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Immunology

Human antibody to OFA-I, a tumor antigen, produced *in vitro* by Epstein–Barr virus-transformed human B-lymphoid cell lines

(IgM/membrane/fetal brain/neuroectodermal origin)

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ABSTRACT **We established two long-term human B-lymphoblastoid cell lines (L55 and L72) transformed by Epstein–Barr virus that produced IgM κ antibodies to the human tumor antigen, OFA-I. Peripheral blood lymphocytes obtained from melanoma patients were used as the source of the B lymphocytes. Antibody specificity was determined by the immune adherence assay using various human cancer and noncancer tissues as targets. L55 antibody (designated anti-OFA-I-1) reacted with a variety of human tumor types whereas L72 antibody (designated anti-OFA-I-2) reacted only with tumor cells of neuroectodermal origin (melanoma, glioma, and neuroblastoma). The levels of IgM detected in the spent medium of 1 × 10^6 L55 and L72 cells were 4 and 9 μg/ml, respectively, by radioimmunoassay.**

Recent advances in methods for *in vitro* antibody production (1) have led to improved definition and characterization of antigen and epitope specificities on human tumor cells (2–6). Nearly all of these antibodies have been produced by hybridomas from mice. Although human monoclonal antibodies have been reported (7), none have been produced against tumor cells. Treatment with human monoclonal antibodies specific for tumor cells could eliminate metastatic spread of residual disease in the postoperative cancer patient. We have investigated the nature of a human tumor-associated fetal antigen, the oncofetal antigen (immunogenic) (OFA-I) by using human anti-OFA-I antibody produced *in vitro* by Epstein–Barr virus (EBV)-transformed lymphoblastoid cell lines established from peripheral blood lymphocytes of melanoma patients.

OFA-I, initially defined by serum antibody from melanoma patients, is a membrane antigen on various histological types of human cancer cells that crossreacts with human fetal brain tissue (8, 9). OFA-I has not been found in fetal liver, spleen, thymus, or small intestine or on adult normal cells. The immunogenic nature of OFA-I was confirmed by its ability to regularly induce an antibody response in melanoma patients exposed to the antigen in an allogenic melanoma cell vaccine (10).

Previously, we reported the establishment of monospecific anti-OFA-I-secreting lymphoblastoid cell lines from the peripheral blood lymphocytes (PBLs) of patients having high titers of circulating anti-OFA-I (11). However, the secretion was not permanent. After 8 wk of culture, anti-OFA-I antibody titers suddenly decreased and, finally, they became undetectable. In the present study, we established two long-term anti-OFA-I-producing cell lines by EBV transformation of an additional 232 PBL specimens: one has produced antibody for more than 12 mo and the other produced anti-OFA-I for more than 6 mo. This *in vitro* technique has now enabled us to detect more than one distinct antigenic specificity that is consistent with the original functional description of OFA-I as defined by serum antibody from cancer patients. In this report, we distinguish two antigenic specificities, OFA-I-1 and OFA-I-2, by their distribution on human cell lines and biopsied tissue. The results indicate that OFA-I-1, like the previously described OFA-I, is found on various histological types of human cancer cells, while OFA-I-2 is detected only on tumor cells of neuroectodermal origin.

MATERIALS AND METHODS

Establishment of Human Lymphoblasts That Produce Antibody to OFA-I. Only two long-term antibody-producing lines were obtained after EBV transformation of 232 PBL specimens. The two cell lines, L55 and L72, were established from the PBLs of two melanoma patients. The method was described earlier (11). The other 230 specimens either failed or were short-term producers (6 days to 6 wk), whereas L55 produced anti-OFA-I for more than 6 mo and L72 continued production for more than 1 yr. The maximum antibody titer from L55 was 1:32 against the OFA-I-positive melanoma cell line M14 and, for L72, the titer was 1:16. A continuous supply of these two antibodies was ensured by cryopreserving both lines (3.2 × 10^8 L55 cells and 2.6 × 10^9 L72 cells divided into several hundred aliquots). When thawed, both continued to produce anti-OFA-I in the spent tissue culture medium with a maximum titer at 4 days.

To maintain cells exhibiting the highest titers from the L55 and L72 lines, these cultures were occasionally cloned by the limiting-dilution method in the presence of irradiated human PBLs as feeder layers. For example, when Costar 96-well microculture plates were seeded with 10 cells per well, each under irradiated PBL feeder layers, more than 90% of the wells had growing cells within 3 wk, and all became antibody producers. The antibody titers from the producer wells varied. Some were 2- to 8-fold higher than the original noncloned cells, but only for a short period. After several passages, the titer dropped to that of the original noncloned cells. Antibodies from cloned cells were characterized for their antibody specificities by absorption and isoelectrofocusing. Results showed that both cloned and noncloned antibodies were identical. On the other hand, if the plates were seeded at 1 cell per well, fewer than 50% of the wells had growing cells within 4 wk. None of the clones produced a higher titer of antibody than original cells.

Human Tumor and Nontumor Cell Lines. The specificity of the antibody produced by the L55 and L72 cell lines was tested by the immune adherence (IA) assay, as described below, against 75 human cell lines: 27 melanomas, 7 gliomas, 8 neuroblastomas, 11 carcinomas, 3 sarcomas, 2 leukemias, 1 Burkitt

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Abbreviations: OFA-I, oncofetal antigen (immunogenic); EBV, Epstein–Barr virus; PBL, peripheral blood lymphocyte; IA assay, immune adherence assay; IMIF, indirect membrane immunofluorescence; P_i/NaCl, phosphate-buffered saline; RIA, radioimmunoassay; FITC, fluorescein isothiocyanate.

lymphoma, 1 myeloma, 10 EBV-transformed lymphoblastoid cell lines, and 5 skin or muscle fibroblasts. All cultured cells were maintained in RPMI 1640 (Microbiological Associates) supplemented with 10–20% fetal calf serum and antibiotics (a combination of penicillin, streptomycin, and Fungizone or a combination gentamycin and Fungizone) in humidified 5% CO_2/95% air at 37°C. Cells were used as targets in the IA assay between the 3rd and 5th day after passage when more than 50% confluent.

IA Assay. The initial determination of anti-OFA-I specificity against the OFA-I-positive melanoma cell line UCLA-SO-M14 (M14) using the IA assay has been described (8). Methods for testing antibody to other target cell lines were essentially the same. The IA assay has been shown by us and others (12) to be an extremely sensitive method for detecting IgM antibody binding to tumor cells. In brief, the assay detects antibody bound to tumor cells by specific rosette formation of human erythrocytes in the presence of complement components 1–3. All monolayer cells were harvested by rinsing once with trypsin diluted to 0.25% with Versene buffer/0.13 M NaCl/0.05 M glucose containing an antibiotic/antimycotic mixture (GIBCO). The OFA-I antigen(s) defined by serum antibody is not affected by this gentle trypsinization. After harvesting, the IA assay was performed using antibody from crude spent medium or partially purified IgM from the spent medium of the L55 and L72 cell lines. Each partially purified preparation was standardized to a concentration of 20 μg of IgM/ml. Antibody reactivity against each target cell line was assessed by its IA titer (defined as the antibody dilution at which 50% of target cells are involved in rosette formation with human erythrocyte indicator cells).

Indirect Membrane Immunofluorescence (IMIF). The IMIF assay was used to identify the immunoglobulin classes of antibody reacting to M14 cells as reported (13).

Absorption Techniques. The reactivity of the L55 and L72 antibody to biopsied melanoma tissue, cultured lymphoblasts and fibroblasts, and autopsied fetal tissues was tested by quantitatively absorbing each antibody with the appropriate tissue followed by testing the resulting activity against M14 cells in the IA assay. Before absorption, the antibody titers were adjusted to 1:16–1:32 against the M14 target cells in this assay. The preparation and storage of tissue and the absorption techniques used have been described (11, 14).

Preparation of IgM from Spent Medium. Fractionation of IgM from the spent culture medium of the L55 and L72 cell lines was carried out as follows. The spent culture medium was concentrated to 1/10th vol with an Amicon filter (cut-off, M_r 50,000) and IgM was precipitated with 50% ammonium sulfate, dialyzed against 0.15 M NaCl/0.1 M Na phosphate, pH 7.2 (P_i/NaCl), and resuspended in 1/10th vol of P_i/NaCl. After P_i/NaCl dialysis, the concentrated fraction was further dialyzed against distilled water (pH 7.0) to obtain a precipitate that contained IgM. The precipitate was suspended in P_i/NaCl and dialyzed against P_i/NaCl. Any undissolved precipitate was pelleted at 100,000 × *g* for 90 min and IgM in the supernate was stored as aliquots in a −190°C nitrogen freezer until used.

Quantitation of IgM Production by Sandwich Radioimmunoassay (RIA). A solid-phase RIA similar to that described by Pierce and Klinman (15) was developed to accurately measure human IgM levels in the L55 and L72 spent culture fluids. The IgG fraction of rabbit anti-human IgM (DAKO) was diluted to 10 μg/ml in 0.06 M $NaHCO_3$ buffer (pH 9.5), and 0.1 ml aliquots were incubated in Costar polyvinylchloride microtiter wells for 18 hr at 4°C. Unbound antiglobulin was aspirated and the wells were washed with 0.05% Triton X-100/2% fetal calf serum in P_i/NaCl (RIA buffer) and incubated at 37°C for 2 hr with this buffer to saturate or "block" any remaining protein binding sites present. Next, duplicate wells were incubated for 2 hr at 37°C with RIA buffer containing 1:2 serial dilutions of antibody from culture fluids. Control wells contained known dilutions of purified IgM from a myeloma patient. Plates were washed three times with RIA buffer, incubated for 2 hr at 37°C with ^{125}I-labeled anti-human IgM (10^5 cpm/0.1 ml; 2×10^7 cpm/μg); and washed four times with RIA buffer, and radioactivity was measured in a Beckman gamma spectrometer. Comparison of the test samples with the IgM standard binding curve provided accurate quantitation down to dilutions containing IgM at less than 5 ng/ml. No crossreactivity with other human Ig was detected in this RIA sandwich technique.

RESULTS

Identification of the OFA-I Specificity of the *in Vitro*-Produced Antibody. The method for determining the specificity of antibody produced by the L55 and L72 cell lines has been described (11). Briefly, cells were subcultured in 2–5% fetal calf serum/RPMI 1640 medium every 3–5 days by adjusting their density to 2×10^5/ml. The spent tissue culture medium was monitored by the IA assay every 3–5 days for the presence of antibody against the OFA-I-positive M14 melanoma cell line. The spent medium from the L55 line achieved peak IA titers of 1:32 after 35 days. The L55 lymphoblasts continued to produce anti-M14 antibody for 28 wk, after which time no activity could be detected. Thus, subsequent experiments were carried out with L55 lymphoblasts cryopreserved at the time of peak (1:16–1:32) antibody reactivity. On the other hand, the L72 lymphoblasts have continued to produce anti-M14 antibody at a titer of 8–16 for more than 1 yr. The antibody secretion was maintained by occasional cloning of the L72 line every 2 to 3 mo by the limiting-dilution method. Table 1 shows that absorption of L55 and L72 antibody by lymphoblasts autologous to M14 (UCLA-SO-L14) did not decrease the titer, confirming that the M14 reactivity was not directed against HLA specificities (16). Possible reactivity against blood group antigen specificities was excluded by the fact that there was no decrease in antibody titer after absorption of the spent medium with human erythrocytes from 20 donors (see Table 3). However, peak anti-M14 activity could be completely abolished from spent medium of both by absorption with OFA-I-positive second trimester human fetal brain tissue. Fetal liver from the same fetus did not reduce the antibody titers significantly, in complete accord with the absorption pattern described previously for anti-OFA-I from patients' sera (8, 9).

Immunoglobulin Class of Anti-OFA-I Antibody. Identification of the immunoglobulin class produced by the L55 and L72 cultures was accomplished by immunofluorescence determination using fluorescein isothiocyanate (FITC)-labeled goat

Table 1. Definition of antibody specificities by absorption of spent tissue culture media from L55 and L72 lymphoblastoid cell lines

Tissues used for absorption	Antibody titer (IA_{50}) after absorption	
	L55	L72
Antibody not absorbed	32	16
M14 target cells (5×10^6)	<2	<2
L14 lymphoblasts (2×10^7)	16–32	8–16
Fetal brain (100 μl)	<2	4
Fetal brain (two 100-μl portions)	<2	<2
Fetal liver (100 μl)	32	16–32

One hundred microliters of spent media was adsorbed on various cells and tissues.

anti-human IgG (γ-chain specific), anti-human IgM (μ-chain specific), anti-human IgA (α-chain specific), anti-human κ, or anti-human λ (DAKO, Denmark) as the second antibody in the IMIF assay. As shown in Table 2, M14 target cells coated with 10-fold concentrated spent medium from both L55 and L72 cultures were stained by goat anti-human IgM and anti-human κ, whereas no cells were reactive with antibodies to human IgG, IgA, or anti-human λ. Therefore, the anti-OFA-I produced in these cultures was limited to the IgM κ class. As a specificity control, an OFA-I-negative melanoma cell line, M15, did not display any second antibody specificity.

To confirm their B-cell origin, the L55 and L72 cells were examined with FITC-labeled goat antibodies to human immunoglobulins. The degree of response and the percentage of positive responses were directly related to the time of subculture. When the cells were tested 1 day after subculture, nearly all were stained by FITC-labeled anti-human IgM (μ-chain specific), but the percentage of positive cells decreased each day thereafter. No cells were stained by FITC-labeled goat antibody to IgG (γ-chain specific) or to IgA (α-chain specific). Light chain specificity was confirmed by anti-κ antibody. By these methods, L55 and L72 cells were shown to be of B-cell origin and to express IgM on their cell membranes.

Quantitation of Antibody Production. The IA assay indicated that maximum antibody titers occurred in the spent medium of the L55 and L72 cultures within 4 to 5 days of subculture, when the cell density was approximately 1×10^6/ml. Increases in cell density did not appreciably increase the antibody titers, and occasionally there was a reduction in antibody titer. The amount of IgM detected by RIA in day 5 spent medium cultures was 4 μg/ml for L55 and 9 μg/ml for L72. Neither IgG nor IgA were detected by this assay. Fig. 1 shows that essentially all IgM from both culture supernatants could be absorbed by M14 cells. The OFA-I-negative cell line M15 did not absorb any antibody. The specific nature of these absorptions was confirmed by the fact that M14 cells did not absorb nonrelevant IgM produced by a human myeloma cell line. This finding, in agreement with the absorption data shown in Table 1, indicates that antibodies produced by both L55 and L72 are directed solely to OFA-I specificities on M14 cells.

Reactivity of L55 and L72 Anti-OFA-I Antibody to Human Cell Lines and Biopsied Tissue. The antibody produced by the L55 and L72 cell lines was tested for specificity against 75 different human cell lines and eight biopsied melanoma tissues. Table 3 shows the distinct differences in the patterns of reactivity of the two antibody preparations. Antibody from L72 (designated anti-OFA-I-2) bound only to cells of neuroectodermal origin (melanoma, neuroblastoma, and glioma), whereas antibody from L55 (designated anti-OFA-I-1) reacted with a variety of histological tumor types, as well as with cultured skin or muscle fibroblastoid cells. For example, anti-OFA-I-1 was strongly reactive to M105 and M24 melanomas, CHP100 and KA neuroblastomas, B-734 breast carcinoma, L6 lung carcinoma, and C474 colon carcinoma cell lines (antibody titers ranged from 1:16 to 1:96 by IA assay), while anti-OFA-I-2 did not react to any of these cell lines. Conversely, anti-OFA-I-2 reacted strongly with M19 and M101 melanoma cell lines (antibody titers were 1:16 and 1:32, respectively) and with one of the biopsied melanoma tissues whereas anti-OFA-I-1 did not bind to these cells. None of the 10 human B lymphoblasts autologous to the OFA-I-positive tumor lines were reactive as target cells.

Table 2. Reactivity of L55 and L72 spent medium to M14 cells (OFA-I positive) by IMIF assay

	% FITC-labeled cells	
	L55	L72
Human IgM (μ)	94	88
Human IgG (γ)	0	0
Human IgA (α)	0	0
κ	98	92
λ	0	0

After concentration to 1/10th vol by 50% ammonium sulfate precipitation, spent tissue culture medium was tested for reaction to FITC-labeled antibodies to various human immunoglobulins. Positive controls for FITC-labeled antibodies included IgM, IgG, and IgA anti-M14 sera obtained from cancer patients immunized with the M14 line (10). Concentrations of the second antibodies were chosen as the lowest dilution with no background fluorescence in the absence of the first antibody.

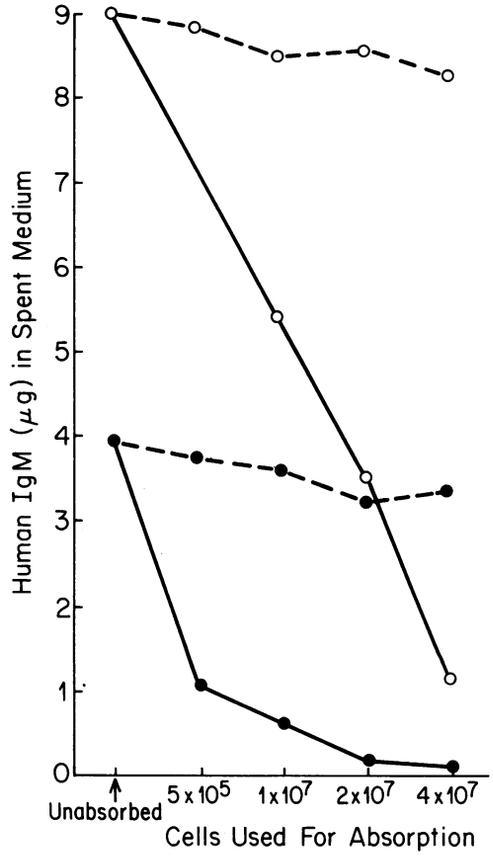

FIG. 1. Quantitative absorption of human IgM in spent tissue culture media of L55 (●; 3.97 μg) and L72 (○; 9 μg) by OFA-I-positive M14 cells (——) and OFA-I-negative M15 cells (– – –). Equal volumes of spent medium (concentrated to half volume) and cell suspension were mixed and incubated at 4°C for 4 hr with agitation. Cells were sedimented by centrifugation at 10,000 × *g* for 30 min, and the quantity of IgM in the supernatant fluid was measured by radioimmunoassay.

Cross-Absorption Assays to Confirm Monospecificity of L72 and L55 Antibodies. To confirm that the positive reactivity of L72 antibody to melanoma, neuroblastoma, and glioma cell lines was due to reaction with an identical antigen, cross-absorption assays were carried out using M14 melanoma, LAN-1 neuroblastoma, and SK-GM-3 glioma cell lines, all of which were OFA-I-2 positive. Antibody titers to each of these lines were adjusted to 1:32 by IA. Cell suspensions containing M14, LAN-1, or SK-GM-3 at 2×10^8/ml were used to absorb an equal volume of L72 antibody. After absorption, the remaining antibody titer of each was determined by the IA assay. As shown in Table 4, reactivities to M14, LAN-1, and SK-GM-3 were

Table 3. Reactivity of L55 (anti-OFA-I-1) and L72 (anti-OFA-I-2) against human tissues in the IA assay or in the IA absorption assay

Tissue	*n*	Tissue reacting, no. L55 and L72	L55 only	L72 only	Tissue not reacting, no.
Cultured					
Melanoma	26	10	4	6	5
Glioma	8	5	1	1	1
Neuroblastoma	8	4	3	1	0
Carcinoma and sarcoma	14	0	10	0	4
Lymphoid tumor	4	0	1*	0	3
Fibroblasts†‡	5	0	5	0	0
B lymphoblasts†§	10	0	0	0	10
Biopsied					
Melanoma†¶	8	3	1	2	2
Erythrocytes†¶	20	0	0	0	20

Partially purified IgM fractions of L55 and L72 culture spent media were used to test the antigenicity of cultured tumor cell lines. Both IgM fractions were adjusted to 20 μg of IgM/ml.

* The K562 myeloid leukemia cell line was allowed to react with L55 antibody. The line is known to be a target for natural killer cytotoxicity.

† By the IA absorption assay.

‡ At 5×10^6 cells/100 μl of antibody.

§ At 2×10^7 cells.

¶ At 100 μl of packed cells.

completely removed by any of these lines. Thus, it appeared that the L72 antibody detected an identical antigen on the three cell lines.

Cross-absorption assays were carried out on L55 antibody using the OFA-I-1-positive M14, CHP-100 neuroblastoma, and B-734 breast carcinoma cell lines. Monospecificity was confirmed in this case also (Table 4).

Detection of Anti-OFA-I-1 and Anti-OFA-I-2 in Patient Sera. A standard serum pool from a melanoma patient who showed strong anti-OFA-I activity by IA was tested along with anti-OFA-I-1 and anti-OFA-I-2 against a variety of cell lines and fetal tissues. Table 5 shows that those cells that were negative for both OFA-I-1 and OFA-I-2 were also negative for OFA-I as detected by the patient's sera. On the other hand, cells that were positive for either OFA-I-1 or OFA-I-2 were also positive with anti-OFA-I serum. This result indicated that both OFA-I-1 and OFA-I-2 were immunogenic in patients and that each represents an antigenic subset of OFA-I on melanoma cells.

DISCUSSION

This paper reports the establishment of two long-term EBV-transformed human anti-OFA-I-producing B-lymphoblastoid cell lines. One line has continued to produce anti-OFA-I for more than 1 yr; this line is thus a permanent one that produces monospecific antibody to a human tumor-associated antigen. These *in vitro*-produced antibodies were used to define two different antigenic specificities of OFA-I on human cell lines and biopsied tissues. OFA-I-1, as we previously reported (8, 9), is found on various histologic types of human cancer cells and on cultured human fibroblasts. Another antigen, OFA-I-2, was identified with IgM antibody produced by the L72 cell line. This antigen has been detected only on tumor cells of neuroectodermal origin (melanoma, neuroblastoma, and glioma). Cross-absorption assays (Table 4) confirmed the presence of a common antigen among these three different histologic types of tumors. Human antibody directed against antigens other than OFA-I were not detected in the culture medium from both the cell lines.

Antigens associated with human tumors of neuroectodermal origin have been reported by several investigators (5, 17–20). However, in those studies, mouse monoclonal antibodies or xenoantisera against human tissues were used to define the antigens whereas, in the present study, *in vitro*-produced human antibodies were tested, thus limiting detection to those antigens immunogenic in man. In fact, as shown in Table 5, sera from patients do contain humoral antibody to the antigen. Previous studies of the clinical significance of the circulating anti-OFA-I antibody in cancer patients showed a definite correlation between survival of postoperative stage 2 and 3 melanoma patients and their serum levels of IgM anti-OFA-I (21, 22). However, as Table 5 indicates, some patients probably responded to both the OFA-I-1 and the OFA-I-2 specificities. Thus, in further investigations, it should be possible to refine such disease survival analysis by monitoring both the broadly crossreactive anti-OFA-I-1 and the neuroectodermal anti-OFA-I-2 levels against those target cells shown to specifically express only OFA-I-1 or OFA-I-2 or alternatively with each purified antigen.

Repeated attempts were made to establish antibody-producing human lymphoblast clones by limiting-dilution methods on various feeder layers, including human PBLs, human skin fibroblasts, mouse macrophages, and a drug-marked hypoxanthine/aminopterin/thymidine-sensitive human lymphoblastoid cell line. No clones, however, produced higher titers of anti-OFA-I than the original noncloned cells. These results suggest that L55 and L72 may have been naturally selected as clones during long-term culture. Alternatively, factors suppressing the selection of anti-OFA-I may have been generated during the cloning process. Similar difficulties in cloning antibody-producing lymphoblastoid cells have been reported by other investigators (23, 24). Thus, in the present system, we cannot definitely exclude the possibility that OFA-I-1 or OFA-I-2 may consist of more than one epitope specificity. However,

Table 4. Cross-absorption of L72 (anti-OFA-I-2) and L55 (anti-OFA-I-1) antibodies by OFA-I-1- and OFA-I-2-positive tumor cell lines

Cells used for absorption	L72 antibody, IA_{50} M14	LAN-1	SK-GM-3	L55 antibody, IA_{50} M14	CHP-100	B-734
Buffer	32	16–32	16	32	32	16–32
M14 (melanoma)	<2	<2	<2	<2	<2	<2
LAN-1 (neuroblastoma)	<2	<2	<2	NT	NT	NT
SK-GM-3 (glioma)	<2	<2	<2	NT	NT	NT
CHP-100 (neuroblastoma)	NT	NT	NT	<2	<2	<2
B-734 (breast carcinoma)	NT	NT	NT	<2	<2	<2

Titers of nonabsorbed antibody to OFA-I-1 and OFA-I-2 to each cell line were adjusted to 32 (IA_{50}). Antibody was mixed with an equal volume of cell suspension (2×10^8 cells/ml) or with buffer and incubated at 4°C overnight. Titers of remaining antibody were assayed by IA with each target. NT, not tested.

Table 5. Comparison of antibody reactivity from melanoma patients' sera to anti-OFA-I-1 and anti-OFA-I-2 produced *in vitro*

Human tissue	Type	IA_{50} Patients' sera*	IA_{50} OFA-I-1	IA_{50} OFA-I-2
UCLA-SO-M14	Cultured melanoma	64	64	32
-M15	Cultured melanoma	<1	<1	<1
-M19	Cultured melanoma	16	<1	16
-M24	Cultured melanoma	8	16	<1
-M101	Cultured melanoma	16	<1	32
-M104	Cultured melanoma	32	32	16
-M108	Cultured melanoma	4	12	<1
-M4	Cultured melanoma	<1	<1	<1
B-734	Cultured breast carcinoma	16	32	<1
CHP-100	Cultured neuroblastoma	64	96	<1
McKinnie	Cultured colon carcinoma	<1	<1	<1
UCLA-SO-L14	Cultured lymphoblasts	<1	<1	<1
Fetal brain†		+	+	+
Fetal liver†		−	−	−

* Sera were first absorbed by pooled human PBLs from 10 donors to remove possible alloreactivities to targets.

† Reactivity to fetal brain and fetal liver was determined by the IA absorption assay using M14 as target cells.

initial data using isoelectrofocusing and the extremely sensitive silver-staining techniques with peptides from purified L55 and L72 IgM preparations showed that these noncloned cultures produce antibody containing only a single light chain, suggesting they are both of monoclonal origin (unpublished results).

Studies to define the chemical nature of the antigen were initiated. Since the immunoglobulin class of natural anti-OFA-I found in patients is exclusively IgM, there was a high probability that the antigen contained or was composed of carbohydrate moieties of glycolipids or lipopolysaccharide that could induce T-cell-independent IgM antibody (25). OFA-I-positive melanoma cells were treated with different glycosidases. The results showed that sialidase abolished OFA-I-2 antigenicity completely but did not affect OFA-I-1. Further characterization of these antigens showed that both are unique membrane glycolipids with distinct characteristics. Details will be provided elsewhere.

Recently, mouse monoclonal antibody to human tumor cell membrane antigens have been used for radioimaging and for therapy (26, 27). Although no serious side effects or allergic reactions have been noted, some potential problems include the induction of anti-mouse immunoglobulin after repeated mouse antibody administration and a potential reaction of monoclonal antibody to certain normal tissues. Furthermore, an accumulation of anti-mouse immunoglobulins in the circulation could neutralize subsequently administered monoclonal antibody, diminishing its effectiveness. Circulating monoclonal antibody antiglobulin complexes could lead to immunologic suppression and enhanced tumor growth or to kidney disfunction. The availability of unlimited quantities of human tumor-associated antibody clearly would provide improved reagents for further therapeutic trials.

In a previous study, it has been shown that IgM anti-OFA-I antibody produced *in vitro* (11) or isolated from patients' sera (28) is highly cytotoxic *in vitro* in the presence of complement. In addition, 5-yr survival for melanoma patients having high titers of serum IgM anti-OFA-I antibody was significantly prolonged compared with that for those having low titers ($P = 0.001$) (21). These findings suggest that the *in vitro*-produced anti-OFA-I is a potential reagent for specific and selective immunotherapy for patients with OFA-I-positive tumors. The application of the antibody to radioimaging of such tumors may be complicated because approximately half of the patients we studied had detectable titers of natural anti-OFA-I antibody that presumably would compete with the radiolabeled anti-OFA-I antibody.

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