on gel electrophoresis followed by blotting on nitrocellulose sheet.** Lane 1, human plasma fibronectin; Lane 2, thermolysin digest of human plasma fibronectin (31); Lane 3, desialylated human plasma fibronectin by hydrolysis in 1% acetic acid. Left margin shows identification of the fragments. FN, intact fibronectin; case (Lane 3). This component was immunostained intensely by the antibody (Lane 7). In addition, slower migrating bands, including the one corresponding to sialosyl-α2→6-lacto-norhexaosylceramide and another with slower mobility, were detected as being stained by the antibody (Lane 7). This type of large accumulation of gangliosides having the NeuAcα2→6Gal residue was not observed in the other case of human cancer (lung cancer metastatic to liver) shown in Lanes 4 and 8. The major gangliosides chemically detectable in this case were GM1 and sialosyl-α2→3-lacto-neotetraosylceramide (Lane 4), which were not immunostained by this antibody (Lane 8). Only a small quantity of sialosyl-α2→6-lacto-neotetraosylceramide was detected, which was weakly immunostained by this antibody (Lane 8).

As an example of glycoproteins having the NeuAcα2→6Gal residue, human plasma fibronectin, its thermolysin digest, and desialylated products were separated on gel electrophoresis and immunostained after blotting on nitrocellulose sheet. An intense band was immunostained for intact fibronectin (Fig. 4, Lane 1), and a doublet with Mr = 140,000–150,000, 105,000, 44,000, and 23,000 was immunostained after thermolysin digestion (Lane 2) (31). An intense fibronectin stain was completely lost after the sialosyl residue of fibronectin was eliminated (Lane 3).

**DISCUSSION**

Profiles of cell surface carbohydrates have been probed and defined by lectins (32). This approach has been strengthened and partially replaced by monoclonal antibodies directed to defined carbohydrate structures. Another potentially important use of anti-carbohydrate monoclonal antibodies is their application in structural analysis of carbohydrates. The im-
munostaining procedure recently developed on thin layer chromatography (10) and blotting of electrophoretic gels on nitrocellulose sheets (11) is highly sensitive and requires only nanogram quantities of material. Immunostaining of complex carbohydrates by multiple monoclonals in combination with enzymatic hydrolysis will be potentially useful to elucidate the structure of nanogram quantities of carbohydrates, if the necessary number of well defined monoclonal antibodies are available. Efforts to gain increasing numbers of well defined monoclonal anti-carbohydrate antibodies are essential to reach such a goal.

The antibody described in this paper defines the terminal residue NeuAcα2→6Gal in glycolipids and glycoproteins and the NeuAcα2→6GalNAC residue in glycoproteins. The antibody does not react with the internal sialosylα2→6GalNAc residue (28) linked to polypeptides since glycophorin, which contains this structure, was not reactive.

The structure NeuAcα2→6Gal (or GalNAc) is known to be widely distributed in a variety of glycoproteins as the terminus of the short O-linked oligosaccharide in mucin-type glycoproteins, i.e. NeuAcα2→6GalNAcα1→O-Ser (Thr), and as the terminus in the side chain of a complex type asparagine-linked structure, i.e. NeuAcα2→6Galβ1→4GlcNAcβ1→2Manα1→R (see for a review Ref. 14), although such a structure has been confirmed only relatively recently based on methylation analysis (15–17, 25). Determination of such structures in glycoproteins requires isolation of oligosaccharides or glycopolypeptides after degradation, and methylation analysis before and after desialylation, and needs at least 0–200 μg of glycopolypeptides. If glycopolypeptides contain 3–5% carbohydrates or glycopolypeptides after degradation, and methylation structures in glycoproteins requires isolation of oligosaccharides or glycopolypeptides after degradation, and methylation analysis before and after desialylation, and needs at least 0–200 μg of glycopolypeptide. The results shown in Figs. 3 and 4 required only 100 ng of the glycoprotein. Since neither a ganglioside having N-glycolylneuraminosylα1→2→6-galactosyl residue nor a glycoprotein exclusively containing this structure have been isolated, we have had no chance to test the reactivity of the IB9 antibody to this structure.

On the other hand, the NeuAcα2→6Gal structure is rarely found in sialosyl glycolipids (gangliosides), and the structure constitutes the terminus of the type 2 N-acetyllactosaminyl chain, i.e. NeuAcα2→6Galβ1→4GlcNAcβ1→3Galβ1→R. Gangliosides with this structure have been isolated and characterized only recently (12, 13). Two types of gangliosides have been isolated and characterized from human erythrocytes (12). Normal colonic mucosal tissue and normal liver contain the total gangliosides of human erythrocytes, respectively (12). Two cases of human cancer are presented in this paper, as shown in Fig. 3. One accumulated sialosylα2→6 gangliosides and the other did not. However, we have observed many other cases, and the results of these studies will be published elsewhere. Since sialosylα2→6-lacto-neotetraosylceramide is the major ganglioside in meconium (13), but represents only a minor component in various normal tissues, the remarkable accumulation of this ganglioside in human cancer may reflect an oncofetal expression of this ganglioside. The antibody is useful in determining the profile of gangliosides and sialoeyl structures in tissues and cells.

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