Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 5392–5396, September 1983
Immunology

Ganglioside GM2 as a human tumor antigen (OFA-I-1)

(monospecific human antitumor antibody/membrane glycolipid/fetal brain/melanoma)

TADASHI TAI*, JAMES C. PAULSON†, LESLIE D. CAHAN‡, AND REIKO F. IRIE*§

Departments of *Surgery/Oncology, †Biological Chemistry, and ‡Surgery/Neurosurgery, University of California, Los Angeles, School of Medicine, Los Angeles, California 90024

Communicated by S. Hagiwara, May 23, 1983

ABSTRACT **A monospecific antibody produced *in vitro* by a B-lymphoblastoid cell line transformed with Epstein–Barr virus has been shown to recognize a membrane antigen (OFA-I-1) on human tumors and fetal brain. This study identifies the chemical nature of OFA-I-1. The glycolipid fraction of antigen-rich spent medium of an OFA-I-1-positive melanoma cell line, M14, was extracted by chloroform/methanol/water, 4:8:3 (vol/vol), and was separated into fractions of neutral glycolipids and gangliosides by DEAE-Sephadex followed by base treatment and Bio-sil A column elution. OFA-I-1 antigens were found exclusively in the ganglioside fraction when assayed with monospecific anti-OFA-I-1 by an immune adherence inhibition test. The results obtained from thin-layer chromatography of the antigenic M14 ganglioside and sequential glycosidase digestion suggested that the antigen was a ganglioside GM2, GalNAcβ1→4(NeuAcα2→3)Galβ1→4Glc→Cer. These results were further supported by testing various authentic gangliosides and neutral glycolipids for OFA-I-1 antigenicity. Only GM2 showed positive reactivity.**

Specific monoclonal antibodies have been used as precise reagents to identify and characterize the molecular properties of various types of membrane antigens of human tumor cells (1–5). Mouse monoclonal antibodies made by hybridoma technology are most commonly used in such studies. However, tumor-associated antigens defined by xenoantibodies may not be as relevant for the recognition of antigens that have immunogenicity in man as human antibodies. During the last several years, we have investigated cell surface antigens that induce immune responses in cancer patients (6–8). In 1975, we described an antigen expressed on a variety of human tumors that reacted with serum antibodies of cancer patients (9). The antigen was named oncofetal antigen-immunogenic (OFA-I) because it was expressed on both tumors and fetal normal brain (10). However, human serum antibodies, because they are polyclonal and contain a relatively low level of specific antibodies, are generally quite difficult to use as reagents for identification of the precise chemical structure of tumor cell antigens, particularly for distinguishing between various subsets of closely related antigenic determinants.

Recently, we produced human monoclonal antibodies *in vitro* against OFA-I by transforming human B lymphocytes with Epstein–Barr virus (11). This technology enabled us to define two different antigenic specificities on the OFA-I target cells. One antigen, OFA-I-2, is expressed only on tumors of neuroectodermal origin, whereas the second, OFA-I-1, is found on a wide variety of human cancer cells, including melanomas, brain tumors, sarcomas, breast carcinomas, and tumors of several other types. The chemical structure of OFA-I-2 has been identified as the ganglioside GD2 [GalNAcβ1→4(NeuAcα2→8NeuAcα2→3)Galβ1→4Glc→Cer] (12). In this report, we describe the chemical nature of OFA-I-1 that binds to monospecific antibody OFA-I-1 produced *in vitro*. By using immune adherence inhibition as the assay, the antigen was successfully purified and characterized as the ganglioside GM2.

MATERIALS AND METHODS

OFA-I-1 Antigen Source. A human melanoma cell line, UCLA-SO-M14 (M14), which expresses OFA-I-1 and OFA-I-2 and sheds both antigens into the culture medium, was maintained in a chemically defined medium supplemented with 0.05% human serum albumin as described (12). Spent cell culture medium of this cell line was collected in plastic flasks when monolayers were >80% confluent. As a source of soluble, crude antigen, this medium was centrifuged at 1,000 × g for 10 min to remove cell debris and was concentrated 100-fold with an Amicon filter.

Fetal Tissue. Human fetal liver and brain tissue were prepared as described (8). Adult brain was obtained from postmortem specimens. Tissues were frozen at −80°C until needed for chemical analysis.

Monospecific Antibody to OFA-I-1. Anti-OFA-I-1 antibody secreted into the spent tissue culture medium of a human lymphoblastoid cell line (L55) was used as the source of antibody. Its immunologic specificities have been described (11).

Antigen Assay. The reactivity of OFA-I-1 antibody with soluble antigen was determined by inhibition of immune adherence (7). In brief, serially diluted antigen or test fractions (50 μl) and 50 μl of anti-OFA-I-1 antibody were mixed and incubated at 4°C overnight. After incubation, 2.5×10^4 M14 cells in 25 μl of barbital-buffered saline were added to the antigen/antibody mixture, and the immune adherence assay was performed.

Glycolipids. Purified human brain gangliosides (GM3, GM1, GD1a, GD1b, GT1b) were generously provided by Lars Svennerholm (University of Goteborg, Sweden). The GD3 of human brain was a gift from John Magnani and Victor Ginsburg (National Institutes of Health, Bethesda, MD). GD2 and GM2 were prepared from GD1b and GM1, respectively, by using bovine testes β-galactosidase as described (12). LacCer, $GgOs_3Cer$, and $GgOs_4Cer$ were prepared from GM3, GM2, and GM1, respectively, by treatment with mild acid hydrolysis

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Abbreviations and nomenclature of neutral glycolipids are according to the recommendation of the Nomenclature Committee, International Union of the Pure and Applied Chemistry (31). GlcCer = Glc→Cer; LacCer = Galβ1→4Glc→Cer; $GbOs_3Cer$ = Galα1→4Galβ1→4Glc→Cer; $GbOs_4Cer$ = GalNAcβ1→3Galα1→4Galβ1→4Glc→Cer; $GgOs_3Cer$ = GalNAcβ1→4Galβ1→4Glc→Cer; $GgOs_4Cer$ = Galβ1→3GalNAcβ1→4Galβ1→4Glc→Cer. Abbreviations of the ganglio series gangliosides are according to the system of Svennerholm (32). As an example, the structure of GT1b is NeuAcα2→3Galβ1→3GalNAcβ1→4(NeuAcα2→8NeuAcα2→3)Galβ1→4Glc→Cer. Other ganglioside structures are given in representational form in Fig. 3. OFA-I, oncofetal antigen-immunogenic.

§ To whom reprint requests should be addressed.

(1% acetic acid at 100°C for 1 hr). The products were later purified by TLC in solvent 1. GlcCer was prepared from GM3 by using sialidase and jack-bean β-galactosidase as described below. $GbOs_3Cer$ and $GbOs_4Cer$ were purchased from Supelco (Bellefonte, PA).

TLC. Merck precoated high-performance TLC plates (silica gel 60, 200 μm in thickness) were employed for fractionation of the glycolipids. Solvents used for developing the chromatograms were composed of chloroform/methanol/0.22% $CaCl_2$, 55:45:10 (vol/vol) (solvent 1), and chloroform/methanol/water, 65:25:4 (vol/vol) (solvent 2). Gangliosides were visualized with resorcinol stain (13) or orcinol stain (14), and neutral glycolipids were visualized with orcinol stain. Polypeptides were visualized by fluorescamine spray (Supelco) (15). TLC plates precoated with silica gel 60 (250 μm in thickness, Merck) were employed when a preparative isolation of the gangliosides was required.

Isolation and Purification of Glycolipids. Glycolipids were extracted from the OFA-I-1-rich M14 spent medium by using the method of Svennerholm and Fredman (16) and separated into neutral and acidic fractions by DEAE-Sephadex chromatography (17). Acidic glycolipids were further purified by base treatment and passage over a Bio-sil A column. Briefly, concentrated M14 spent medium (20 ml) was mixed with 54 ml of methanol at room temperature under constant stirring before 27 ml of chloroform was added. The mixture was stirred for 30 min and was centrifuged at 3,000 × *g* for 30 min. The supernatant was clarified by filtration. The precipitate was reextracted and the two extracts were combined and evaporated to dryness. The sample was dissolved in 2 ml of water, dialyzed overnight at 4°C against water, lyophilized, and dissolved in chloroform/methanol/water, 30:60:8 (vol/vol). The precipitate was removed by centrifugation, and the supernatant was applied to a column (7 ml) of DEAE-Sephadex A-25, equilibrated and washed with 200 ml of chloroform/methanol/water, 30:60:8 (vol/vol), to elute neutral glycolipids. The fraction containing gangliosides was then eluted with 200 ml of chloroform/methanol/0.8 M sodium acetate, 30:60:8 (vol/vol). The eluate was evaporated to dryness, dialyzed against water, and lyophilized. The ganglioside fraction was further treated with 0.1 M NaOH in methanol for 3 hr at 37°C, then dialyzed against cold water, evaporated, and dissolved in chloroform/methanol, 4:1 (vol/vol). This solution was applied to a column (5 ml) of Bio-sil A, equilibrated and washed with 100 ml of chloroform/methanol, 4:1 (vol/vol). Gangliosides were eluted with 100 ml of chloroform/methanol, 1:2 (vol/vol). The isolation of gangliosides from brain and liver tissues was carried out in a similar manner. Final purification of the gangliosides was achieved by preparative TLC. After development with solvent 1, the silica gel was scraped off the plate in 2-mm-wide sections and gangliosides were extracted from the silica by sonication in chloroform/methanol/water by using the method of Ledeen and Yu (17).

Carbohydrate Analysis. Lipid-bound sialic acid was determined after hydrolysis in 50 mM H_2SO_4 at 80°C for 1 hr by the thiobarbituric acid assay (18). Gangliosides were visualized with resorcinol stain on TLC and the content of each component ganglioside was determined by densitometric scanning (17, 19).

Enzyme Treatment of OFA-I-1. The OFA-I-1 ganglioside purified by the TLC plate was subjected to sequential degradation by using various glycosidases. Neuraminidase of *Arthrobacter ureafaciens* was purchased from Calbiochem–Behring. Jack-bean β-*N*-acetylhexosaminidase and β-galactosidase were from Sigma. β-Galactosidase of bovine testes was a gift from George W. Jourdian (University of Michigan) (20). The following enzyme reactions were performed with the addition of sodium taurocholate (Sigma) at a 3.0 molar ratio of detergent to substrate and was incubated at 37°C overnight. After the reaction with an enzyme, products were visualized on TLC in solvent 2, unless otherwise noted. One unit of glycosidase was defined as the amount of enzyme required to hydrolyze 1 μmol of the substrate per min.

OFA-I-1 (120 nmol) was incubated with 2 units of *A. ureafaciens* sialidase in 0.05 M sodium acetate buffer (pH 4.5) to a total volume of 2.0 ml at 37°C for 2 days (21). Asialo-OFA-I-1 (60 nmol) was incubated with 2 units of N-*acetyl*-β-hexosaminidase of jack bean in 0.05 M sodium citrate buffer (pH 5.0) (400 μl) (22). After the removal of one sialic acid and one *N*-acetylhexosamine, OFA-I-1 (33.3 nmol) was incubated with 2 units of jack-bean β-galactosidase in 0.05 M sodium citrate buffer (pH 3.5) (480 μl) (22).

RESULTS

Detection of OFA-I-1 in Ganglioside Fraction of M14 Cells. In a previous study, the chloroform/methanol/water extraction of OFA-I-2 (ganglioside GD2) from M14 cells that expressed both OFA-I-1 and OFA-I-2 antigens suggested that OFA-I-1 was a glycolipid (12). Although the OFA-I-1 antigen was detected in the filtrate after the chloroform/methanol/water extraction of the cells, its antigenicity could not be destroyed by treatment with sialidase alone. However, in subsequent studies we found that OFA-I-1 could be completely destroyed by sialidase treatment in the presence of a detergent, sodium taurocholate, an indication that it was a ganglioside which contained sialic acid attached to the inner galactose of a ganglioside (23).

To confirm the possibility that OFA-I-1 was a ganglioside, M14 cell gangliosides were isolated from the M14 spent medium as described in *Materials and Methods*. All fractions obtained by this isolation process, including precipitated proteins and neutral glycolipids, were tested for OFA-I-1 antigenic activity. Only fractions containing gangliosides were positive. The recovery of antigen in the final purified ganglioside fraction ranged from 30% to 80%. The antigenic determinant(s) of OFA-I-1 appeared to be carbohydrate related, because (*i*) heat treatment (100°C, 5 min) did not change the antigenicity, (*ii*) chemical *O*-acetylation (24) destroyed the antigen, and (*iii*) periodate treatment (25) also eliminated the antigenicity.

Isolation of OFA-I-1 from the Ganglioside Fraction. The ganglioside fraction was fractionated by TLC to separate each component ganglioside from the M14 cells. The M14 cell gangliosides had four prominent bands on the TLC corresponding to the standard gangliosides GM3, GM2, GD3, and GD2, as shown in Fig. 1. Each ganglioside appeared as a doublet. Antigen activity of OFA-I-1 was determined from an unstained sample in an adjacent lane of the same chromatogram that was divided into 2-mm-wide sections and scraped from the plate. Antigenic activity was detected only in the fractions that comigrated with the standard GM2 in the two peaks corresponding to doublet bands (Fig. 1). The antigen-positive doublet bands were isolated from the TLC, rechromatogramed, and tested for the involvement of polypeptides in the purified antigen. No visible band was detected in the area of the antigen GM2 after staining with fluorescamine spray, suggesting that the antigenic carbohydrate was not an amphipathic glycopeptide. Thus, OFA-I-1 was tentatively identified as ganglioside GM2. The chromatographic doublet was presumed to be caused by the ceramide moiety, as has been reported by other investigators (26).

Sequence Analysis of OFA-I-1 with Glycosidases. The OFA-I-1 ganglioside was subjected to sequential glycosidase digestion and the reaction mixtures at each step were analyzed on

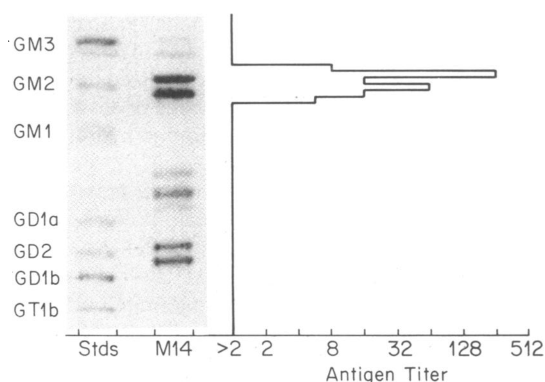


FIG. 1. Detection of OFA-I-1 in the ganglioside fraction of M14 cells. Selected ganglioside standards (Stds) and gangliosides from M14 cells were developed on TLC in solvent 1. One of two lanes containing duplicate samples of M14 ganglioside was sectioned and assayed for OFA-I-1 by inhibition of monospecific anti-OFA-I-1 binding to M14 cells by immune adherence with serially diluted antigen. Gangliosides in the remaining lanes were visualized by the resorcinol stain. Antigen titer is expressed as the reciprocal of the dilution that gave 50% inhibition of immune adherence.

TLC. When OFA-I-1 was incubated with *A. ureafaciens* sialidase in the presence of sodium taurocholate, it comigrated with standard $GgOs_3Cer$ (asialo GM2), as shown in Fig. 2. In the absence of detergent, no changes in the migration were observed. This result was consistent with OFA-I-1 being a ganglio series ganglioside, because bacterial sialidase cannot cleave the sialic acid from a branched galactose of this ganglioside (21, 23). Asialo-OFA-I-1 was incubated with jack-bean β-*N*-acetylhexosaminidase. The reaction mixture comigrated with authentic LacCer, indicating that one *N*-acetylhexosamine had been removed from the asialo-OFA-I-1. The remaining disaccharide glycolipid was further digested with jack-bean β-galactosidase.

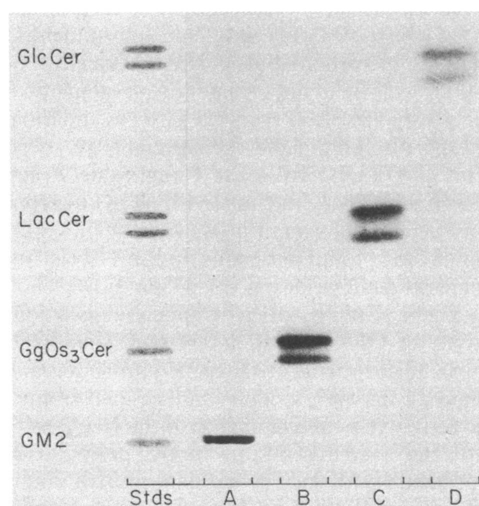


FIG. 2. Sequence analysis of OFA-I-1 with glycosidases. The ganglioside doublet recognized as an antigen by anti-OFA-I-1 was isolated by preparative TLC. This sample was subjected to sequential glycosidase digestion and the reaction mixtures at each step were analyzed on TLC in solvent 2. Glycolipids were stained with orcinol. Lane A, purified OFA-I-1; lane B, OFA-I-1 incubated with *A. ureafaciens* sialidase in the presence of sodium taurocholate; lane C, the reaction product from lane B after incubation with jack-bean β-*N*-acetylhexosaminidase; lane D, the reaction product C after treatment with jack-bean β-galactosidase. Standard glycolipids (Stds) were chromatographed with each product (lanes A–D) separately.

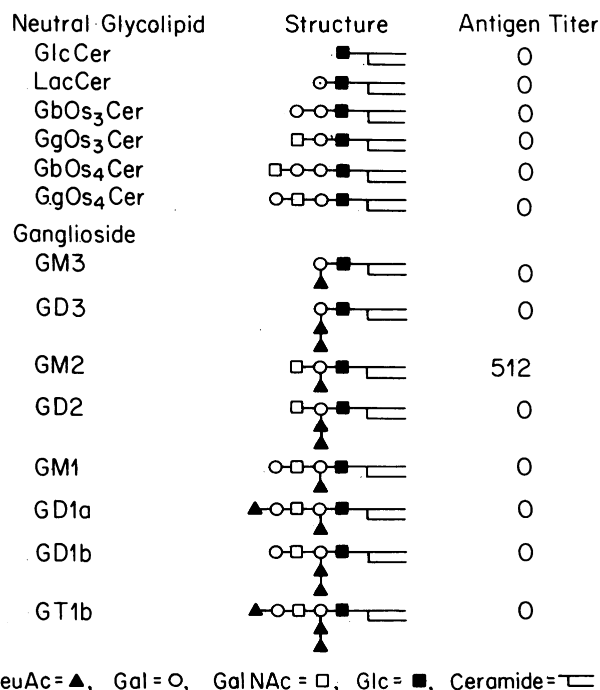
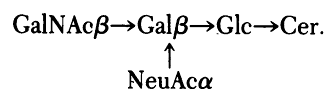


FIG. 3. Reactivity of anti-OFA-I-1 antibody with various glycolipids. Glycolipids (≈6 nmol) were serially diluted in phosphate-buffered saline and tested for inhibition of anti-OFA-I-1 binding to M14 cells in the immune adherence assay. Antigen titer is given as the reciprocal of the dilution that gave 50% inhibition of rosette formation (0 = <2).

This final reaction product then migrated with standard GlcCer, an indication that one galactose had been cleaved from the disaccharide glycolipid. These results are consistent with the carbohydrate structure of ganglioside GM2:



Reactivity of Anti-OFA-I-1 Antibody with Various Authentic Glycolipids. Authentic glycolipids (6 nmol), including GM2 and 14 other acidic glycolipids and neutral glycolipids, were tested for OFA-I-1 antigen activity by using anti-OFA-I-1 antibody in the immune adherence inhibition test (Fig. 3). Only ganglioside GM2 showed antigenic activity. GD2, identified as OFA-I-2, was completely negative for OFA-I-1 antigenic activity. It is noteworthy that the structural difference between GM2 and GD2 involves only one sialic acid residue. Other glycolipids that only differed from GM2 by the addition of one galactose (GM1) or the loss of one *N*-acetylgalactosamine (GM3) or sialic acid ($GgOs_3Cer$) were also devoid of antigen activity.

GM2 in Human Adult Brain, Fetal Brain, and Fetal Liver. Initially, OFA-I-1 was defined as an antigen present on human cancer cells and fetal brain tissues (7, 8). The antigen was not detected in fetal liver. To verify OFA-I-1 as a fetal brain-associated antigen, the ganglioside patterns of human adult brain, fetal brain, and fetal liver were analyzed by TLC. The ganglioside fractions from these organs were extracted and compared after separation by TLC. GM2 was identified as one of the major gangliosides in fetal brains as shown in Fig. 4. The proportion of GM2 in fetal brains was 13.4% of total sialic acid as calculated by densitometric scanning. In contrast, the proportion of GM2 in fetal liver and adult brain was only 2.4% and 3.6%, respectively. GM3 (92.4%) was the prominent ganglioside in fetal liver.

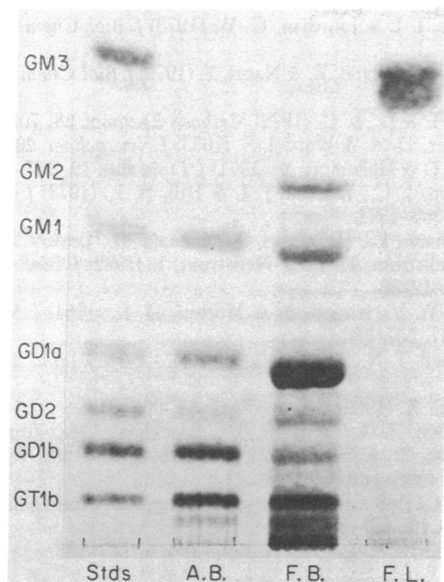


FIG. 4. Gangliosides of human adult and fetal brains and fetal liver. Each sample that contained 10 μg of sialic acid was developed on TLC in solvent 1 and stained with resorcinol. Stds, gangliosides as standards; A.B., adult brain; F.B., fetal brain; F.L., fetal liver.

DISCUSSION

The present study describes the molecular property of a membrane antigen that is reactive with a human monospecific antibody to OFA-I-1 produced *in vitro* (11). The antigen was detected by the antibody on various types of human tumors. Based on the results of our investigation, we concluded that OFA-I-1 is the ganglioside GM2, because (*i*) the OFA-I-1 ganglioside comigrates with authentic GM2 in TLC; (*ii*) results obtained by sequential cleavage of OFA-I-1 with glycosidases are those predicted for GM2; and (*iii*) anti-OFA-I-1 antibody reacts only with authentic GM2 but with no other acidic or neutral glycolipid analog tested.

In our previous studies, we identified the chemical nature of a membrane antigen that was reactive with a human monospecific antibody to OFA-I-2 as the ganglioside GD2 (12). The two monospecific antibodies not only recognize either cell membrane GM2 or GD2 but clearly distinguish the difference of one sialic acid residue in the one ganglioside (see Fig. 3), suggesting the crucial importance of the development of monoclonal antibodies for precise characterization of antigen determinants and for specific clinical application in treatment and diagnosis of cancer.

OFA-I-1 is similar to the OFA-I that we initially described, because it is widely distributed on human tumor, fetal brain, and cultured fibroblasts (7, 8, 27). In those earlier studies, we used an immunologic assay to define OFA-I as follows: (*i*) the antigen was expressed on human tumor but was not expressed on noncancerous adult tissues; (*ii*) a crossreactive antigen was expressed by fetal brain tissues but not by fetal liver from the same fetus; and (*iii*) the antigen was immunogenic in man and was detectable by human serum antibody. To verify this immunologic definition biochemically, the quantity of GM2 in fetal brain and fetal liver was compared. In fetal brain, GM2 was detected as one of the four major gangliosides (13.4%). In contrast, fetal liver contained only 2.4% of GM2 compared to the total liver gangliosides. Because the difference in ganglioside content between liver and brain was more than 1:10 (19, 28), the amount of GM2 in fetal liver was estimated to be less than 2% of fetal brain. The immunologic assay used in our earlier study could not detect this small level of OFA-I-1 in fetal liver. However, in the ganglioside-rich tissue of adult brain (twice that of fetal brain), the GM2 content was 3.6% of the total brain gangliosides. Our immunologic assays have detected OFA-I in some adult brain tissues, but not in every case (7, 27). According to Ando *et al.* and Suzuki, the amount of GM2 in adult brain varies with the area or the tissues being studied (19, 29). For example, the content of GM2 in grey matter is 6 times greater than that of white matter. Alternatively, the differences in the ceramide portion of GM2 ganglioside in fetal and adult brains may be responsible for the binding of the anti-OFA-I-1 antibody. Recently, Nudelman *et al.* reported that ganglioside GD3 obtained from malignant melanoma contained a different ceramide composition than that of brain and suggested that the ceramide portion might influence the antigen activity of the carbohydrate structure of the ganglioside (26). Although GM2 seems to be preferentially synthesized by fetal brain and tumor tissues, the synthesis is not exclusive to such tissues. Mechanisms of GM2 biosynthesis and its significance in malignant cells remain to be studied.

Several mouse monoclonal antibodies that recognize gangliosides on human tumor cells have recently been described (3, 5, 26, 30). Magnani *et al.* (3, 30) identified a colon carcinoma antigen as a fucosylated ganglioside [NeuAcα2→3Galβ1→3(Fucα1→4)GlcNAcβ1→3Galβ1→4Glc→Cer]. Pukel *et al.* (5) found a monoclonal antibody against human melanoma cells that recognized ganglioside GD3 (NeuAcα2→8NeuAcα2→3Galβ1→4Glc→Cer). Nudelman *et al.* (26) also reported a monoclonal antibody produced against the same human melanoma cell line as Pukel *et al.* (above) that binds to GD3. These antigen/antibody systems may be useful for imaging tumors *in vivo* in cancer patients or for detecting circulating gangliosides secreted by tumor cells or early diagnosis of cancer recurrence. In contrast, the gangliosides identified by our human monospecific antibodies (GM2 and GD2) could induce immune responses in the autochthonous host, as evidenced by the fact that lymphocytes from cancer patients produced antibodies to these gangliosides without any additional foreign immune stimulation. Because of their immunogenic capacity to induce IgM antibodies that are cytotoxic to tumor cell with complement, these antigens may be useful for treatment of cancer patients via immunization. Future efforts must be directed towards the induction of anti-GM2 antibodies in man by combining purified GM2 with a highly immunogenic adjuvant. If these efforts are successful, then active specific immunotherapy with OFA-I-1, which is expressed on many types of human cancer cells, may become a most powerful weapon against the metastatic spread of human cancer cells.

We thank Drs. Donald L. Morton, Eugene W. Stern, and Romaine E. Saxton for their helpful discussion and suggestions. We also thank Mrs. Lan Sze and Mrs. Estella Farmatiga for their technical assistance and Ms. E. Jane Shaw for her editorial assistance. This work was supported by Grants CA00543, CA30647, and CA12582, awarded by the National Cancer Institute. J.C.P. is a recipient of an American Cancer Society Faculty Research Award; L.D.C. is a recipient of National Institute of Neurological and Communicative Disorders and Stroke Teacher Investigator Development Award NS00544; and R.F.I. is a recipient of a National Cancer Institute Research Career Development Award.

1. Mitchell, K. F., Fuhrer, J. P., Steplewski, Z. & Koprowski, H. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7287–7291.
2. Woodbury, R. C., Brown, J. P., Yeh, M. Y., Hellstrom, L. & Hellstrom, K. E. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2183–2187.
3. Magnani, J. L., Brockhaus, M., Smith, D. F., Ginsburg, V., Blaszczyk, M., Mitchell, K. F., Steplewski, Z. & Koprowski, H. (1981) *Science* **212**, 55–56.
4. Bumol, T. F. & Reisfeld, R. A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1245–1249.

5. Pukel, C. S., Lloyd, K. O., Travassos, L. R., Dippold, W. G., Oettgen, H. T. & Old, L. J. (1982) *J. Exp. Med.* **155,** 1133–1147.
6. Irie, K., Irie, R. F. & Morton, D. L. (1974) *Science* **186,** 454–456.
7. Irie, R. F., Irie, K. & Morton, D. L. (1974) *Cancer Res.* **36,** 3510–3517.
8. Irie, R. F., Giuliano, A. E. & Morton, D. L. (1979) *J. Natl. Cancer Inst.* **63,** 367–373.
9. Irie, R. F. & Morton, D. L. (1975) *Proc. Am. Assoc. Cancer Res.* **16,** 171.
10. Irie, R. F. (1980) in *Serologic Analysis of Human Cancer Antigens*, ed. Rosenberg, S. (Academic, New York), pp. 493–513.
11. Irie, R. F., Sze, L. L. & Saxton, R. E. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5666–5670.
12. Cahan, L. D., Irie, R. F., Singh, R., Cassidenti, A. & Paulson, J. C. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7629–7633.
13. Svennerholm, L. (1957) *Biochim. Biophys. Acta* **24,** 604–611.
14. Svennerholm, L. (1956) *J. Neurochem.* **1,** 42–53.
15. Sherma, J. & Touchstone, J. C. (1974) *Anal. Lett.* **7,** 279–287.
16. Svennerholm, L. & Fredman, P. (1980) *Biochim. Biophys. Acta* **617,** 97–109.
17. Ledeen, R. W. & Yu, R. K. (1982) *Methods Enzymol.* **83,** 139–191.
18. Warren, L. (1959) *J. Biol. Chem.* **234,** 1971–1975.
19. Ando, S., Chang, N. C. & Yu, R. K. (1978) *Anal. Biochem.* **89,** 437–450.
20. Distler, J. J. & Jourdian, G. W. (1973) *J. Biol. Chem.* **248,** 6772–6780.
21. Saito, M., Sugano, K. & Nagai, Y. (1979) *J. Biol. Chem.* **254,** 7845–7854.
22. Li, Y. T. & Li, S. C. (1972) *Methods Enzymol.* **28,** 702–713.
23. Wegner, D. A. & Wardel, S. (1973) *J. Neurochem.* **20,** 607–612.
24. Saito, T. & Hakomori, S. (1971) *J. Lipid Res.* **12,** 257–259.
25. Paulson, J. C., Rearick, J. I. & Hill, R. L. (1977) *J. Biol. Chem.* **252,** 2363–2371.
26. Nudelman, E., Hakomori, S., Kannagi, R., Levery, S., Yeh, M. Y., Hellstrom, K. E. & Hellstrom, I. (1982) *J. Biol. Chem.* **257,** 12752–12756.
27. Rees, W. V., Irie, R. F. & Morton, D. L. (1981) *J. Natl. Cancer Inst.* **67,** 557–562.
28. Seyfried, T. N., Ando, S. & Yu, R. K. (1978) *J. Lipid Res.* **19,** 538–543.
29. Suzuki, K. (1965) *J. Neurochem.* **12,** 969–979.
30. Magnani, J. L., Nilsson, B., Brockhaus, M., Zopf, D., Steplewski, Z., Koprowski, H. & Ginsburg, V. (1982) *J. Biol. Chem.* **257,** 14365–14369.
31. IUPAC-IUB Commission on Biochemical Nomenclature (1977) *Lipids* **12,** 455–468.
32. Svennerholm, L. (1964) *J. Lipid Res.* **5,** 145–155.