Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 3333–3337, May 1990
Immunology

Human monoclonal antibody with dual GM2/GD2 specificity derived from an immunized melanoma patient

(cancer immunology/Epstein–Barr virus transformation/neuraminidase)

HIROSHI YAMAGUCHI, KOICHI FURUKAWA, SHEILA R. FORTUNATO, PHILIP O. LIVINGSTON, KENNETH O. LLOYD, HERBERT F. OETTGEN, AND LLOYD J. OLD

Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Contributed by Lloyd J. Old, January 2, 1990

ABSTRACT **GM2 ganglioside is a common cell surface constituent of human melanoma and other tumors of neuroectodermal origin, and vaccination with GM2 ganglioside results in high levels of anti-GM2 antibodies in patients with melanoma. Lymphocytes from a GM2-vaccinated patient (VS) were transformed by Epstein–Barr virus and tested for production of antibodies with reactivity for GM2-positive tumor cells. A high percentage of antibody-producing B cells was detected, but antibody reactivity was generally lost during culture expansion. Two cultures, however, remained stable for antibody productivity and one was used to develop a stable hybrid line with mouse myeloma. The monoclonal antibody (designated 3-207) derived from patient VS has dual specificity for GM2 and GD2, despite the fact that only GM2 antibody could be detected in the patient's serum. Monoclonal antibody 3-207 shows high-titered reactivity with a range of melanoma, astrocytoma, neuroblastoma, and leukemia cell lines, cells with prominent cell surface expression of GM2 and GD2. The cell surface reactivity of monoclonal antibody 3-207 was not abolished by treatment of target cells with neuraminidase, as the enzyme converted GD2 to GM2, which was still detected by monoclonal antibody 3-207.**

Ganglioside antigens have become the focus of much interest for cancer immunologists studying human malignant melanoma (1). Gangliosides are prominent constituents displayed on the surface of melanoma cells and a number of mouse monoclonal antibodies (mAbs) recognizing cell surface gangliosides have been generated and are being used in clinical trials (2–6). The analysis of the humoral immune reaction of melanoma patients, first with serum antibodies and then with human mAbs, has substantiated the important fact that gangliosides, such as GM2, GD2, and GD3,* are immunogenic in humans (8–14). This finding provides the rationale for the use of ganglioside vaccines in the immunotherapy of human melanoma. By following the strategy we have developed for the construction of melanoma vaccines, the immunogenicity of each vaccine is monitored by specific antibody response in vaccinated patients; only vaccines eliciting consistent and high-titered responses are tested in controlled trials for therapeutic benefit. The study of GM2 vaccines with bacillus Calmette–Guérin (BCG) as the adjuvant is furthest advanced (12). These studies have given us the opportunity to analyze the GM2 response at the clonal level, using Epstein–Barr virus (EBV) transformation of peripheral blood lymphocytes (PBLs) from patients with high serum titers of anti-GM2 antibody. A fine specificity analysis of one such mAb (3-207) is the subject of the present report.

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.

MATERIALS AND METHODS

Tissue Culture. The derivation and maintenance of melanomas and other cell lines were described (15). To remove heterologous fetal bovine serum (FBS) components, SK-MEL-93-II melanoma cells and CCRF-HSB-2 T-cell leukemia cells were grown in ITS medium (Collaborative Research).

GM2 and BCG Vaccines. Approximately 1×10^7 viable units of BCG (Tice strain, University of Illinois) were suspended in distilled water by sonication and added to tubes containing 100 μg of dried GM2. The suspension was lyophilized and resuspended in isotonic phosphate-buffered saline (PBS) shortly before vaccine administration (12).

EBV Transformation. PBLs from a patient were separated from heparinized venous blood by Ficoll/Hypaque (Pharmacia) gradient centrifugation. The B95-8 marmoset lymphoblastoid cell line (16) was grown in RPMI 1640 medium (containing 2 mM glutamine, 1% nonessential amino acids, penicillin at 100 units/ml, streptomycin at 1 μg/ml), and 10% (vol/vol) FBS. EBV-containing supernatants from this cell line were collected and passed through a 0.45-μm (pore size) filter and stored at −80°C. PBLs were adjusted to 2×10^6 cells per ml in RPMI 1640 medium containing 10% FBS and mixed with B95-8 supernatants at a ratio of 5:1 (vol/vol). After overnight incubation, cells were washed once and plated in 96-well plates (Falcon 3072). Cells containing antibodies reactive to cell surface antigens were expanded and later subcloned by limiting dilution.

Fusion Procedure. EBV-transformed B cells and mouse myeloma NS-1 (17) or 653 (18) cells were fused at ratios of 1:1 or 2:1 for 1.5 min at 37°C in 0.4 ml of 42% (wt/vol) polyethylene glycol (M_r, 3000–3700; J. T. Baker) containing 15% (vol/vol) dimethyl sulfoxide. After fusion, cells were washed once and resuspended in RPMI 1640 medium containing 15% FBS, 0.2 mM hypoxanthine, 0.4 μM aminopterin, and 32 μM thymidine (HAT medium). Cells were then plated at 2×10^5 cells per well into 96-well plates, preseeded with feeder layers prepared from BALB/c or C57BL/6 peritoneal cells ($1–3 \times 10^4$ cells per well). After overnight incubation, 100 μl of HAT medium with 20 μM ouabain (Sigma) was added to the wells. Cells were maintained in HAT medium for 2 weeks and then in RPMI 1640 medium containing 15% FBS, 0.2 mM hypoxanthine, and 32 μM thymidine.

Serological Assays. Immune adherence (IA) and protein A and absorption assays were performed as described (15, 19,

Abbreviations: mAb, monoclonal antibody; BCG, bacillus Calmette–Guérin; EBV, Epstein–Barr virus; FBS, fetal bovine serum; NeuAc, *N*-acetylneuraminic acid; NeuGc, *N*-glycolylneuraminic acid; IA, immune adherence; PBL, peripheral blood lymphocyte.

*Gangliosides are designated according to the nomenclature of Svennerholm (7). Unless otherwise stated, gangliosides contain sialic acid of the *N*-acetylneuraminic acid (NeuAc) type.

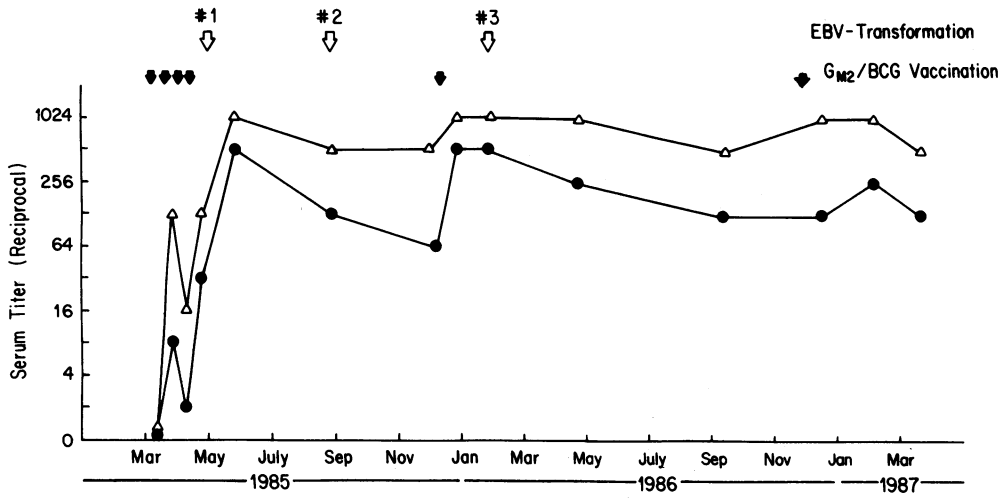

FIG. 1. Reactivity of sequential serum specimens from patient VS with cultured tumor cells. ●, SK-MEL-31 (melanoma); △, CCRF-HSB-2 (T-cell leukemia). The serological assay was IA. Titer refers to highest serum dilution showing 20% positive target cells. Solid arrows indicate vaccination with GM2 and BCG. Open arrows indicate EBV transformation.

20). Mouse mAbs 5-3 (IgM) and 3F8 (IgG3) were used to detect GM2 and GD2, respectively (4, 3). Neuraminidase treatment was performed as described (20), using neuraminidase from *Vibrio cholerae*, *Arthrobacter ureafaciens* (Calbiochem–Behring), and *Clostridium perfringens* (type X, Sigma).

Gangliosides. Gangliosides were extracted by chloroform/methanol and isolated as described (2). GM3 and GD3 were purified in our laboratory from dog erythrocytes and human melanoma cells, respectively. GM2 was prepared as described (4). GM1, GD1a, and GT1b were purchased from Supelco. GD2 was a gift from H. Wiegandt (University of Marburg, Marburg, F.R.G.). GD1b and *N*-glycolylneuraminic acid (NeuGc)-GM2 were provided by R. Yu (Yale University). The sialic acid concentration was determined by thiobarbituric acid assay.

Analysis of Reactivity with Gangliosides. Thin layer chromatography (TLC) was performed on plates coated with silica gel 60 (Merck) with chloroform/methanol/2.5 M NH_4OH, 60:35:8 (vol/vol). Gangliosides were visualized by a spray of resorcinol-hydrochloric acid. Immunostaining on TLC plates was done according to the method of Magnani (21) with modifications (22). ELISAs were performed as described (4). Antibody inhibition assays using gangliosides were carried out as described (13).

Neuraminidase Treatment of Gangliosides. The ganglioside mixture (approximately 20 μg) from neuroblastoma cell line IMR-32 was treated with 15 μl of 0.1 M sodium acetate (pH 4.9) with or without taurocholate (2 mg/ml; Sigma) and 15 μl of neuraminidase (500 units/ml) from *C. perfringens* or *A. ureafaciens* at 37°C for 48 hr. Enzyme (15 μl) was added at 12-hr intervals and taurocholate was also added to maintain the concentration of 1 mg/ml. After adding an equal volume of methanol, the reaction mixture was desalted on a C_{18} reverse phase column (Waters) and the products were analyzed by TLC–resorcinol spray and immunostaining.

RESULTS

Clinical History of Patient VS. Patient VS was a 35-year-old woman who was well until December 1984 when a Clark's level IV (2.9-mm depth) primary melanoma was removed from the left temple. A neck dissection performed at that time revealed 2 of 23 lymph nodes were positive for melanoma. Between March 12 and April 23, 1985, the patient received four immunizations at 2-week intervals of a vaccine consisting of 100 μg of GM2 plus 1×10^7 BCG injected on a rotating basis to extremities. Booster immunizations were administered on December 5, 1985, and December 16, 1986 (Fig. 1). The patient had a recurrence adjacent to the primary site, which was resected February, 1986, and then remained well until August 1987 when a bone scan was performed because of back pain. Multiple bone metastases were detected. The patient died in January 1988.

Reactivity of Sera from Patient VS. Two cell lines expressing high levels of GM2 were selected for monitoring the serum reactivity of GM2-vaccinated patients; SK-MEL-31 melanoma and CCRF-HSB-2 T-cell leukemia. Before immunization, VS serum showed no reactivity in IA (detecting IgM) or protein A (detecting IgG) assays. After immunization, there was a pronounced increase in IA reactivity for both cell lines, but no change in protein A reactivity. IA reactivity remained high over the 2-year observation period (Fig. 1).

The specificity of the serum antibody response was analyzed using purified gangliosides in an ELISA (Fig. 2) and TLC immunostaining (data not shown). Before immunization there was no reactivity with GM2, GD2, NeuGc-GM2, and GD1a. After immunization, VS sera showed strong reactivity with NeuAc-GM2 and NeuGc-GM2, but no detectable reactions with GD2 or GD1a.

EBV Transformation of PBLs from Patient VS. EBV transformation of PBLs from patient VS was carried out on three

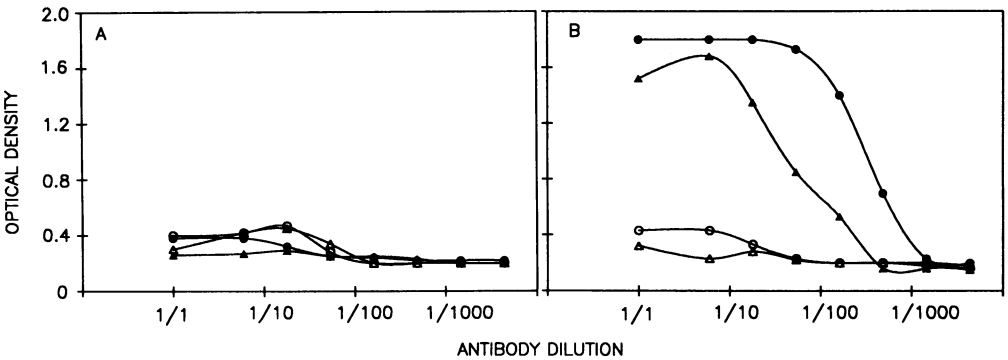

FIG. 2. Specificity analysis (ELISA) of sera from patient VS with purified gangliosides. ●, GM2; ▲, NeuGc-GM2; ○, GD2; △, GD1a. (*A*) Before immunization (March 1985). (*B*) After immunization (May 1985).

Table 1. EBV transformation of PBLs from melanoma patient VS after vaccination with GM2 and BCG

EBV transformation	No. of PBLs	No. of cells per well in 96-well plates	No. of positive wells/no. of wells screened	No. stable EBV-B-cell cultures
1	44×10^6	7.3×10^4	82/600 (13.7)	1
2	36×10^6	6.7×10^4	38/540 (7.0)	0
3	23×10^6	5.5×10^4	16/420 (3.8)	1 (EB3-207)

A positive well was a well with growing B cells having undiluted supernatants giving >50% rosette formation with SK-MEL-31 and/or CCRF-HSB-2 target cells. The serological assay was IA. Numbers in parentheses are percentages. EBV transformation 1 was done in May 1985, EBV transformation 2 was done in August 1985, and EBV transformation 3 was done in January 1986.

separate occasions: twice after the initial set of vaccinations and once after the first booster immunization (Fig. 1). As reported (13), T cells in PBLs were not eliminated and cells were plated at $5.5–7.3 \times 10^4$ cells per well in 96-well plates (Table 1). After 3–5 weeks, virtually all wells had vigorous cell growth. Supernatants were screened for IA reactivity using SK-MEL-31 and CCRF-HSB-2 as target cells. The highest percentage of positive wells (13.7%) was found with PBLs obtained 1 week after the fourth immunization, whereas subsequent transformations showed a lower percentage of positive wells. During expansion of positive cultures (from 96-well plates to 24-well plates and then subsequently at limiting dilution in 96-well plates), only two of the cultures continued to produce antibody. One of these (EB3-207) produced the highest titer of antibody and was, therefore, selected for further study.

Fusion of EB3-207 with Mouse Myeloma NS-1 or 653. To develop stable hybridoma lines with good cloning efficiency, EB3-207 cells were fused with mouse myeloma lines NS-1 or 653 (Table 2). In each of five fusions, hybridoma growth was detected, with a fusion frequency of 27–75 hybrids per 1×10^7 EB3-207 cells. The number of hybridoma-containing wells with IA-reactive antibody varied from 9% to 100%. One NS-1 hybrid and one 653 hybrid were selected for subcloning and two stable hybridomas, designated 3-207 (NS-1) and 3-207 (653), were established.

Specificity Analysis of Antibody Produced by EB3-207 and the 3-207 (NS-1) Hybrid and Comparison with Patient VS Serum. Table 3 summarizes typing results on an 83-cell panel. Without exception, antibodies produced by EB3-207 cells and the 3-207 (NS-1) hybrid cotyped on the panel. Reactivity was particularly strong for tumor cell lines of neuroectodermal origin. Only 3 of 24 cancer cell lines of epithelial origin showed reactivity. Absorption analysis with 3-207 (NS-1) and VS serum gave concordant results and, with few exceptions, confirmed the results of direct serological assays. Cell lines

Table 2. Fusion of EB3-207 with mouse myeloma NS-1 or 653

Fusion trial	No. of EB3-207 cells	No. of myeloma cells	No. of wells with hybridoma growth	No. of positive wells
1	40×10^6	$40 \times 10^{6*}$	114 (28.5)	40 (35.1)
2	40×10^6	$40 \times 10^{6*}$	186 (46.5)	52 (28.0)
3	20×10^6	$20 \times 10^{6*}$	150 (75.0)	150 (100.0)
4	27×10^6	$15 \times 10^{6\dagger}$	73 (27.0)	22 (30.1)
5	15×10^6	$15 \times 10^{6\dagger}$	44 (29.3)	4 (9.1)

For wells with hybridoma growth, numbers are number of wells with hybridoma growth per 1×10^7 EBV-B cells. Positive wells are wells with growing hybrids that have undiluted supernatants giving >50% rosette formation with SK-MEL-31 and/or CCRF-HSB-2 target cells. The serological assay was IA. The numbers in parentheses are percentages.
*NS-1 myeloma cells were used.
†653 myeloma cells were used.

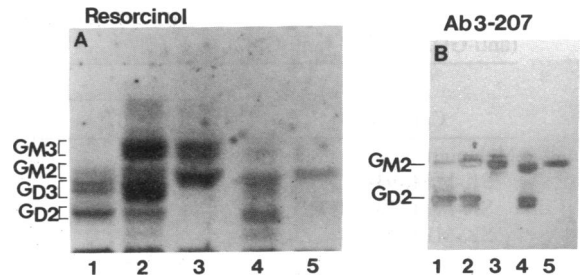

FIG. 3. TLC patterns of gangliosides (resorcinol spray) from five cancer cell lines (*A*) and immunostaining with mAb 3-207 (NS-1) (*B*). Lanes: 1, SK-MEL-31 (melanoma); 2, SK-MEL-173 (melanoma); 3, JB-RH (mouse melanoma); 4, IMR-32 (neuroblastoma); 5, SK-N-SH (neuroblastoma). A 1:5 dilution of supernatant was used.

reactive with 5-3, the mouse IgM mAb detecting GM2, were also positive with EB3-207, but many more cell lines were positive with EB3-207. No simple relation could be discerned between 3F8 and EB3-207 typing, except that the antigens detected by these antibodies were well represented on tumors of neuroectodermal origin. To rule out the contribution of heterologous serum components used in cell culture, SK-MEL-93-II and CCRF-HSB-2 cells were cultured in ITS medium; reactivity with EB3-207 was not significantly reduced. Pretreatment of target cells with neuraminidase from three separate sources did not change reactivity with EB3-207.

Immunochemical Analysis of the Antigen Detected by Human mAb 3-207. Total ganglioside fractions were prepared from five cell lines (two melanomas, one mouse melanoma, and two neuroblastomas) and fractionated by TLC (Fig. 3*A*). Various amounts of four gangliosides (GD2, GD3, GM2, and GM3) were evident. Immunostaining on TLC plates with the same samples showed that antibody 3-207 (NS-1) reacted strongly with GD2 and GM2 (Fig. 3*B*). The reactivity of antibody 3-207 (NS-1) with various purified gangliosides was tested by ELISA (Fig. 4). mAb 3-207 (NS-1) showed strong reactivity with GD2, GM2, and NeuGC-GM2. No reactions were seen with GM3, GD3, GM1, GD1b, GD1a, and GT1b. These ELISA reactions were confirmed using immunostaining of purified gangliosides separated by TLC (Fig. 5), and inhibition studies (data not shown). Reactivity was strongly inhibited by GD2 and NeuGc-GM2, inhibited less by NeuAc-GM2, and not inhibited by GM3, GD3, GM1, GD1b, GD1a, or GT1b.

Effect of Neuraminidase Treatment on the Reactivity of mAb 3-207 with Isolated Gangliosides. Isolated gangliosides were

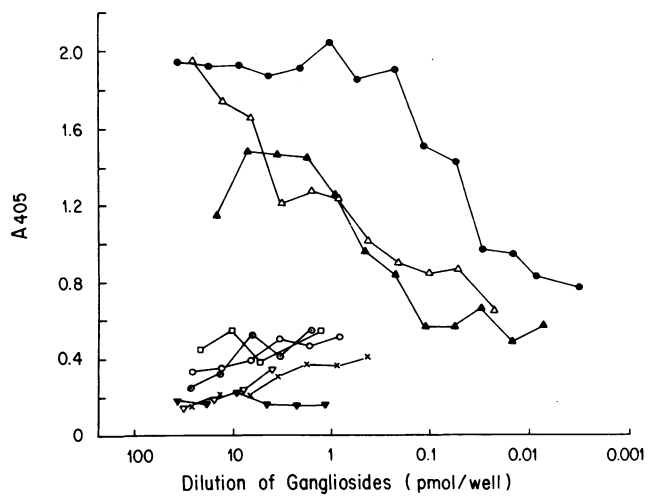

FIG. 4. Reactivity of EB3-207 with purified gangliosides in ELISA. ●, GD2; △, GM2; ▲, NeuGc-GM2; ○, GM3; □, GD3; ▽, GM1; ×, GD1b; ⊙, GD1a; ▼, GT1b. A 1:5 dilution of supernatant was used.

Table 3. Serological analysis of EB3-207, human mAb 3-207 (NS-1), patient VS serum, and mouse mAbs 3F8 (anti-GD2) and 5.3 (anti-GM2)

Cell line	Serological activity				
	EB 3-207*	3-207 (NS-1)*	VS serum†	3F8*	5-3*
Melanoma					
SK-MEL-93-II	512	128 (+)†	+	1024	−
SK-MEL-93-II (ITS)	256	32			
SK-MEL-31	256	128 (+)	+	32	512
SK-MEL-94	128	64 (+)	+	256	−
SK-MEL-173	64	16 (+)	+	512	±
SK-MEL-13	±	± (+)	+	1024	−
SK-MEL-23	±	± (+)	+	1024	−
SK-MEL-29	±	± (+)	−	128	−
SK-MEL-37	±	± (+)	+	512	−
SK-MEL-61	−	− (+)	+	64	−
SK-MEL-28	−	− (−)	−	64	−
SK-MEL-130	−	− (−)	−	±	−
SK-MEL-189	−	− (−)	−	256	−
MeWo	−	− (−)	−	64	−
Astrocytoma					
SK-MG-1	256	64 (+)	+	±	128
SK-MG-4	64	32 (+)	+	32	±
U-251-MG	64	16 (+)	+	128	2
SK-MG-6	−	− (−)	−	64	−
Neuroblastoma					
IMR-32	8000	2000 (+)	+	1024	512
SK-N-SH	8000	2000 (+)	+	±	1024
SK-N-BE(2)	8000	2000 (+)	+	±	1024
LA-N-1	1024	512 (+)	+	2000	1024
SMS-SAN	1024	512		2000	−
SK-N-MC	128	64 (+)	+	−	−
Leukemia					
K-562	32	16 (+)	+	−	±
HL-60	−	− (−)	−	−	−
CCRF-HSB-2	2000	1024 (+)	+	128	128
CCRF-HSB-2(ITS)	1024	256			
CCRF-CEM	−	− (−)	−	−	−
T-45	−	− (−)	−	−	−
P-12/Ichikawa	−	− (−)	−	−	−
NALL-1	−	− (+)	−	−	−
NALM-1	−	− (−)	−	−	−
BALL-1	1024	128 (+)	+	−	−
Daudi	−	− (−)	−	−	−
SK-LY-16	−	− (−)	−	−	−
Xenogeneic cells					
JB-RH	1024	128 (+)	+	−	1024
B16	128	64 (+)	+	−	±
Sheep erythrocytes	−	− (−)	−	−	−

The following cell lines were unreactive or very minimally reactive with the antibodies: renal cancer (SK-RC-6, -7, -8, -9, and -45); lung cancer (SK-LC-2, -6, -8, and -15); breast cancer (MDA-MB-231, -361, and MCF-7); colon cancer (HT-29, SW480, and SW1417); bladder cancer (T-24, 235J, and 5637); ovarian cancer (2774, A-10, and SK-OV-3); other cancers (CAPAN-2, ME-180, and GCC-SV), three EBV-transformed B-cell lines and cultured normal fibroblasts (n = 3), kidney epithelium (n = 4), and melanocytes (n = 3). PBLs (n = 3) and types A, B, AB, and O erythrocytes were unreactive.

*Data are reciprocal supernatant dilution showing 50% erythrocyte-rosette formation with target cells; −, no reaction at a dilution of 1:2; or ±, less than 50% reactivity at a 1:2 dilution. Data in parentheses are results of absorption tests.

†NS-1 hybrid supernatant and patient serum were absorbed with indicated cell types and tested for residual activity for SK-MEL-31 target cells. +, Complete absorption; ±, partial absorption; −, no absorption.

treated with neuraminidases and retested for reactivity with antibody. After treatment with neuraminidase from *C. perfringens*, with or without taurocholate, the GD2 band disappeared but the GM2 band persisted and could be detected with mAb 3-207 (data not shown). The neuraminidase from *A. ureafaciens* gave a different result. This neuraminidase, when used in the absence of taurocholate, abolished the GD2 band but did not reduce the GM2 band (Fig. 6*A*, lane 2). When taurocholate was added to the *A. ureafaciens* neuraminidase, the GM2 band also disappeared (Fig. 6*A*, lane 3). Immunostaining showed that mAb 3-207 reacted with GM2 after treatment with neuraminidase from *A. ureafaciens* in the absence of taurocholate but did not detect any gangliosides after the treatment with this neuraminidase in the presence of taurocholate (Fig. 6*B*, lane 3). These results indicate that *A. ureafaciens* neuraminidase in the presence of detergent cleaves the sialic acid of GM2, converting it to asialo-GM2 and that mAb 3-207 does not react with asialo-GM2.

DISCUSSION

The number of human mAb detecting ganglioside antigens is growing rapidly. Our group has isolated human antibodies

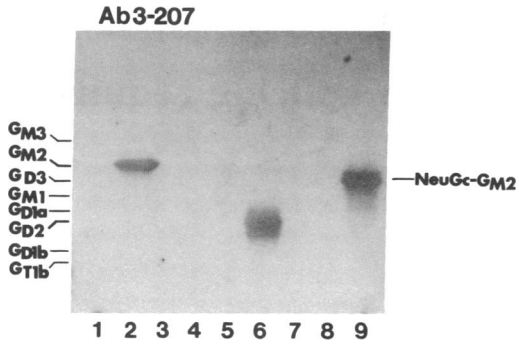

FIG. 5. TLC immunostaining of purified gangliosides with 3-207 (653). Lanes: 1, GM3; 2, GM2; 3, GD3; 4, GM1; 5, GDIa; 6, GD2; 7, GDIb; 8, GTIb; 9, NeuGc-GM2. Ganglioside amount was 0.2 nmol. A 1:5 dilution of supernatant was used.

recognizing GM3, GD3, and their NeuGc forms (13, 14, 23), and Irie and her colleagues (9, 10) have isolated antibodies detecting GM2 and GD2. Although these antibodies were isolated from patients with melanoma, the nature of the immunogenic stimulus that elicits them is unknown. Antibodies recognizing gangliosides can also be isolated from normal individuals, although it is too soon to say whether the frequency and specificity of ganglioside-reactive antibodies from normal individuals differ from those of melanoma patients. Techniques for analyzing the humoral immune response of humans to antigens such as gangliosides at the clonal level have improved greatly, although several technical problems remain that need resolution. One of the most vexatious is the loss of antibody reactivity that frequently accompanies the expansion of EBV-transformed cell cultures. Whether this is due to clonal loss of antibody secretion or to an overgrowth of unrelated B-cell clones is unknown. One strategy that we and others have used to capture and stabilize antibody-secreting EBV-transformed cells is hybridization with mouse fusion partners. In our past studies, we observed that the fusion frequency between EBV-transformed cells and NS-1 was quite low. In the present study, a much higher fusion frequency was observed, and we ascribed the difference to the addition of ouabain on the day after the fusion procedure rather than on the same day.

The human mAb 3-207 isolated from patient VS has exceptionally strong reactivity for neuroblastoma cells, and to a lesser degree, for astrocytoma and melanoma cells. This reactivity pattern undoubtedly reflects the dual specificity that mAb 3-207 has for GM2 and GD2, although the reactivity

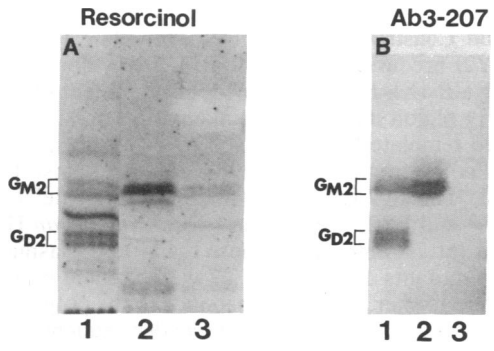

FIG. 6. Effect of *A. ureafaciens* neuraminidase on gangliosides. (*A*) Resorcinol spray. (*B*) Immunostaining. Lanes: 1, untreated gangliosides from IMR-32 neuroblastoma cells; 2, treated without taurocholate; 3, treated with taurocholate. A 1:5 dilution of EB 3-207 supernatant was used.

of this antibody with individual cell lines could not be predicted from the titers observed with 3F8 (anti-GD2) and 5-3 (anti-GM2) mouse mAbs. It may be that antibodies with dual specificity such as mAb 3-207 show synergistic complement-fixing properties. The dual specificity of mAb 3-207 for GM2 and GD2 distinguishes this antibody from the human anti-GM2 and anti-GD2 mAbs isolated by Irie and her colleagues (9, 10) and certain anti-ganglioside mAbs isolated from the mouse (3, 4). We have isolated (13, 14) other human mAbs with cross-reactive specificity (i.e., FCM1 cross-reacted with GM3 and GD1a and HJM1 cross-reacted with GD3 and GD2), and it will be interesting to determine the clonal frequency of anti-ganglioside antibodies with single, dual, or multiple specificities and whether this frequency is altered by vaccination.

This work was supported in part by grants from the National Cancer Institute (CA-08748, CA-47427, and CA-33049) and the Aaron Diamond Foundation.

1. Lloyd, K. O. & Old, L. J. (1989) *Cancer Res.* **49,** 3445–3451.
2. Pukel, C. S., Lloyd, K. O., Travassos, L. R., Dippold, W. G., Oettgen, H. F. & Old, L. J. (1982) *J. Exp. Med.* **155,** 1133–1147.
3. Cheung, N. V., Saarinen, U. M., Neely, J. E., Landmeier, B., Donovan, D. & Coccia, P. F. (1985) *Cancer Res.* **45,** 2642–2649.
4. Natoli, E. J., Jr., Livingston, P. O., Pukel, C. S., Lloyd, K. O., Wiegandt, H., Szalay, J., Oettgen, H. F. & Old, L. J. (1986) *Cancer Res.* **46,** 4116–4120.
5. Houghton, A. N., Mintzer, D., Cordon-Cardo, C., Welt, S., Fliegel, B., Vadhan, S., Carswell, E., Melamed, M. R., Oettgen, H. F. & Old, L. J. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1242–1246.
6. Cheung, N. K., Medof, M. E. & Munn, D. (1988) *Prog. Clin. Biol. Res.* **271,** 619–632.
7. Svennerholm, L. (1963) *J. Neurochem.* **10,** 613–623.
8. Tai, T., Cahan, L. D., Tsuchida, T., Saxton, R. E., Irie, R. F. & Morton, D. L. (1985) *Int. J. Cancer* **35,** 607–612.
9. Tai, T., Paulson, J. C., Cahan, L. D. & Irie, R. F. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5392–5396.
10. Cahan, L. D., Irie, R. F., Singh, R., Cassidenti, A. & Paulson, J. C. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7629–7633.
11. Watanabe, T., Pukel, C. S., Takeyama, H., Lloyd, K. O., Shiku, H., Li, L. T. C., Travassos, L. R., Oettgen, H. F. & Old, L. J. (1982) *J. Exp. Med.* **158,** 1884–1889.
12. Livingston, P. O., Natoli, E. J., Jr., Calves, M. J., Stockert, E., Oettgen, H. F. & Old, L. J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2911–2915.
13. Yamaguchi, H., Furukawa, K., Fortunato, S. R., Livingston, P. O., Lloyd, K. O., Oettgen, H. F. & Old, L. J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2416–2420.
14. Furukawa, K., Yamaguchi, H., Oettgen, H. F., Old, L. J. & Lloyd, K. O. (1989) *Cancer Res.* **49,** 191–198.
15. Carey, T. E., Takahashi, T., Resnick, L. A., Oettgen, H. F. & Old, L. J. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3278–3282.
16. Miller, G. & Lipman, M. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 190–194.
17. Kohler, G., Howe, C. S. & Milstein, C. (1976) *Eur. J. Immunol.* **6,** 292–295.
18. Kearney, J. F., Radbruch, A., Liesegang, B. & Rajewsky, K. (1979) *J. Immunol.* **123,** 1548–1550.
19. Shiku, H., Takahashi, T., Oettgen, H. F. & Old, L. J. (1976) *J. Exp. Med.* **144,** 873–881.
20. Real, F. X., Mattes, M. J., Houghton, A. N., Oettgen, H. F., Lloyd, K. O. & Old, L. J. (1984) *J. Exp. Med.* **160,** 1219–1233.
21. Magnani, J. L., Smith, D. F. & Ginsberg, V. (1980) *Anal. Biochem.* **109,** 399–402.
22. Furukawa, K., Clausen, H., Hakomori, S., Sakamoto, J., Lundblad, A., Mattes, M. J. & Lloyd, K. O. (1985) *Biochemistry* **24,** 7820–7826.
23. Furukawa, K., Yamaguchi, H., Oettgen, H. F., Old, L. J. & Lloyd, K. O. (1988) *J. Biol. Chem.* **263,** 18507–18512.