# Characterization of a Glycosphingolipid Antigen Defined by the Monoclonal Antibody MBr1 Expressed in Normal and Neoplastic Epithelial Cells of Human Mammary Gland*

Eric G. Bremer‡, Steven B. Levery‡, Sandro Sonnino§, Riccardo Ghidoni§, Silvana Canevari¶, Reiji Kannagi‡, and Sen-itiroh Hakomori‡

From the ‡Program of Biochemical Oncology and Membrane Research, Fred Hutchinson Cancer Research Center and Departments of Pathobiology, Microbiology, and Immunology, University of Washington, Seattle, Washington 98104, the §Department of Biological Chemistry, Medical School, University of Milan, Milan, Italy and the ¶Division of Experimental Oncology A, Instituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian 1, 20133 Milan, Italy

The antigen defined by a monoclonal antibody, MBr1, was found to be expressed in normal human mammary gland epithelia and human mammary carcinoma cells (Ménard, S., Tagliabue, E., Canevari, S., Fossati, G., and Colnaghi, M. I. (1983) *Cancer Res.* 43, 1295–1300). The antigen has been isolated from breast cancer cell line MCF-7, which was used as immunogen, and its structure was determined by methylation analysis, NMR spectroscopy, direct probe mass spectrometry, and enzymatic degradation as identified below.

Fucα1→2Galβ1→3GalNAcβ1→3Galα1
$$\qquad\qquad\qquad\qquad\qquad →4Galβ1→4Glcβ1→1Cer$$
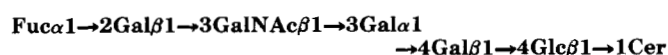
The antibody cross-reacted weakly with fucosylasialo-G_{M1} (IV^2FucGg_4), which shares the same terminal sequence, Fucα1→2Galβ1→3GalNAc, with this antigen. However, various other structures, including lacto-series H structure (Fucα1→2Galβ1→4/or3GlcNAcβ1→3Gal), did not show any reactivity with this antibody. Therefore, this antigen represents a blood group H antigen with a globo-series structure which is abundant in human teratocarcinoma (Kannagi, R., Levery, S. B., Ishigami, F., Hakomori, S., Shevinsky, L. H., Knowles, B. B., and Solter, D. (1983) *J. Biol. Chem.* 258, 8934–8942), although its presence must be limited in normal adult human tissue.

The presence of cell type-specific glycosphingolipid antigens expressed on tumor cells or on normal cells at a certain stage of development has been increasingly apparent (1) since the monoclonal antibody approach (2) has been applied to the analysis of cell surface structures. A breast cancer-associated antigen has been defined by the monoclonal antibody MBr1, although the antibody reacted with normal mammary gland epithelial cells and postpartum milk as well (3). The antibody did not react with a large variety of other tumor cell lines, tumor cells from surgical specimens, nor a large variety of normal human tissues including normal blood cells (4, 5). The antigen was found to be soluble in chloroform/methanol or tetrahydrofuran phosphate buffer extract and was assumed to be a glycosphingolipid, although a large variety of known glycosphingolipids did not show any reactivity with the anti-

body (5). Because of the highly restricted distribution of the antigen, the elucidation of the chemical properties of this antigen was of great interest. This paper describes the isolation and characterization of the glycosphingolipid antigen (termed MBr1 antigen) from the human breast cancer cell line MCF-7.

MATERIALS AND METHODS

Cells—Human breast cancer cell line MCF-7 (6) was cultured in suspension in the medium HB101TM (Hana Media, Inc., Berkeley, CA). Cells were harvested by centrifugation, and 40 ml of packed cells were collected for glycosphingolipid extraction.

Extraction and Fractionation of Glycosphingolipids—Lipids were extracted according to the procedure of Tettamanti *et al.* (7). Namely, lyophilized cells were suspended in 5 ml of 0.01 M potassium phosphate buffer, pH 6.8, and homogenized for 5 min with 20 ml of tetrahydrofuran. The pellet was recovered by centrifugation and extracted three times with 2 ml of the same buffer and 8 ml of tetrahydrofuran. The four extracts were combined and evaporated to dryness in a rotary evaporator. The total lipid extract thus obtained was suspended in water and dialyzed against water for three days. After dialysis, the suspension was lyophilized and the residue was solubilized in methanol and subjected to ion-exchange chromatography on a DEAE-Sepharose column (30 × 1 cm) according to the method of Yu and Ledeen (8). All the antigenicity was recovered in the nonacidic glycosphingolipid fraction. Therefore, this fraction was further separated into components by chromatography on silica gel 100 (70–230 mesh ASTM, Merck, Darmstadt, West Germany). The column (2 × 50 cm) was equilibrated with a mixture of chloroform/methanol/water (60:35:5, v/v/v), eluted with the same solvent mixture, and 20-ml fractions were collected. The fractions which reacted with the MBr1 antibody (tubes 30–40) were pooled and rechromatographed on a long column of silica gel 100 (1 × 100 cm) equilibrated with chloroform/methanol/water (110:40:6), eluted with the same solvent mixture, and 20-ml fractions were collected. Three fractions showing antigenicity were eluted and termed fractions 1 (800–1200 ml), 2 (1200–1600 ml), and 3 (1600–2000 ml). The major activity was found in fraction 3, and the activity associated with fractions 1 and 2 was due to the presence of a small quantity of the same antigen as was found in fraction 3. The active component, designated as "MBr1 antigen," migrated between H_1- and H_2-glycolipids (9) on HPTLC.[1] Fraction 3 was further purified by HPLC on Iatrobeads RS-8010 with a gradient solvent system of isopropanol/hexane/water (50:45:3 to 50:25:10) according to the method originally described by Watanabe and Arao (10) and modified by Kannagi *et al.* (11, 12). The antigen finally purified from fraction 3 was homogeneous on HPTLC, as shown in Fig. 1.

Antibodies and Immunological Reactivities of Glycosphingolipids—Monoclonal antibody MBr1, directed against human breast cancer cell line MCF-7, was established as previously described and was of

---

[1] The abbreviations used are: HPTLC, high-performance thin-layer chromatography; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; GC-MS, gas chromatography-mass spectrometry; Cer, ceramide; Fuc, fucose; Hex, hexose; G_{M1}, Galβ1→3GalNAcβ1→4(NeuAcα2→3)Galβ1→4Glcβ1→1Cer.

the IgM class (3). The reactivity of the glycosphingolipids with the antibody was determined by three different methods. A preliminary screening of the crude glycosphingolipid fraction was performed by absorption assay as described previously (5). The second method was TLC immunostaining of each glycosphingolipid subfraction separated on HPLC according to a modification (11, 12) of the procedure originally described by Magnani *et al.* (13). Briefly, the glycosphingolipids were chromatographed on HPTLC plates (SI-HPF plates, J. T. Baker Chemical Co., Phillipsburg, NJ) and reacted successively with 1:500 diluted purified stock solution of monoclonal MBr1, 1:1,000 diluted rabbit anti-mouse IgM (μ-chain specific), and ^{125}I-labeled protein A solution. The concentration of the purified stock solution of MBr1 antibody was 2.5 mg/ml. The third method was solid phase radioimmunoassay performed on vinyl strips (Costar, Cambridge, MA) according to the method previously described by Kannagi *et al.* (14).

Methylation Analysis and Direct Probe Mass Spectrometry—The purified glycosphingolipid (100 μg) was permethylated (15) and purified on an LH-20 column (16). A portion of glycosphingolipids (about 15 μg) was analyzed on direct-probe electron-impact mass spectrometry as previously described (17, 18) on a Finnigan 3000 quadrupole mass spectrometer. Conditions are given in the legend to Fig. 4. The major portion of permethylated glycosphingolipids (85 μg) was subjected to hydrolysis, and the partially methylated alditol acetates were analyzed by gas chromatography-chemical ionization mass spectrometry. Identification of partially methylated alditol acetates by chemical ionization mass spectrometry was described previously (19, 20). Details of the entire procedure will be described elsewhere.[2] Briefly, this involved hydrolysis in 300 μl of 0.5 N sulfuric acid in 90% acetic acid at 80 °C for 7 h, neutralization with 0.5 N sodium hydroxide, treatment of the dried residue with NaBD$_4$ (10 mg/ml) in 0.025 N NaOH at 4 °C, treatment with a small amount of acetic acid, drying of the residue under a nitrogen stream, co-distillation of the excess borate with methanol (5 times), and acetylation with acetic anhydride for 2 h at 100 °C. After removal of the excess acetic anhydride with toluene under nitrogen, the partially methylated alditol acetates were isolated from the salts by chloroform-water partition. After washing several times with water, the chloroform layer was concentrated under nitrogen and analyzed by gas chromatography-mass spectrometry on a Finnigan 3300 GC-MS adapted for use with fused silica capillary columns. Columns used were 30-m DB-5 and DB-225 (J & W Scientific, Rancho Cordova, CA) bonded phase fused silica capillaries, with outer diameter 0.32 mm and 0.25-μm coating thickness. Oven temperature was programmed from 140–250 °C at 4 °C/min.

The chemical ionization source (methane, 300 μ) was maintained at 100 °C (electron energy, 120 V; emission current, 0.5 mA; coll 35 eV). The 6110 data system scanned the mass range 60–450 atomic mass units every 2 s, beginning at 2 min after injection.

In order to confirm identification of 4,6-OMe-GalNAcMe, the acetylated reduced hydrolysate of the MBr1 glycolipid was mixed with that of type 1 chain paragloboside isolated from meconium and separated on a DB-5 column.

Fatty Acid Analysis—Fatty acids were analyzed as methyl esters by methods previously described (21). Briefly, 50 μg of a glycosphingolipid was hydrolyzed in a sealed tube with 1 ml of 0.5 N HCl in anhydrous methanol for 18 h at 80 °C. The MeOH was extracted 3 times with hexane, and the combined extracts were carefully dried under an N$_2$ stream just to dryness. Fatty acid methyl esters were analyzed by the same GC-MS system as for partially methylated alditol acetates (DB-5 column), but using a temperature program of 150–260 °C at 4 °C/min. This system was sufficient to separate and identify the methyl esters of hydroxy fatty acids without further derivatization. Quantitation was obtained using response factors for standard fatty acid methyl esters (National Institutes of Health fatty acid standards, plus C16:0, C18:0, C20:0, and C22:0 hydroxy fatty acids) injected under identical conditions.

^{1}H NMR Spectroscopy—500-MHz ^{1}H NMR spectra were obtained on a Bruker (W. Germany) WM-500 spectrometer equipped with an Aspect 2000 data system, operating in the Fourier-transform mode and using quadrature detection. The glycosphingolipid sample (approximately 200 μg) was deuterium-exchanged three times by dissolving in a 1:1 mixture of CDCl$_3$/CD$_3$OD, followed by evaporation under dry N$_2$ in a warm water bath, and dissolved in 0.4 ml of [^{2}H$_6$]dimethyl sulfoxide containing 2% D$_2$O (22) and 1% tetramethylsilane as chem-

ical shift reference. 400–600 free induction decays were collected for each spectrum, using a 90° excitation pulse angle; a spectral width of 5000 Hz on 16K data points gave an an acquisition time of 1.638 s, to which was added a 2-s relaxation delay. Resolution enhancement was obtained by applying a Lorentzian to Gaussian function (23), included in the Bruker software package, to the free induction decays before transformation.

Enzymatic Degradation—Fucosidase digestion was carried out as previously described by Schwarting *et al.* (24). Approximately 20 μg of the MCF-7 glycolipid (fraction 3) was dissolved in 400 μl of 0.05 M sodium citrate buffer (pH 5.0) and 300 μg of sodium deoxytaurocholate. The reaction was started by addition of 0.1 unit of α-fucosidase from bovine kidney (Sigma) and allowed to proceed for 16 h at room temperature. The reaction was stopped by dilution of the reaction mixture in an equal volume of methanol (400 μl). Glycolipids were recovered from the reaction mixture by reversed-phase chromatography (Analytachem International, Harbor City, CA) (25) to remove salts followed by DEAE-Sephadex chromatography to remove detergent. The DEAE pass-through was collected and evaporated to dryness. Analysis of the α-fucosidase-treated products was performed by HPTLC in chloroform/methanol/water (60:35:8).

RESULTS

Isolation of the Glycosphingolipid Antigen from MCF-7 Cells Defined by the Antibody MBr1—The major antigen reactivity was found to be associated with fraction 3 eluted from silicic acid chromatography, and the antigen was obtained as a single band on further purification by HPLC in an isopropranyl alcohol/hexane/water system, as shown in Fig. 1. HPTLC immunostaining of fraction 3 indicated that MBr1-positive material co-migrated with the orcinol-positive band (Fig. 1, *lanes 4* and *6*). Activity associated with fractions 1 and 2 was due to the presence of a small quantity of the same antigen that was present in fraction 3, as indicated by HPTLC migration. The major orcinol-positive component purified from fraction 2 (Fig. 1, *lane 3*) did not co-migrate with the MBr1 staining (Fig. 1, *lane 5*). The antigen in fraction 3 was also weakly reactive with SSEA-3 antibody, which defines the terminal or subterminal structure R→Galβ1→3GalNAcβ1→3Galα1→4Gal (12) (see Fig. 1); however, SSEA-3 stained material migrating slightly faster than the MBr1 staining. Fraction 2 was strongly reactive with SSEA-3 antibody and is assumed to be a galactosyl globoside as previously described (12).
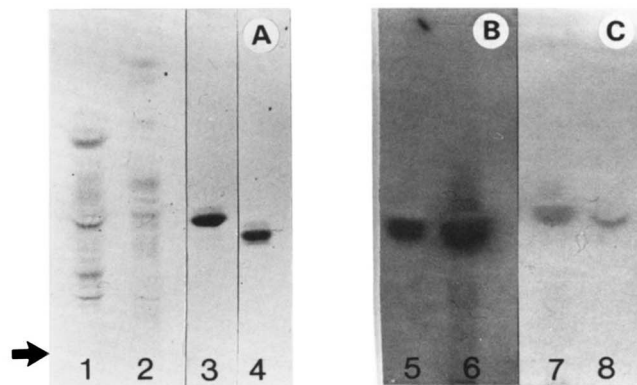

FIG. 1. **HPTLC of fraction 2 and 3 glycosphingolipids from human breast carcinoma cell line MCF-7 and the immunostaining pattern with the antibody MBr1.** *Panel A*, HPTLC pattern revealed by orcinol-sulfuric acid. *Lane 1*, total ganglioside fraction; *lane 2*, total neutral glycolipid fraction; *lane 3*, fraction 2 glycosphingolipid after HPLC purification (this is identified as galactosylgloboside, VI^{3}GalGb$_4$) (12); *lane 4*, fraction 3 glycosphingolipid (MBr1 antigen) after HPLC purification. *Panel B*, immunostaining pattern of fractions 2 and 3 by the antibody MBr1. *Lane 5*, fraction 2; *lane 6*, fraction 3. *Panel C*, immunostaining pattern of fractions 2 and 3 by anti-SSEA-3 antibody. *Lane 7*, fraction 2; *lane 8*, fraction 3.

TABLE I

Glycosyl H-1 chemical shifts (parts/million from tetramethylsilane) and $^3J_{1,2}$ coupling constants (Hz) of glycolipids from breast carcinoma and teratocarcinoma cells

					J		
		Fucα1→2	Galβ1→3	GalNAcβ1→3	Galα1→4	Galβ1→4Cer	Glcβ1→1Cer
	K						
GL6[a]	302	4.954 (2.5)[b]	4.459 (7.3)[b]	4.459 (7.3)[b]	4.801 (3.5)	4.258 (6.3)	4.165 (7.8)
MBr1	300	4.955 (2.5)[b]	4.463 (7.9)[b]	4.463 (7.9)[b]	4.802 (4.3)	4.257 (7.9)	4.171 (7.9)[c]
							4.204 (7.9)[c]
	308	4.961 (2.5)[b]	4.464 (6.7)	4.479 (7.9)	4.813 (4.3)	4.261 (7.9)	4.170 (7.9)
							4.202 (7.9)
	338	4.982 (2.5)[b]	4.481 (6.7)	4.520 (7.9)	4.846 (4.2)	4.280 (8.0)	4.179 (7.9)
							4.214 (7.9)

[a] Data from Ref. 11 but with third decimal added.
[b] Approximate value for $^3J_{1,2}$.
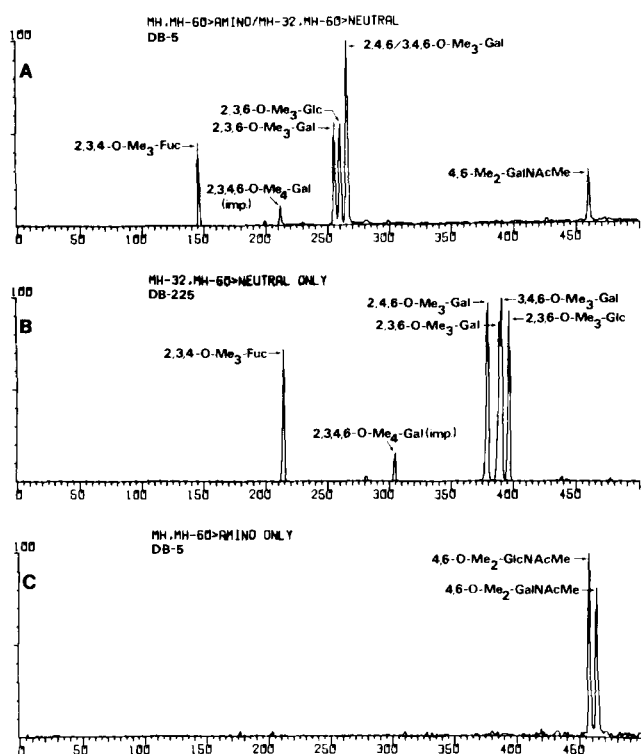[c] Confirmed by decoupling from Glc H-2 resonance.

FIG. 2. **Partially *O*-methylated alditol and hexosaminitol acetates detected in the hydrolysate of permethylated MBr1 antigen (fraction 3 glycosphingolipid).** *Panel A,* separation on DB-5 column. *Panel B,* separation on DB-225 column. *Panel C,* separation of 4,6-di-*O*-Me-GlcNAcMe and 4,6-di-OMe-GalNAcMe on DB-5 column. The sample was a mixture of acetylated reduced hydrolysate of type 1 chain paragloboside (Lc₄) and that of MBr1 antigen. The GC-MS are plotted as composite "limited mass chromatograms": *ordinate,* sum of ion intensities of MH⁺, (MH-60)⁺ for *N*-acetylhexosaminitols and (MH-32)⁺, (MH-60)⁺ for neutral alditol derivatives; *abscissa,* scan number.

Chemical Characterization of the Glycosphingolipid Antigen Defined by MBr1 Antibody—The antigen was identified as giving the same NMR resonances as "GL6 glycolipid" isolated from human teratocarcinoma with the structure Fucα1→ 2Galβ1→3GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer (12) (see Table I). The ¹H-NMR spectrum of the antigen showed anomeric resonances identical to those of GL6 glycolipid, as shown in Fig. 7*b* of Ref. 12, *i.e.* two anomeric resonances of the IVth sugar (GalNAcβ) and Vth sugar (Galβ) which coincide at 4.46 ppm. These resonances can be separated into their respective doublets by increasing the temperature (Table

I). The extreme downfield position of the signal at 4.95 ppm and the vicinal coupling constant ($J_{1,2} = 2.5$ Hz) is consistent with a terminal Fucα1→2 residue, and the resonances at 4.80, 4.26, and 4.17 ppm were identified as originating from internal αGal, βGal, and βGlc, respectively, as previously published (12). Further details of NMR assignment of this glycosphingolipid and other glycosphingolipids with extended globo-series structure will be published elsewhere.[3]

Gas chromatography-mass spectrometry of permethylated alditol acetates showed the presence of 2,3,4-tri-OMe-Fuc (terminal Fuc), 4,6-di-OMe-GalNAcMe (→3GalNAc1→), 2,3,6-tri-OMe-Glc (→4Glc1→), 2,3,6-tri-OMe-Gal (→ 4Gal1→), and a larger peak corresponding to 2,4,6-tri-OMe-Gal(→3Gal1→) and/or 3,4,6-tri-OMe-Gal(→2Gal1→) (Fig. 2*A*). The last two compounds were clearly separated on a DB-225 capillary column, from which 2,4,6-tri-OMe-Gal elutes significantly earlier than 3,4,6-tri-OMe-Gal (Fig. 2*B*). Since the retention times of 4,6-di-OMe-GalNAcMe and -Glc-NAcMe are very close, the identity of 4,6-di-OMe-GalNAcMe was demonstrated unambiguously by co-injection of permethylated alditol acetates from type 1 chain lactotetraosylceramide containing 4,6-di-OMe-GlcNAcMe (Fig. 2*C*). A small quantity of 2,3,4,6-OMe₄Gal was detected which was due to the occurence of an impurity (galactosyl-globoside). In other samples, such an impurity was not detectable.

The presence of terminal fucose was further confirmed by incubation of the antigen with beef kidney fucosidase under the conditions as previously described (24). The hydrolysis product was examined on TLC immunostained with MBr1 antibody as well as with SSEA-3 antibody. The MBr1 glycolipid was hydrolyzed to give a doublet having a similar TLC mobility as galactosyl globoside (*lane 5*). The resulting doublet was stained by SSEA-3 antibody (*lane 10*) but not by MBr1 antibody (*lane 15*) (Fig. 3).

Direct probe mass spectrometry of the permethylated glycosphingolipid showed the terminal deoxyhex (m/z 189→157), deoxyhex-OHex (m/z 393→361), deoxyhex-OHex-OHexNAc (m/z 638→606/638→228) and deoxyhex-OHex-OHexNAc-OHex (m/z 842→810) (Fig. 4). This is in agreement with the structure assigned by NMR. In addition, the Hex1→3HexNAc linkage is indicated by the diagnostic ion at m/z 228 in the absence of ions at m/z 260 and 182. This is analogous to the fragmentation of type 1 chain lactotetraosyl derivatives, with the ion at m/z 228 resulting from preferential loss of the 3-*O*-substituent from the HexNAc of the parent at m/z 638 (26). The spectrum was similar to that of GL6 of human teratocarcinoma as previously described (12), with some variation due

FIG. 3. **High-performance thin-layer chromatography of MBr1 glycolipid antigen and its degradation product with α-L-fucosidase of bovine epididymis and its immunostaining pattern.** *Panel A,* orcinol-sulfuric acid stain. *Panel B,* Immunostaining pattern of a similar plate as in *Panel A* by anti-SSEA-3 antibody. *Panel C,* Immunostaining pattern of a plate prepared under the same condition as in *Plate A* and *B* and stained by MBr1 antibody. *Lanes 1, 6,* and *11,* lactoneotetraosylceramide (paragloboside). *Lanes 2, 7,* and *12,* lactotetraosylceramide (type 1 chain, paragloboside). *Lanes 3, 8,* and *13,* MBr1 antigen. *Lanes 4, 9,* and *14,* galactosylgloboside (IV^3GalGb_4) (12). *Lanes 5, 10,* and *15,* MBr1 antigen treated with bovine epididymis α-fucosidase.
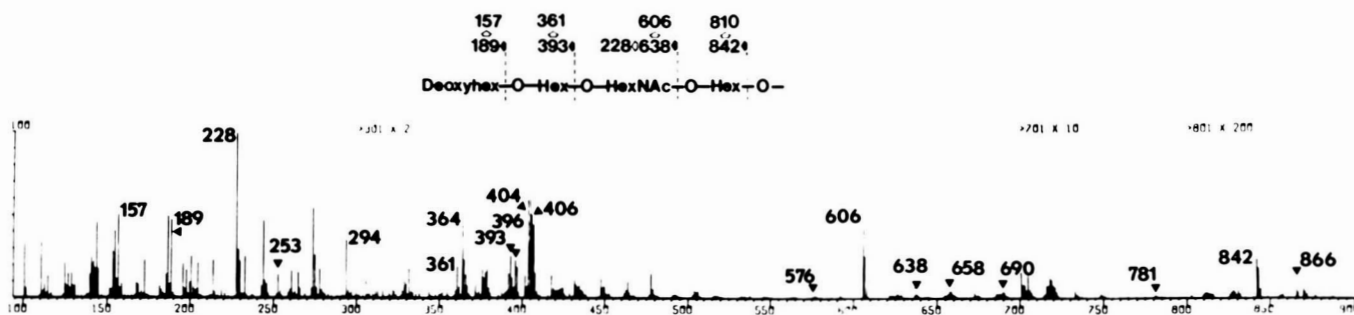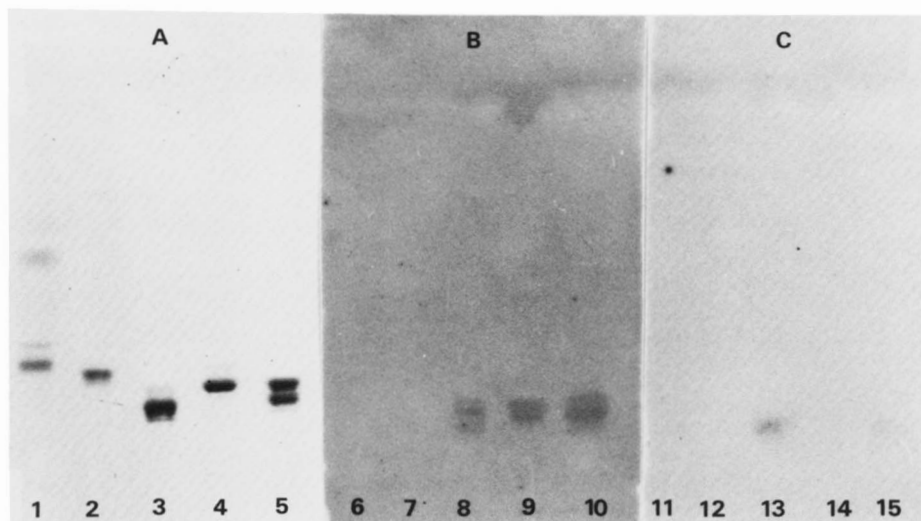

FIG. 4. **Direct-probe mass spectrum of permethylated MBr1 antigen.** Mass spectrometry was performed with a Finnigan 3000 mass spectrometer with 6110 data system under the following conditions: electron energy, 35 V; ion energy programmed from +5.5 V; extractor, +7.7 V; lens, 30 V; emission, +0.5 mA; electron multiplier, 2200 V; sensitivity, 10^{-7} A/V.

TABLE II
Fatty acid analysis of MBr1 antigen glycolipid

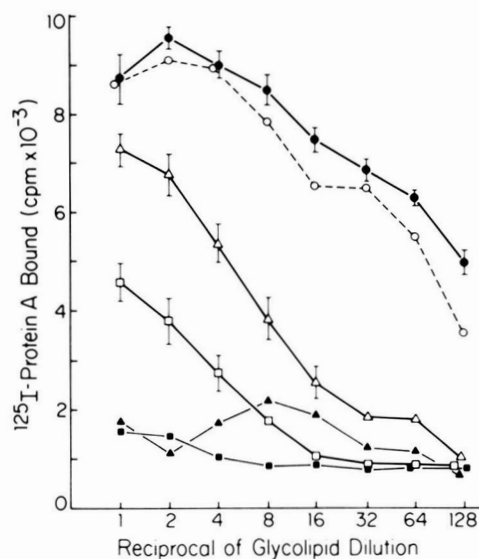|	%
14:0	7.3
16:1	4.0
16:0	33.4
17:0	0.7
18:2	1.1
18:1	14.1
18:1	5.1
18:0	13.5
20:0	0.4
22:1	0.3
22:0	1.7
24:1	12.8
24:0	4.1
h24:1	1.3
26:0	0.4
Total	100.2
% unsaturated	37.4
% saturated	61.5
% OH	1.3

FIG. 5. **The reactivity of the MBr1 antigen as compared with various other glycosphingolipids on solid-phase radioimmunoassay according to the procedure described by Kannagi *et al.* (13).** ●, MBr1 antigen; ○, GL6 ($IV^3Fuc\alpha1 \rightarrow 2Gal\beta Gb_4$) of human teratocarcinoma; △, fucosylasialo-G_{M1} (IV^2FucGg_4); □, GL5 ($IV^3\beta GalGb_4$) of human teratocarcinoma; ▲, α-galactosylfucosyl G_{M1} ($IV^2FucIV^3\alpha GalGg_4$) of rat hepatocytes; ■, H_1 glycolipid ($IV^2FucnLc_4$) of human erythrocytes.

to differences in the running conditions. The ions at *m/z* 253 and 364 are derived from octadecasphingenine, and that at *m/z* 396 has been attributed to 4-D-hydroxysphinganine (26). The most prominent fatty acid ions are at *m/z* 294 (16:0), *m/z* 404 and 406 (24:1 and 24:0), and *m/z* 320 and 322 (18:1 and 18:0). Ceramide B fragments containing C24:1 fatty acid in

combination with sphingenine and 4-D-hydroxysphinganine can be seen at m/z 658 and 690, respectively. A fragment at m/z 576 can be attributed to 18:0 fatty acid with sphingosine. Ceramide A fragments (with glycosyl moiety) are found at m/z 781, 749 (781-32) (18:0 fatty acid with sphingenine) and at m/z 831 (863-32) (C24:1 fatty acid with sphingenine).

Fatty acid composition of the MBr1 antigen is shown in Table II. C16:0, C18:1, C18:0, and C24:1 represent the majority. It should be noted that the antigen contained significant amounts of unsaturated fatty acids, which is unusual for glycosphingolipids. In addition, fatty acid analysis is consistent with the predicted sphingosines from mass spectrometry.

Immunological Reactivity of MBr1 Antibody with Glycosphingolipids with Various Structures—TLC immunostaining with 1:500 dilutions of MBr1 antibody showed selective reactivity with GL6 glycolipid as well as the MBr1 glycosphingolipid antigen. The reactivity was further confirmed by solid-phase radioimmunoassay with various glycosphingolipids, and the results are shown in Fig. 5. The antibody reacted identically with equal molar amounts of GL6 isolated from human teratocarcinoma and the fraction 3 glycosphingolipid, thus confirming the structure as the true antigen. No reactivity was observed with type 1 or type 2 chain H_1 glycosphingolipid or α-galactosylfucosylasialo-G_{M1} ($IV^3\alpha GalIV^2\alpha FucGg_4$). A weak cross-reactivity was observed with "GL5" of human teratocarcinoma ($IV^3\beta GalGb_4$). A significant cross-reactivity was observed with fucosylasialo-G_{M1} (IV^2FucGg_4).

DISCUSSION

With the application of the monoclonal antibody approach, a few hitherto unknown glycosphingolipid structures have been disclosed as tumor-associated or developmentally regulated markers (1), such as di- or trifucosyl type 2 chain (21, 27), sialosyl-Le^a (28), and gangliosides with sialosyl $2\rightarrow6Gal$ linkage (29) or O-acetyl sialic acids (33). A large quantity of glycosphingolipids having Le^x (X), Le^a, and Le^b determinants have been found to accumulate in a large variety of human adenocarcinoma (30, 31). This could be due to the fact that many monoclonal antibodies which were prepared against human cancer cells and tissues and which have been claimed to be "tumor-specific" are in fact directed to Le^x, Le^a, and Le^b structures (32). In contrast to these antigens, which belong to lacto series, the MBr1 antigen defined by MBr1 antibody is now clearly identified as a member of the "extended globo series" (12).

The antigen defined by the monoclonal antibody MBr1 is not only present in human breast cancer cells, but is also present in normal mammary gland epithelia and in apocrine sweat glands (3). The distribution of this antigen in other normal and neoplastic human tissues, studied by the highly sensitive avidin-biotin-peroxidase technique (4), was quite complex but restricted to normal and malignant epithelia. The antigen has now been clearly identified as a unique globo-series glycosphingolipid having an H-like determinant at the terminus, which is identical to GL6 of human teratocarcinoma cells as previously characterized (12). The structure was identified by methylation analysis, NMR spectroscopy, direct probe mass spectrometry, and enzymatic degradation. The epitope structure recognized by the antibody, postulated by cross-reactivity of analogous glycolipids, was Fuc$\alpha 1\rightarrow$ $2Gal\beta 1\rightarrow3GalNAc\beta 1\rightarrow3$. It is of particular interest to note that the antibody does not cross-react with the normally present H antigen carried by lacto-series structure (Fuc$\alpha 1\rightarrow$ $2Gal\beta 1\rightarrow4/or3GlcNAc\beta 1\rightarrow3Gal$), although the terminal tri-

saccharides of MBr1 and type 1 chain H differ only in the configuration of the HexNAc 4-hydroxyl group (axial *versus* equatorial), thus establishing the importance of that orientation to the specificity of the antibody. In addition, the presence of globo-series H is limited in normal human tissue. Nevertheless, globo-series H is the major H antigen in human teratocarcinoma as previously described (12). It is possible that globo H is also present in human embryonic tissue and its synthesis is developmentally regulated. The H antigen carried by globo structure could be uniquely distinguishable from the H antigen carried by lacto structure, which is indicated by the lack of cross-reactivity by their respective antibodies. On the other hand, a degree of cross-reaction with ganglio-series H structure was observed, indicating a similarity which should be based on the common structure Fuc$\alpha 1\rightarrow$ $2Gal\beta 1\rightarrow3GalNAc$ between globo-H and ganglio-H. The ganglio-H structure is highly limited in normal human tissues, and has been found mainly in outer mammalian cells and tissues. Further systematic studies on blood group determinants carried by globo and ganglio series *versus* lacto series may bring us important information on the distribution of aberrant blood group antigens associated with human embryogenesis and oncogenesis.

REFERENCES

1. Hakomori, S., and Kannagi, R. (1983) *J. Natl. Cancer Inst.* **71**, 231–251
2. Köhler, G., and Milstein, C. (1975) *Nature (Lond.)* **256**, 495–497
3. Ménard, S., Tagliabue, E., Canevari, S., Fossati, G., and Colnaghi, M. I. (1983) *Cancer Res.* **43**, 1295–1300
4. Mariami-Costantini, R., Colnaghi, M. I., Leoni, F., Menard, S., Cerasoli, S., and Rilke, F. (1984) *Virchows Arch. Pathol. Anat. Physiol. Klin. Med.* **402**, 389–404
5. Canevari, S., Fossati, G., Balsari, A., Sonnino, S., and Colnaghi, M. I. (1983) *Cancer Res.* **43**, 1301–1305
6. Fogh, J., and Trempe, G. (1975) in *Human Tumor Cells in Vitro* (Fogh, J., ed) Plenum Press, New York
7. Tettamanti, G., Bonali, F., Marchesini, S., and Zambotti, V. (1973) *Biochim. Biophys. Acta* **296**, 160–170
8. Yu, R. K., and Ledeen, R. W. (1972) *J. Lipid Res.* **13**, 680–686
9. Hakomori, S. (1981) *Semin. Hematol.* **18**, 39–62
10. Watanabe, K., and Arao, Y. (1981) *J. Lipid Res.* **22**, 1020–1024
11. Kannagi, R., Nudelman, E., Levery, S. B., and Hakomori, S. (1982) *J. Biol. Chem.* **257**, 14865–14874
12. Kannagi, R., Levery, S. B., Ishigami, F., Hakomori, S., Shevinsky, L. H., Knowles, B. B., and Solter, D. (1983) *J. Biol. Chem.* **258**, 8934–8942
13. Magnani, J. L., Smith, D. F., and Ginsburg, V. (1980) *Anal. Biochem.* **109**, 399–402
14. Kannagi, R., Stroup, R., Cochran, N. A., Urdal, D. L., Young, W. W., Jr., and Hakomori, S. (1983) *Cancer Res.* **43**, 4997–5005
15. Hakomori, S. (1964) *J. Biochem. (Tokyo)* **55**, 205–208
16. Stellner, K., Saito, H., and Hakomori, S. (1973) *Arch. Biochem. Biophys.* **155**, 464–472
17. Ledeen, R. W., Kundu, S. K., Price, H. C., and Fong, J. W. (1974) *Chem. Phys. Lipids* **13**, 429–446
18. Karlsson, K.-A., Pascher, I., Pimlott, W., and Samuelsson, B. E. (1974) *Biomed. Mass Spectrom.* **1**, 49–56
19. McNeil, M., and Albersheim, P. (1977) *Carbohydr. Res.* **56**, 239–248
20. Laine, R. A. (1980) in *Twenty-Seventh International Congress of Pure and Applied Chemistry* (Varmavuori, A., ed) pp. 193–198, Pergamon Press, New York
21. Hakomori, S., Nudelman, E., Levery, S. B., and Kannagi, R. (1984) *J. Biol. Chem.* **259**, 4672–4680
22. Dabrowski, J., Hanfland, P., and Egge, H. (1980) *Biochemistry* **19**, 5652–5658
23. Ernst, R. R. (1966) *Adv. Magn. Reson.* **2**, 1–135
24. Schwarting, G. A., Carroll, P. G., and DeWolf, W. C. (1983) *Biochem. Biophys. Res. Commun.* **112**, 935–940
25. Williams, M. A., and McCluer, R. H. (1980) *J. Neurochem.* **35**, 266–269
26. Egge, H., and Hanfland, P. (1981) *Arch. Biochem. Biophys.* **210**, 396–404
27. Fukushi, Y., Hakomori, S., Nudelman, E., and Cochran, N. (1984) *J. Biol. Chem.* **259**, 4681–4685
28. Magnani, J. L., Nilsson, B., Brockhaus, M., Zopf, D., Steplewski, Z., Koprowski, H., and Ginsburg, V. (1982) *J. Biol. Chem.* **257**, 14365–14369
29. Hakomori, S., Patterson, C. M., Nudelman, E., and Sekiguchi, K. (1983) *J. Biol. Chem.* **258**, 11819–11822
30. Yang, H., and Hakomori, S. (1971) *J. Biol. Chem.* **246**, 1192–1200
31. Hakomori, S., and Andrews, H. (1970) *Biochim. Biophys. Acta* **202**, 225–228
32. Brockhaus, M., Magnani, J. L., Herlyn, M., Blaszczyk, M., Steplewski, Z., Koprowski, H., and Ginsburg, V. (1982) *Arch. Biochem. Biophys.* **217**, 647–651
33. Cheresh, D. A., Varki, A. P., Varki, N. M., Stallcup, W. B., Levine, J., and Reisfeld, R. A. (1984) *J. Biol. Chem.* **259**, 7453–7459