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A Murine Monoclonal Antibody Detecting *N*-Acetyl- and *N*-Glycolyl-G_{M2}: Characterization of Cell Surface Reactivity[1]

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ABSTRACT

Antisera reactive with the ganglioside G_{M2} were raised by immunizing C57BL/6 mice with the C57BL/6 melanoma JB-RH. Fusion with NS-1 was performed using splenic mononuclear cells from a mouse with high antibody titer. An immunoglobulin M monoclonal antibody (monoclonal antibody 5-3) was identified which was reactive with an antigen that was resistant to heat, trypsin, and Pronase. A panel of purified glycolipids was used to determine the specificity of monoclonal antibody 5-3. Reactivity was restricted to *N*-acetyl- and *N*-glycolyl-G_{M2}. No reactivity was detected with asialo-G_{M2} or other gangliosides. Monoclonal antibody 5-3 was used to define the expression of G_{M2} on the cell surface of cultured human normal and malignant cells. Reactivity was seen with cell lines derived from 8 of 8 astrocytomas, 5 of 5 neuroblastomas, 7 of 9 sarcomas, 4 of 18 human melanomas, 2 of 4 murine melanomas, 4 of 37 epithelial cancers and with 0 of 6 skin fibroblast and 0 of 2 brain fibroblast lines. G_{M2}, like G_{D2} and G_{D3}, appears to be a differentiation antigen largely restricted to cells of neuroectodermal origin.

INTRODUCTION

In serological studies of cell surface antigens expressed by malignant melanomas and other human cancers of neuroectodermal origin, glycosphingolipids have turned out to be of particular interest (1–3). First identified in this context was the disialoganglioside G_{D3}, recognized by the mouse monoclonal IgG3 antibody (MoAb) R24 and the IgM monoclonal antibody 4.2 (4–6). Another disialoganglioside, G_{D2}, was initially detected on melanomas by autologous and allogeneic sera (7, 8) and recently also by mouse and human monoclonal antibodies (9, 10). From the pattern of cellular distribution in normal and malignant tissues, G_{D3} and G_{D2} have the characteristics of differentiation antigens of neuroectodermal origin. Interest in ganglioside antigens has been intensified by the observation that some patients treated with MoAb R24 showed regression of melanoma metastases (11), indicating that cell surface gangliosides are potential targets for cancer therapy.

Efforts to raise other monoclonal antibodies against components of the glycosphingolipid-biosynthetic pathway have been slow to succeed, probably because gangliosides in general are poorly immunogenic. A mouse monoclonal antibody reactive with *O*-acetylated G_{D3} (12) and human sera and monoclonal antibodies reactive with G_{M2} (13) have been described. The latter has been derived from Epstein-Barr virus-transformed B-cell lines; while it is useful for serological typing of cultured cells, it is much more difficult to produce than mouse monoclonal antibodies at high concentration and in large amounts for clinical application.

We report here the establishment of a mouse hybridoma clone which produces an IgM antibody detecting G_{M2}. Immunoadherence assays on cultured cells and absorption studies with this antibody indicate that G_{M2}, like G_{D2} and G_{D3}, is a differentiation antigen largely restricted to cells of neuroectodermal origin.

MATERIALS AND METHODS

Tissue Culture Lines. Cultures of adherent cells were maintained in minimal essential medium supplemented with 7.5% fetal calf serum, penicillin-streptomycin (100 μg/ml), 2 mM L-glutamine, and 1% nonessential amino acids as described previously (4). Suspension cultures were maintained in RPMI supplemented with 10% fetal calf serum, 1% nonessential amino acids, and penicillin-streptomycin (100 μg/ml).

Generation of Monoclonal Antibody. The chemically induced C57BL/6 melanoma JB-RH was selected for immunization because it expresses high levels of gangliosides in general and G_{M2} in particular. JB-RH melanoma cells were kindly provided by Dr. Jane Berkelhammer (14). JB-RH was cloned by limiting dilution, and subclone JB-RH-16 was selected for rapid growth and high G_{M2} expression. All further reference to JB-RH in this paper indicates this subclone.

To confirm that the cells expressed G_{M2}, ganglioside extracts treated with formic acid to remove sialic acid (see below) were analyzed by thin layer chromatography. The major component of JB-RH gangliosides showed the same migration pattern as the asialo-G_{M2} standards. For the purpose of immunization, JB-RH cells were grown in tissue culture, collected by scraping, and irradiated (10,000 rads) with a ^{60}Co source. A variety of immunization procedures was used in attempts to immunize C57BL/6 mice with these cells. Significant serological reactivity was developed only by mice pretreated with cyclophosphamide and then given a s.c. injection of JB-RH cells mixed with monophosphoryl lipid A as described previously (15). One mouse in this group was given an i.p. injection of JB-RH cells and sacrificed 3 days later. The spleen cells were fused with NS-1 cells, and antibody-producing hybridomas were developed and screened according to previously described procedures (2).

Gangliosides. G_{M1}, G_{D1a}, G_{T1}, and G_{b3}, and G_{b4} were purchased from Supelco, Inc. (Bellefonte, PA). G_{D3} was purified from human melanoma as described below. G_{M3} was purified from dog erythrocytes (16) and G_{D2} was purified from human brain as described below. G_{M2} was prepared by treating G_{M1} with β-galactosidase purchased from Dr. George W. Jourdian (Michigan State University, Ann Arbor, MI). The enzyme treatment was performed essentially as described by Cahan *et al.* (10) for the production of G_{D2}, using 0.2 M sodium acetate (pH 5.0) with 1% sodium taurocholate plus 1% BSA and overnight incubation at 37°C. For the production of asialo-G_{M2}, 1 ml of formic acid was added to 500 mg of G_{M2} and incubated for 0.5 h at 100°C (13). The mixture was allowed to cool to room temperature and placed on a Sep-

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[3] The abbreviations used are: G_{D3}, II^{3}NeuAc$_2$-LacCer or NeuNAcα2-8NeuNAcα2-3-Galβ1-4GlcCer; MoAb, monoclonal antibody; C:M, chloroform:methanol; IA, immunoadherence; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; TLC, thin layer chromatography; ITLC, immune thin layer chromatography; G_{M3}, II^{3}NeuAc-LacCer or NeuNAcα2-3Galβ1-4GlcCer; G_{M2}, II^{3}NeuAc-Gg$_3$Cer or GalNAcβ1-4[NeuNAcα2-3]Galβ1-4GlcCer; G_{D2}, II^{3}NeuAc$_2$-GgCer or GalNAcβ1-4[NeuNAcα2-8NeuNAcα2-3]Galβ1-4GlcCer; G_{D1a}, IV^{3}NeuAc-II^{3}Neu-Gg$_4$Cer or NeuNAcα2-3Galβ1-3GalNAcβ1-4[NeuNAcα2-3]Galβ1-4GlcCer; G_{T1a}, IV^{3}NeuAc$_2$-II^{3}NeuAc-Gg$_4$Cer or NeuNAcα2-8NeuNAcα2-3Galβ1-3GalNAcβ1-4[NeuNAcα2-3]Galβ1-4GlcCer; G_{M1}, II^{3}NeuNAc-Gg$_4$Cer or Galβ1-3-GalNAcβ1-4[NeuNAcα2-3]Galβ1-4GlcCer; Gb3, Galα1-4Galβ1-4-Glc-1Cer; Gb4, GalNacβ1-3Galα1-4-Galβ-1-4Glcβ1Cer; BSA, bovine serum albumin.

Pak column (Waters, Inc., Milford, MA) for the removal of acid, washing with distilled water. The sample was then eluted, dried, and subjected to DEAE-Sephadex column chromatography. G_{M2}, asialo-G_{M2}, *N*-glycolyl-G_{M2}, and *N*-glycolyl-G_{M3} (for use as standards) were generously provided by Dr. Robert Yu, Yale University, New Haven, CT. All gangliosides contain *N*-acetylneuraminyl- as opposed to *N*-glycolylneuraminylsialic acid unless indicated.

Isolation of Glycolipids. Glycolipids were isolated by a modification of the method described by Saito and Hakomori (17). Briefly, cells were homogenized in C:M (2:1) and extracted in a 100-fold volume of C:M (2:1). The homogenate was then filtered and reextracted in a 10-fold volume of C:M (1:1) and filtered and extracted again in a 10-fold volume of C:M (1:2). All filtrates were combined and evaporated in a rotary evaporator. The sample was acetylated, subjected to Florisil chromatography to remove phospholipids, deacetylated, and dialyzed against water for 24 h. After dialysis, the sample was evaporated, resuspended in C:M:water (30:60:8), and applied to a DEAE-Sephadex column equilibrated with C:M:0.8 M sodium acetate (30:60:8). The neutral glycolipid fraction was eluted with C:M:water (30:60:8); the acidic fraction was eluted with C:M:0.8 M sodium acetate (30:60:8).

TLC. Silica gel plates (Analtech, Newark, DE) were activated by heating at 100°C for 1 h (18). The solvent used for developing chromatographs was *N*-propyl alcohol:ammonium hydroxide:water (60:95:11.4). When the solvent had migrated 12 cm from the origin, the plate was removed and air dried, placed in an oven at 120°C for 10 min, cooled to room temperature, and sprayed with resorcinol-HCl. Quantitation was achieved by densitometric scanning with a Shimadzu (Kyoto, Japan) CS-930 thin layer chromatography scanner as described by Ando *et al.* (19).

IA Assay. The assay was performed as described previously (7).

Absorption Tests. Cell lines were grown in T-75 (Corning Glassware, Corning, NY) flasks, scraped off, and washed three times in VBM. The pellet was divided into 30-μl aliquots and incubated for 1 h at 4°C with 45 μl of the MoAb 5-3 at a dilution of 1:1000. Tubes were centrifuged at 1000 rpm for 10 min, and the supernatant was tested in IA assays on JB-RH cells.

Inhibition Tests. Inhibition tests were performed as described previously (5). Ganglioside (10 μg) was dried in dilution tubes (6 x 50 mm) overnight in a desiccator. MoAb 5-3 was diluted 1:1000, and 30 μl were added to the ganglioside preparation. The mixture was kept at 4°C for 1 h and tested in IA assays on JB-RH cells for residual antibody.

ELISA. Glycolipids were serially diluted 2-fold in microtest titration plates, starting at 5 μg/well. The plates were air dried for 2 h and blocked with 1% BSA for 2 h. MoAb 5-3, 10 μl diluted 1:1000, was added to each well, and the plates were incubated for 1 h at room temperature. The plates were washed and the second antibody, anti-mouse IgM (μ-specific) conjugated with alkaline phosphatase (Sigma Chemical Co., St. Louis, MO), was added at a dilution of 1:200. The plates were incubated for 45 min and washed. Diethanolamine substrate was added, the plates were incubated for 20 min at 37°C, and the reaction was quantitated on an ARTEC (ARTEC Labs Corp., NY) reader.

ITLC. Immune staining was done as described previously (20). Gangliosides were placed on TLC aluminum sheet Silica Gel 60 F-254 (Merck, Darmstadt, Germany) and allowed to migrate 12 cm from the origin. The plate was air dried and exposed to 0.02% isobutyl methacrylate in hexane for 30 s, air dried again, and immersed in a 2% BSA solution for 2 h. The plate was exposed overnight to MoAb 5-3 at a dilution of 1:1000 in PBS free of calcium and magnesium with 2% BSA and exposed to 20 ml of anti-mouse IgM μ-chain specific peroxidase conjugated (Sigma) for 1 h. The plate was washed five times in PBS free of calcium and magnesium and exposed to 25 ml of PBS free of calcium and magnesium containing 2 g of 3,3′-diaminobenzidine (Sigma) and 5 μl of 30% hydrogen peroxide (Sigma, St. Louis, MO) for 15 min. The plate was then washed for 5 min with water and air dried.

RESULTS

Generation of MoAb 5-3

One of the primary hybrid clones tested by IA on JB-RH cells was found to be reactive and was subsequently subcloned three times by limiting dilution. The supernatant showed a titer of 1:64, and the antibody was shown by immunodiffusion to be of the IgM class; it was designated MoAb 5-3. The hybridoma was expanded in tissue culture and inoculated into Swiss *nu/nu* mice. Sera from these mice showed an antibody titer of 1:65,610 and the ascites titer was 1:21,870.

Reactivity of MoAb 5-3 with Cell Surface Antigens of Cultured Cells

Direct IA Tests. The reactivity of MoAb 5-3 with cultured cells is shown in Table I. Two of 4 mouse melanomas were positive, with JB-RH cells showing the highest titer. Of 18 human melanomas tested, 4 were positive. With other human cell lines, positive tests were seen as follows: astrocytoma, 8 of 8; neuroblastoma, 2 of 2; fibrosarcoma, 1 of 3; rhabdomyosarcoma, 0 of 2; osteosarcoma, 2 of 3; liposarcoma, 1 of 1; renal cancer, 0 of 6; colon cancer, 0 of 5; bladder cancer, 1 of 6; breast cancer, 1 of 6; lung cancer, 1 of 5; ovarian cancer, 1 of 7; skin fibroblasts, 0 of 6; and SV40-infected skin fibroblasts, 2 of 5. Normal kidney epithelium and fetal and adult brain fibroblasts did not show reactivity.

Qualitative and Quantitative Absorption Tests. Results are summarized in Table 1. In general, the results seen in absorption tests corresponded with those seen in direct tests. Two sarcomas, SW98 and K 165, were exceptions in that they showed no reactivity in direct tests but gave positive absorption tests. Normal fetal brain and liver tissue were not reactive. Quantitative absorption tests showed that JB-RH cells completely absorbed the reactivity of MoAb 5-3 at a dilution of 1:1000 with pellets as small as 1 μl. At the same antibody dilution, a pellet of 10 μl was required for SK-MEL-31 cells to accomplish complete absorption, and a pellet of 30 μl was required for SK-MEL-173 cells.

Biochemical and Serological Characterization of the Antigen Recognized by MoAb 5-3

TLC. Total ganglioside fractions were prepared from various melanoma cell lines and analyzed by TLC. The major ganglioside component of JB-RH comigrated with a G_{M2} standard provided by Dr. Robert Yu, and with G_{M2} prepared in our laboratory (Fig. 1). Ganglioside extracts of SK-MEL-179-IV (a melanoma cell line), SK-Mg-6 (an astrocytoma cell line), and SK-N-Mc (a neuroblastoma cell line) which showed substantial reactivity with MoAb 5-3 in IA assays also contained components which comigrated with G_{M2}. By contrast, extracts of the human melanoma cell lines SK-MEL-23 and SK-MEL-174, fresh human melanoma tissue, and the mouse melanoma cell line S-91 which were nonreactive with MoAb 5-3 contained no ganglioside comigrating with G_{M2}.

ITLC. The reactivity of MoAb 5-3 with various purified gangliosides was tested by ITLC. As shown in Fig. 2, reactivity was restricted to G_{M2}. There was intense staining of both *N*-acetyl-G_{M2} and *N*-glycolyl-G_{M2}. G_{M3}, G_{M1}, G_{D3}, and G_{D1a} in this experiment and asialo-G_{M2}, G_{T1a}, and neutral glycolipids Gb4 and Gb3 in other experiments showed no reactivity with MoAb 5-3.

ELISA. The results confirmed that the reactivity of MoAb 5-3 is restricted to *N*-acetyl- and *N*-glycolyl-G_{M2} (Fig. 3). As in the ITLC analysis, the gangliosides G_{M3}, G_{M1}, G_{D2}, G_{D3}, G_{D1a}, and G_{T1a} and the neutral glycolipids G_{b4} and G_{b3} showed no reactivity.

Inhibition Tests. Various glycolipids were tested for their ability to inhibit the reaction of MoAb 5-3 with JB-RH cells (Fig. 4). G_{M2} alone was inhibitory. The gangliosides G_{M1}, G_{M3},

Table 1 *IA reactivity of MoAb 5-3*

Cell line	Antibody titer	Absorption test
Mouse melanoma		
JB-RH	65,610	+
B16	650	
S-91	0	
K1735	0	
Human melanoma		
SK-MEL-31	21,870	+
SK-MEL-13, -33	810	+
SK-MEL-173-IV	650	+
SK-MEL-37, -178	0	−
SK-MEL-23-II, -28, -29, -44, -75, -88, -93, -109, -131, -169, -174	0	
Astrocytoma		
SK-MG-6, -14	65,000	+
SK-MG-3, -15	21,000	+
U-373-MG	810	+
SK-MG-1, -7	270	+
U-251-MG	270	+
Neuroblastoma		
SK-N-BE_2	2,000	
SAM, MCMB-1, SK-M-MC	810	
Kaw	270	
Sarcoma		
TE-85	21,870	+
Saoes-1, SW872, Wlsp-160	2,430	
U-206, KS-165, SW982	0	+
RD_2	0	−
SW594	0	
Renal cancer		
SK-RC-1, -10, -12, -15, -29, -31	0	
Colon cancer		
SW837, HT29, SK-CO-10, SW480	0	
SW417	0	
Bladder cancer		
T24	2,560	+
JON	0	−
VM-CUB-1, SW1710, VM-CUB-2, SW780	0	
Breast cancer		
MDA-MD-157	2,560	+
CaMa	0	−
MCF-7, MDA-MB-361, MDA-MB-231	0	
SK-Br-5	0	
Lung cancer		
SK-LC-6	2,560	+
SK-LC-21	0	−
SK-LC-9, -15, -17	0	
Ovarian cancer		
OV2774	2,560	+
A-10	0	−
SK-OV-4, -6, -10	0	
QU-3, SW676, A7	0	
Fibroblasts		
135-52-56, 135-57-18	0	−
F4000	0	−
SF123, SF1142	0	
F135-60, F135-86	0	
SV40 infected fibroblasts		
SVJOJ722	3,645	
H(as)	1,215	
A268N, W18Vas, SV40W130	0	
EBV transformed lymphocytes		
AH, AZ, HJ	0	
Fetal tissues		
Brain		−
Liver		−
Normal tissues in tissue culture		
Kidney	0	

G_{D1a}, G_{D3}, and G_{T1a} and the neutral glycolipids Gb4 and Gb3 did not inhibit the reaction.

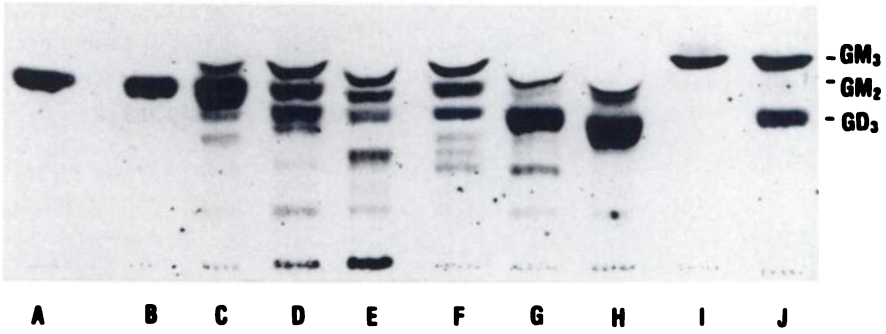
DISCUSSION

Considering the difficulties generally encountered in inducing an antibody response against gangliosides, we attribute the success in producing MoAb 5-3 to two factors, the selection of the immunogen and the method of immunization. Prior study of 7 mouse melanomas showed that JB-RH expressed considerably higher amounts of gangliosides in general and of G_{M2} in particular. Regarding the immunization procedure, we have shown previously that pretreatment with cyclophosphamide (15 mg/kg) and the use of monophosphoryl lipid A as an adjuvant were most consistently effective in inducing a serological response to antigens of chemically induced sarcomas in mice (15, 21). The use of a syngeneic tumor-host system provided the additional convenience of simplifying the screening of sera and hybridoma supernatants.

The results show that MoAb 5-3 reacts with both *N*-acetyl- and *N*-glycolyl-G_{M2}. No other glycolipid tested, acidic or neutral, showed any reactivity in ITLC, ELISA, or inhibition tests (Table 2). Initially, the fact that Mo-Ab 5-3 reacted with both *N*-acetyl- and *N*-glycolyl-G_{M2} led us to believe that the sialic acid moiety was not involved in the antigenic determinant and that the antibody would also react with asialo-G_{M2}. Lack of reactivity with asialo-G_{M2}, however, shows that the sialic acid residue is important for antibody binding. It appears that the antibody either does not distinguish between *N*-acetyl and *N*-glycolyl groups or does not react with this plane of the sialic acid structure. It remains to be determined whether this pattern of reactivity extends to antibodies reactive with other gangliosides, because few antibodies have been studied thoroughly and tested with the *N*-glycolyl and asialo derivatives of the gangliosides they recognize.

Judging from its distribution on various cell lines, G_{M2} appears to be a differentiation antigen largely restricted to cancer cells of neuroectodermal origin. Expression on astrocytomas and neuroblastomas is frequent and expression on melanomas less common, the opposite of the distribution of G_{D3}, another marker of neuroectodermal differentiation (22). The frequent appearance of G_{M2} in soft tissue sarcoma cell lines need not cause concern regarding the implied lineage preference because some sarcomas (with cell types of origin that are often obscure) have also been found to show other characteristics of tumors derived from the neuroectoderm, including expression of G_{D3} (22). Epithelial cancers showed G_{M2} expression only rarely. While normal epithelial kidney cultures and fibroblasts were consistently negative in tests with MoAb 5-3, some fibroblasts transformed by SV40 showed reactivity. Overall, the distribution of the antigen recognized by MoAb 5-3 is very similar to the distribution of the antigen detected by the human monoclonal antibody L55, also reactive with G_{M2} (23), with the exception that fibroblasts were reactive with L55. A possible explanation is that the fibroblasts used were in fact transformed by SV40. An increase of G_{M2} expression after transformation by SV40 has been documented before (1, 24) and is again supported by our results. It is of interest that MoAb 5-3 reacts with both mouse and human melanoma cell lines. This interspecies reactivity is a feature in common with a recently discussed mouse monoclonal antibody to G_{M3} (25), also raised by syngeneic immunization.

With these specificity characteristics, MoAb 5-3 is of considerable interest for the study of normal neuroectodermal differ-



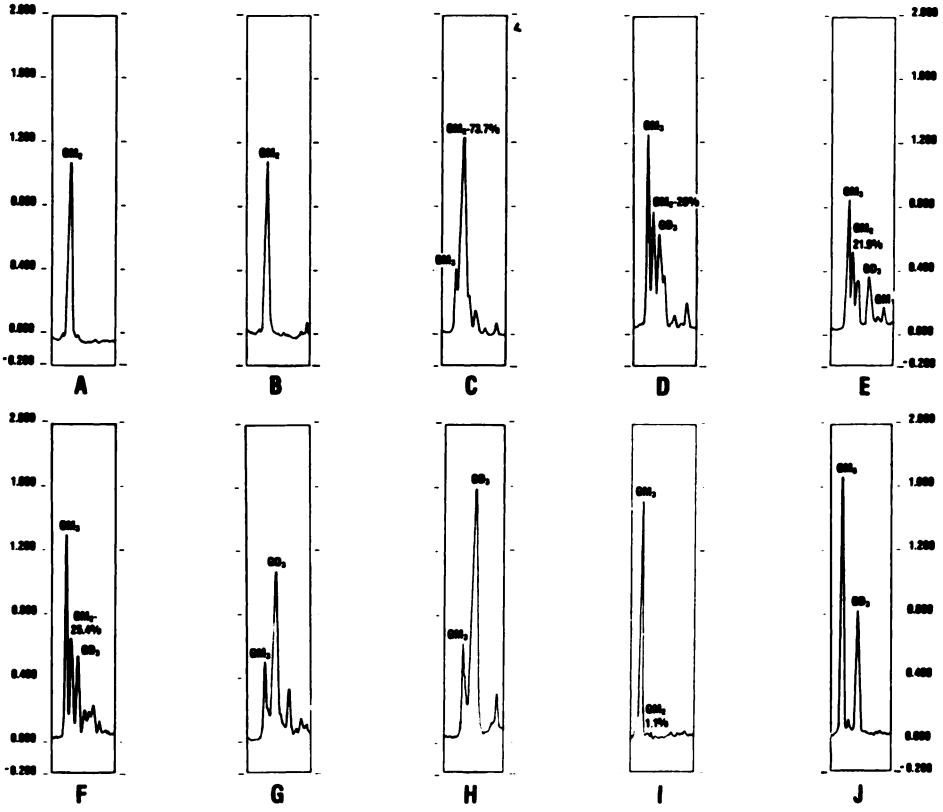


Fig. 1. TLC and densitometric scanning of ganglioside fractions. *A*, G_{M2}, (*N*-acetyl-, R. Yu); *B*, G_{M2} (*N*-acetyl-, prepared from G_{M1}); *C*, JB-RH-16 (mouse melanoma); *D*, SK-MG-6 (human astrocytoma); *E*, SK-N-MC (human neuroblastoma); *F*, SK-MEL-173-IV (human melanoma); *G*, SK-MEL-23 (human melanoma); *H*, SK-MEL-174 (human melanoma); *I*, S91 (mouse melanoma); *J*, biopsy specimen of a human melanoma. The amount of G_{M2} as percentage of total ganglioside fraction was calculated from the areas under the peaks.

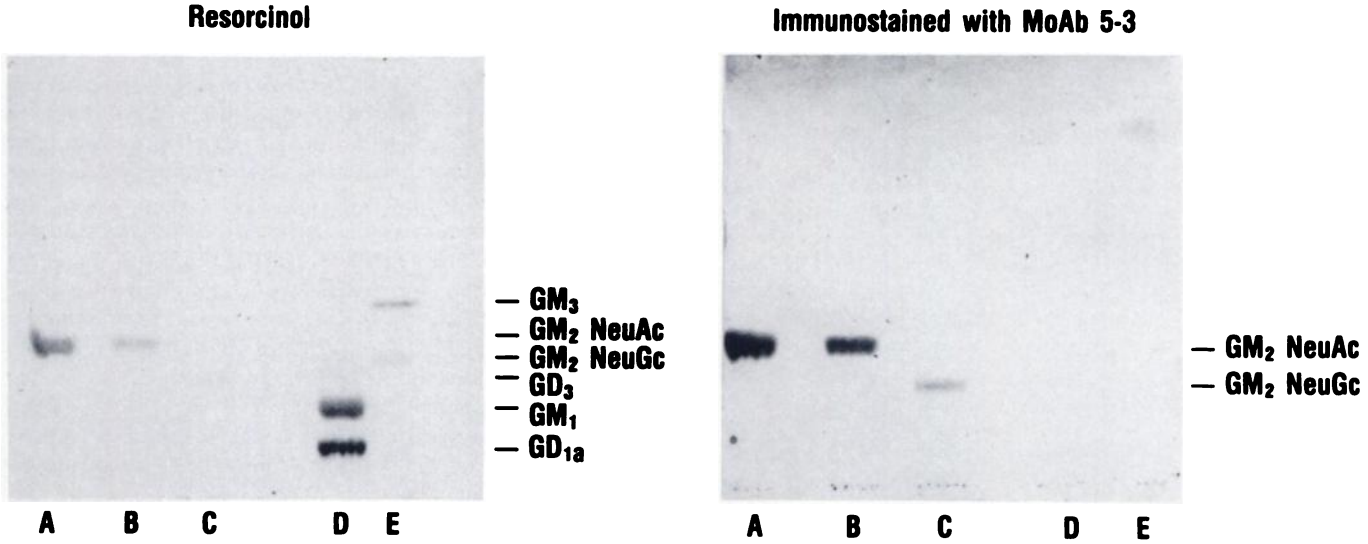


Fig. 2. Detection of G_{M2} ganglioside on TLC plate by immunostaining with MoAb 5-3. Two identical TLC plates were stained, one with resorcinol (*left*), the other with MoAb 5-3 and peroxidase (*right*). *A*, G_{M2} (*N*-acetyl- R. Yu); *B*, G_{M2} (*N*-acetyl-, prepared from G_{M1}); *C*, G_{M2} (*N*-glycolyl); *D*, G_{M1} and G_{D1a}; *E*, G_{M3} and G_{D3}.

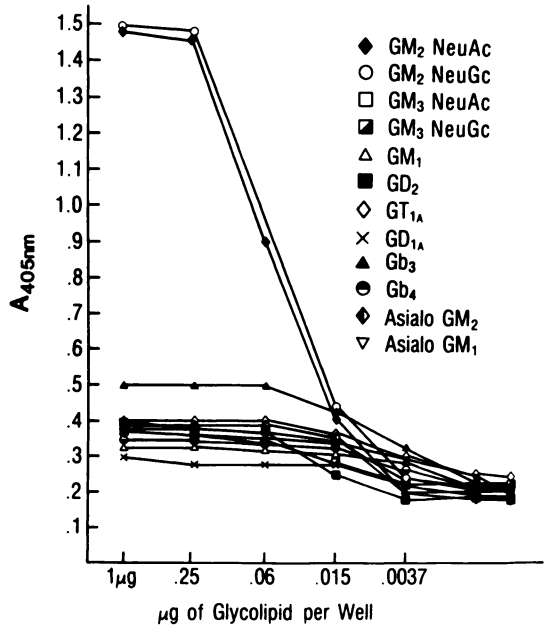


Fig. 3. ELISA reactivity of MoAb 5-3 with purified glycolipids. *NeuAc*, *N*-acetyl-; *NeuGc*, *N*-glycolyl-.

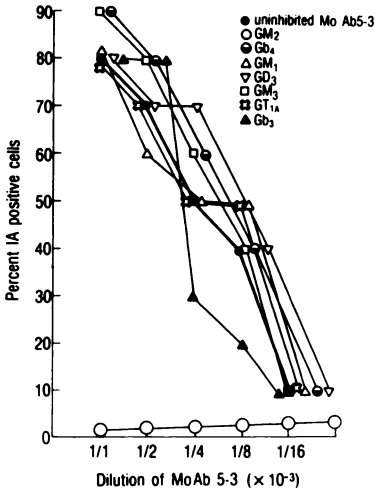


Fig. 4. Inhibition of MoAb 5-3 reactivity (IA) with JB-RH by glycolipids.

Table 2 *Reactivity of MoAb 5-3 with glycolipids*

Glycolipid	Structure	Reactivity by ELISA	Reactivity by ITLC
G_{M3}	Gal-Glc-Cer[a] | NeuAc	–	–
G_{M3} NeuGc	Gal-Glc-Cer | NeuGc	–	–
G_{M2}	GalNAc-Gal-Glc-Cer | NeuAc	+	+
G_{M2} NeuGc	GalNAc-Gal-Glc-Cer | NeuGc	+	+
Asialo-G_{M2}	GalNAc-Gal-Glc-Cer	–	–
G_{D2}	GalNAc-Gal-Glc-Cer | NeuAc | NeuAc	–	–
G_{M1}	Gal-GalNAc-Gal-Glc-Cer | NeuAc	–	–

[a] Gal, galactose; Glc, glucose; Cer, ceremide; NeuAc, *N*-acetyl-; NeuGc, *N*-glycolyl-; GalNAc, *N*-acetylgalactosamine.

entiation, for the analysis of tumors, and possibly for tumor localization and therapy.

REFERENCES

1. Hakomori, S., and Kannagi, R. Glycosphingolipids as tumor-associated and differentiation markers. J. Clin. Immunol., *71:* 231–251, 1983.
2. Carubig, J. M., Yu, R. K., Magala, L. J., Kirkwood, J. M., and Vargy, J. M. Ganglioside of normal and neoplastic human melanocytes. J. Biol. Chem., *120:* 500–504, 1984.
3. Sculz, G., Cheresh, D. A., Varki, N. M., Yu, A., Staffileno, L. K., and Reisfeld, R. A. Detection of ganglioside G_{D2} in tumor tissues and sera of neuroblastoma patients. Cancer Res., *44:* 5914–5920, 1984.
4. Dippold, W. G., Lloyd, K. O., Li, L. T. C., Ikeda, H., Oettgen, H. F., and Old, L. J. Cell surface antigens of human malignant melanoma: definition of six antigenic systems with mouse monoclonal antibodies. Proc. Natl. Acad. Sci. USA, *77:* 6114–6118, 1980.
5. Pukel, C. S., Lloyd, K. O., Travassor, L. R., Dippold, W. G., Oettgen, H. F., and Old, L. J. G_{D3}, a prominent ganglioside of human melanoma. Detection and characterization by mouse monoclonal antibody. J. Exp. Med., *155:* 1133–1147, 1982.
6. Nudelman, E., Hakomori, S., Kannagi, R., Levery, S., Yeh, M-H., Hellström, K. E., and Hellström, I. Characterization of a human melanoma-associated antigen defined by a monoclonal antibody, 4.2. J. Biol. Chem., *257:* 12752–12756, 1982.
7. Watanabe, T., Pukel, C. S., Takeyama, H., Lloyd, K. O., Shiku, H., Li, L. T. C., Travassos, L. R., Oettgen, H. F., and Old, L. J. Human melanoma antigen AH is an autoantigenic ganglioside related to G_{D2}. J. Exp. Med., *156:* 1884–1889, 1982.
8. Irie, R. F., Giuliano, A. E., and Morton, D. L. Oncofetal antigen: a tumor-associated fetal antigen immunogenic in man. J. Clin. Immunol., *63:* 367–373, 1979.
9. Cheung, N-K V., Saarinen, U. M., Neely, J. E., Landmeier, B., Donovan, D., and Coccia, P. F. Monoclonal antibodies to a glycolipid antigen on human neuroblastoma cells. Cancer Res., *45:* 2642–2649, 1985.
10. Cahan, L. D., Irie, R. F., Singh, R., Cassidenti, A., and Pauslon, J. C. Identification of a human neuroectodermal tumor antigen. (OFA-1-2) as ganglioside G_{D2}. Proc. Natl. Acad. Sci. USA, *79:* 7629–7633, 1982.
11. Houghton, A. N., Mintzer, D., Cordon-Cardo, C., Welt, S., Fliegel, B., Vadhan, S., Carswell, E., Melamed, M. R., Oettgen, H. F., and Old, L. J. Mouse monoclonal IgG3 antibody detecting G_{D3} ganglioside: a phase I trial in patients with malignant melanoma. Proc. Natl. Acad. Sci. USA, *82:* 1242–1246, 1985.
12. Cheresh, D. A., Varki, A. P., Varki, N. M., Stallcup, W. B., Levine, J., and Reisfeld, R. A. A monoclonal antibody recognizes an *O*-acetylated sialic acid in human melanoma associated ganglioside. J. Biol. Chem., *259:* 5453–5459, 1984.
13. Tai, T., Paulson, J. C., Cahan, L. D., and Irie, R. F. Ganglioside G_{M2} as a human tumor antigen (OFA-1-1). Proc. Natl. Acad. Sci. USA, 280: 5392–5396, 1983.
14. Berkelhammer, J., Oxenhandler, R. W., Hook, R. R. Jr., and Hennessy, J. M. Development of a new melanoma model in C57BL/6 mice. Cancer Res., *42:* 3157–3163, 1982.
15. Livingston, P. O., Jones, M., DeLeo, A. B., Oettgen, H. F., and Old, L. J. The serologic responses to Meth A sarcoma vaccines after cyclophosphamide treatment is further increased by various adjuvants. J. Immunol., *135:* 1505–1509, 1985.
16. Hashimoto, Y., Otsuka, H., and Yamakawa, T. The occurrence of GM4 and G_{M2} in erythrocytes from inbred strains of mice. J. Biochem. (Tokyo), *91:* 1039–1046, 1982.
17. Saito, T., and Hakomori, S. Quantitative isolation of total glycolipids from animal cells. J. Lipid Res., *12:* 257, 1971.
18. Ledeen, R. W., and Ru, R. K. Gangliosides: structure, isolation and analysis. Methods Enzymol., *83:* 139–191, 1982.
19. Ando, S., Chang, N-C., and Yu, R. K. High-performance thin-layer chromatography and densitometric determination of brain ganglioside compositions of several species. Anal. Biochem., *89:* 437–450, 1978.
20. Kasai, N., Naiki, M., and Yu, R. K. Autoradiography of ganglioside antigens separated by high-performance thin-layer chromatography with their antibodies. J. Biochem. (Tokyo), *96:* 261–264, 1984.
21. Livingston, P. O., DeLeo, A. B., Jones, M., and Oettgen, H. F. Comparison of approaches for augmenting the serologic response to the individually specific methylcholanthrene-induced sarcoma Meth A: pretreatment with cyclophosphamide is most effective. J. Immunol., *131:* 2601–2605, 1983.
22. Real, F. X., Houghton, A. N., Albino, A. P., Cordon-Cardo, C., Melamed, M. R., Oettgen, H. F., and Old, L. J. Surface antigens of melanomas and melanocytes defined by mouse monoclonal antibodies: specificity analysis and comparison of antigen expression in cultured cells and tissues. Cancer Res., *45:* 4401–4411, 1985.
23. Irie, R. F., Sze, L. L., and Saxton, R. E. Human antibody to OFA-1, a tumor antigen, produced *in vitro* by Epstein-Barr virus-transformed human B-lymphoid cell lines. Proc. Natl. Acad. Sci. USA, *79:* 5666–5670, 1982.
24. Hoffman, L. M., Brooks, S. E., and Schneck, L. Human fetal brain cells in culture—increase in G_{M2} ganglioside after SV-40 transformation. Biochim. Biophys. Acta, *665:* 359–361, 1981.
25. Hirabayashi, Y., Hamaoka, A., Matsumoto, M., Matsubara, T., Tagawa, M., Wakabayashi, S., and Taniguchi, T. Syngeneic monoclonal antibody against melanoma antigen with interspecies cross-reactivity recognizes G_{M3}, a prominent ganglioside of B16 melanoma. J. Biol. Chem., *260:* 13328–13333, 1985.