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Common Antigenic Determinants on Human Melanoma, Glioma, Neuroblastoma, and Sarcoma Cells Defined with Monoclonal Antibodies[1]

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ABSTRACT

Antigenic determinants that are common to melanomas, gliomas, neuroblastomas, and sarcomas but that are minimally or not detectably expressed by adult tissues were defined with monoclonal antibodies. Quantitative absorption of monoclonal antibody (Ab 165) with adult tissues followed by testing on antigen-positive UCLA-SO-M14 melanoma cells did not demonstrate antigenic determinant (Ag 165) in brain, lung, liver, kidney, intestine, adrenal, and muscle. Absorption of Ab 376 demonstrated Ag 376 in adult lung but minimal or no antigen in other tissues. Both antigens were associated with a variety of fetal tissues. Assessment of 28 human tumor cell lines with the ^{131}I-staphylococcal Protein A-binding test demonstrated that Ab 165 reacted strongly with melanomas and gliomas and weakly with sarcomas. Ab 376 reacted strongly with melanomas, gliomas, neuroblastomas, and sarcomas. Neither of these antibodies reacted appreciably with carcinoma or teratoma cell lines. Absorption of Ab 165 and Ab 376 with noncultured tumors demonstrated that melanomas, sarcomas, and neuroblastomas can have greater quantities of these antigens *in vivo* than do normal adult tissues. Qualitative and quantitative antigenic heterogeneity within positive classes of tumors was demonstrated for both cultured and noncultured tumors. The differences in antigen expression *in vivo* between normal and neoplastic cells suggest potential value for these antibodies in immunodiagnosis and possibly immunotherapy.

INTRODUCTION

Various types of tumor-associated antigens have been identified on human melanoma cells serologically. Autologous sera, alloantisera, and xenoantisera have demonstrated antigens that are unique to individual melanomas, others that are common to melanomas and other tumors, and still others that are common to a variety of neoplastic and normal cell types (3, 9, 10, 13, 14). More recently, monoclonal antibodies to melanoma-associated antigens (1, 2, 6, 8, 15) have been developed using the lymphocyte hybridoma technique (7). Two monoclonal antibodies that we have produced react with melanoma cell lines but not appreciably with normal lymphocytes, erythrocytes, and fibroblasts (6). The aim of the present study was to assess whether the determinants recognized by these monoclonal antibodies are expressed by normal adult and fetal tissues, by other tumors originating from neuroectodermal cells, and by tumors *in vivo*.

MATERIALS AND METHODS

Cell Lines and Cell Culture. Established human tumor-derived cell lines used in this study included gliomas, neuroblastomas, a medulloblastoma, osteogenic sarcomas, a rhabdomyosarcoma, a leiomyosarcoma, a teratoma, melanomas, transitional cell carcinomas of the bladder, carcinomas of the colon, and a squamous cell carcinoma of the lung. Details of these cell lines and of their propagation have been reported previously (12).

Monoclonal Antibodies to Human Melanoma-associated Antigens. BALB/c mice were immunized with cultured melanoma cells, and their splenocytes were fused with Sp2/O-Ag14 myeloma cells as described (6). Antibody-secreting hybridomas 165 and 376 were cloned twice by limiting dilution, and antibodies secreted by clones 165.30 and 376.80 were utilized in the experiments reported here. These antibodies (Ab) and their respective antigenic determinants (Ag) are designated 165 and 376. Ab 165.30 is IgG_1 κ, and Ab 376.80 is IgG_{2a} λ.

^{131}I-SPA[6] Test for Cell Surface-bound Antibodies. Details of this microassay for antibodies bound to the surface of adherent tumor cells have been reported (12, 16). Target cells in 0.1 ml of Eagle's minimal essential medium with 10% fetal calf serum containing 0.2 μCi [^{125}I]iododeoxyuridine were plated into wells of Microtest II plates (Falcon Plastics, Oxnard, Calif.) and allowed to adhere for 18 to 24 hr. After plates were washed by immersion in D-PBS, appropriately diluted lymphocyte hybridoma culture supernatant was added followed by a 1:100 dilution of rabbit anti-mouse immunoglobulin (Cappel Laboratories, Inc., Cochranville, Pa.) and then ^{131}I-SPA. Each incubation step was 45 min at 37°, and plates were washed by immersion in D-PBS between steps. After the plates were dried, individual wells were counted for ^{125}I and ^{131}I simultaneously in a dual-channel γ counter. The cpm of ^{131}I per 5 μg of target cell protein were calculated for 3 replicate wells, and the mean and standard deviation were determined.

Quantitative Absorption Test. Details of the absorption procedure have been published (12). Absorbents were prepared by homogenizing normal or tumor tissues in D-PBS and then by washing them twice in D-PBS by centrifugation (15 min,

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[6] The abbreviations used are: ^{131}I-SPA, ^{131}I-labeled staphylococcal Protein A; D-PBS, Dulbecco's complete phosphate-buffered saline; ED_{50}, estimated dose of absorbent necessary to remove 50% of monoclonal antibody activity.

48,000 x g). The last centrifugation served to pack the whole-tissue homogenate, and 5 doses (1 to 150 mg) were weighed with an analytical balance. Each weighed dose of absorbent was mixed with 0.5 ml of diluted hybridoma supernatant medium and continually rotated at 4° for 90 min. Then the mixture was centrifuged (15 min, 48,000 × g), and 0.4 ml of the supernatant was removed from the absorbent pellet and assessed for the presence of antibody activity with the ^{131}I-SPA test using antigen-positive UCLA-SO-M14 (M14) melanoma cells as targets.

Appropriate dilutions of hybridoma supernatants were established by titering each antibody against M14 cells. A final dilution of antibody that was approximately midway down the slope and 4 doubling dilutions from the end-point titer was used. The titers of Antibodies 165 and 376 were 1:500 and 1:10,000; the dilutions used in the absorption test were 1:30 and 1:500, respectively.

From the cpm of ^{131}I-SPA bound to M14 cells, a computer program calculated (*a*) the percentage of antibody activity remaining after absorption with each dose, (*b*) the best curve to fit the 5 data points based upon the logistic regression model, (*c*) the estimated dose of absorbent required to remove 50% of the antibody activity (ED_{50}); (*e*) an estimated standard deviation of the data points around the calculated curve which reflects the variability of the data points about the predicted values of the curve; this was <15%; and (*f*) the standard error of the ED_{50} values; this was <10%.

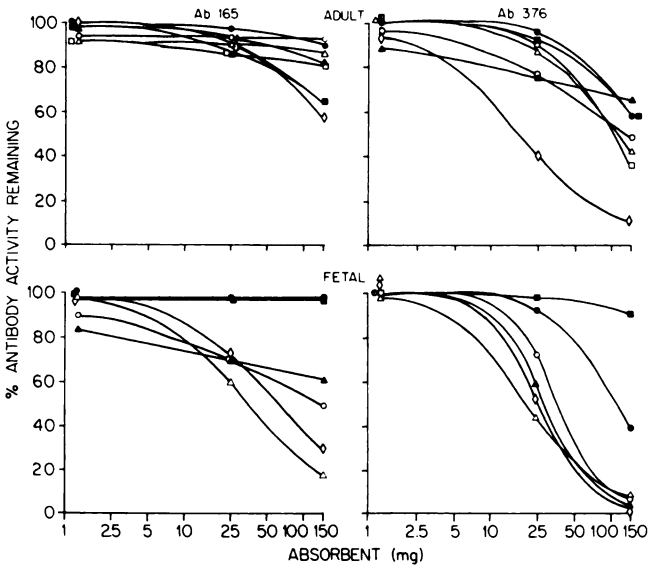

Chart 1. Quantitative absorption of Ab 165 and Ab 376 with adult and fetal tissues. Culture supernatants of the hybridomas (0.5 ml of a 1:50 dilution of Ab 165 and 0.5 ml of a 1:500 dilution of Ab 376) were absorbed with weighed quantities of tissue homogenates, and then residual antibody activity against UCLA-SO-M14 cells was determined with the ^{131}I-SPA test. Five doses of each homogenate between 1 and 150 mg were used. Tissues used were brain (■), lung (◇), liver (●), kidney (○), colon (△), adrenal (□), and muscle (▲). Fetal adrenal was not tested because of insufficient tissue. The best curve to fit the 5 data points was calculated with a computer using the logistic regression model. The estimated standard deviation of the data points around the calculated curves was <15%, which indicates the variability of the actual data points about the computed values.

RESULTS

Reactivity of Antibodies 165 and 376 with Normal Adult and Fetal Tissues. Normal adult and fetal tissues were tested by quantitative absorption (Chart 1). For Ab 165, the ED_{50} values for all adult tissues were all >171. The ED_{50} values for Ab 376 with adult tissues were all >101 except for lung which was 17. Most fetal tissues expressed more of both antigens than did adult tissues. It is noteworthy that neither adult nor fetal brain had detectable amounts of these antigens.

Reactivity of Antibodies 165 and 376 with Tumor Cell Lines. The antibodies were tested against 28 tumor-derived cell lines using the ^{131}I-SPA test (Table 1). Ab 165 reacted strongly with melanomas M7, M10, and M14 and with gliomas D54 and A172. It was minimally reactive with melanoma M20 and sarcomas UCLA-SO-S1, MT, HT-1080, and RD. Ab 376 had a different pattern of reactivity. It bound to all 3 gliomas and to melanomas M7 and M14 but minimally to melanomas M10 and M20. Ab 376 was quite reactive with neuroblastomas (LA-N-2, LA-N-5, SK-N-SH, and KA) and sarcomas (UCLA-SO-S1, HT-1080, and RD). Neither antibody reacted appreciably with carcinoma or teratoma cell lines.

Reactivity of Antibodies 165 and 376 with Melanoma, Neuroblastoma, and Sarcoma Tissues. To determine if antigenic determinants 165 and 376 are expressed by tumors *in vivo*, each antibody was absorbed with homogenates of surgically removed tumors and then tested against M14 melanoma cells (Chart 2). For Ab 165, 5 melanomas had ED_{50} values of 14, 40, 76, 82, and 150, and 3 others had values of >150. For Ab 376, 6 melanomas had ED_{50} values of 4, 5, 6, 30, 34, and 59, and 2 melanomas had values of >150. Thus, noncultured melanomas can have greater amounts of these antigens than do normal adult tissues.

Of 5 neuroblastomas, one had an ED_{50} value of 87 with Ab 165 and 2 had values of 150 with Ab 376. Values for the other neuroblastomas were >150 for both antibodies. Three of 6 sarcomas had more Ag 165 than did adult tissues with ED_{50} values of 48, 48, and 138. With Ab 376, ED_{50} values were 22, 73, 90, and 90 for 4 sarcomas and >150 for 2 others.

DISCUSSION

This investigation demonstrated that tumor cell lines derived from human melanomas, gliomas, neuroblastomas, and sarcomas share antigenic determinants. These determinants also are expressed by fetal tissues, but they are minimally or not detectably expressed by normal adult tissues. Initial studies of noncultured melanoma, sarcoma, and neuroblastoma tissues indicate that these determinants can be expressed in greater quantity by neoplasms *in vivo* than by normal adult tissues.

Ag 165 is expressed strongly by cell lines derived from melanomas and gliomas, weakly by those derived from sarcomas, and minimally or not at all by others. The antigen is not detectable in normal adult tissues and is essentially absent from lymphocytes, erythrocytes, and fibroblasts (6); this indicates that it is associated with few types of mature cells. However, its expression by fetal tissues suggests the possibility that it may be associated with subpopulations of normal stem cells or their immature derivatives. Although this possibility will need to be resolved by analysis of individual cells, our initial studies of melanoma, sarcoma, and neuroblastoma tissues indicate that major differences between normal tissues and neoplasms can occur *in vivo*.

Ag 376 has a different cell distribution pattern than does Ag

Table 1
Binding of antibodies 165 and 376 to tumor cell lines

Target cell		% of maximum binding activity[a] AB 165	AB 376
Melanoma			
UCLA-SO-M7		*63*	*30*
UCLA-SO-M10		*35*	16
UCLA-SO-M14		*100*	*57*
UCLA-SO-M20		15	15
Glioma			
A172 MG		*57*	*100*
D54 MG		*81*	*93*
U251 MG		<1	*44*
Neuroblastoma			
LA-N-1		<1	15
LA-N-2		2	*34*
LA-N-4		3	18
LA-N-5		2	*34*
CHP-100		<1	18
CHP-134		9	19
IMR-32		<1	18
SK-N-SH		1	*26*
SK-N-MC		1	10
KA		3	*56*
Medulloblastoma	TE-671	7	2
Leiomyosarcoma	UCLA-SO-S1	13	*51*
Osteogenic sarcoma			
MT		*21*	9
HT-1080		14	*21*
Rhabdomyosarcoma	RD	19	*34*
Teratoma	Tera-1	1	7
Colon carcinoma			
SK-CO-1		8	7
HT-29		9	6
Transitional cell carcinoma of bladder			
T24		1	11
J82		3	10
Squamous cell carcinoma of lung	SK-MES-1	3	14

[a] Data are the average of 2 experiments in which the binding of each antibody to different tumor cell lines was assessed with the ^{131}I-SPA-binding test, as described in "Materials and Methods." The cell line binding the most antibody, as indicated by the highest cpm of ^{131}I-SPA, was taken as the maximum binding (100%), and binding by all other cell lines was expressed relative to that level. The maximum cpm ^{131}I-SPA (per 5-μg target cell protein) that bound in the 2 experiments were as follows: Ab 165, 12,435 and 7,118 cpm; Ab 376, 4780 and 5674 cpm. All values >20% are italicized to facilitate comparison of the patterns of reactivity for the antibodies.

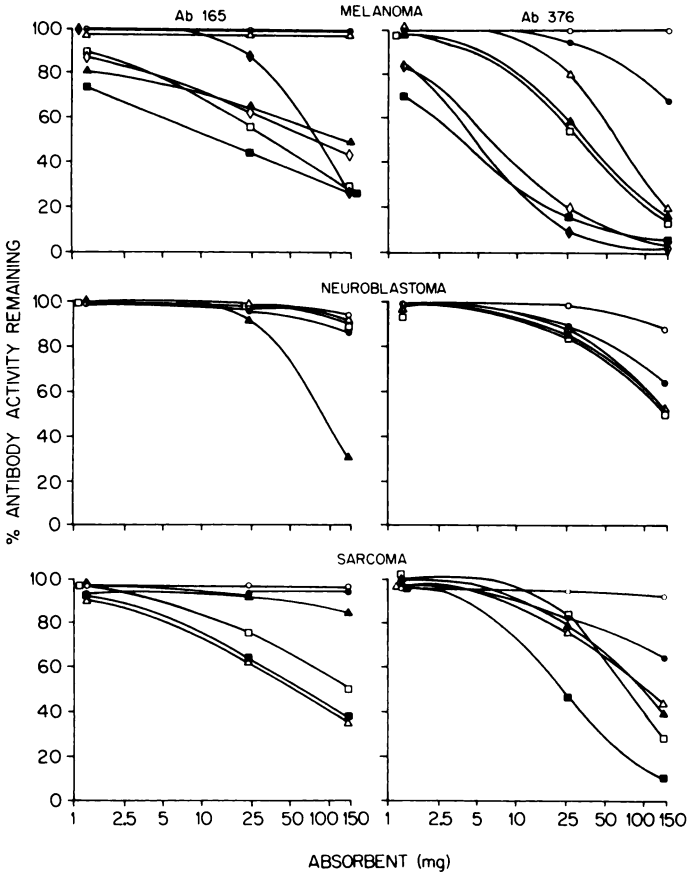

Chart 2. Quantitative absorption of Ab 165 and Ab 376 with homogenates of noncultured melanomas, neuroblastomas, and sarcomas. The absorption test and data analysis were performed as described in Chart 1. Each *curve* is for an individual tumor; corresponding *curves* for the 2 antibodies are indicated by the same symbols. Melanomas tested were locally invasive, Clark Level IV (○, ◇), or metastatic (●, ▲, ■, ◆, △, □). One of the melanomas was amelanotic (●). Histological types of sarcomas were osteogenic sarcoma (○), liposarcoma (●), rhabdomyosarcoma (△), undifferentiated sarcoma (▲), leiomyosarcoma (□), and chondrosarcoma (■). Neuroblastomas tested were all metastatic (Clinical Stage IV).

165. In addition to melanoma and glioma cell lines, it clearly is expressed by neuroblastoma and sarcoma cell lines. *In vivo*, Ag 376 is associated with adult lung. Like Ag 165, Ag 376 is not associated with other adult tissues in significant amounts but is expressed by a number of fetal tissues. Initial absorption analysis of noncultured tumors demonstrated that melanomas can have more of this antigen *in vivo* than do all normal tissues and that sarcomas can have more than do all tissues except lung. The neuroblastomas tested had only minimal amounts of Ag 376.

The reaction patterns of antibodies 165 and 376 with cell surface determinants parallels those of 2 other monoclonal antibodies and a human serum. The 2 monoclonal antibodies reacted with melanoma and glioma cell lines but not with lymphocytes, erythrocytes, or fibroblasts (5). The human serum, which was obtained from a patient with a glioma, reacted with glioma, melanoma, neuroblastoma, and sarcoma cells but not with normal cells including fetal brain (11). Comparative studies will be necessary to define molecular relationships between the determinants reacting with these antibodies. The expression of Ag 165 by melanomas and gliomas also parallels that of S100, a nervous system-specific cytoplasmic protein (4). However, S100 is strongly expressed in brain, whereas Ag 165 is not detectable in this tissue.

It was postulated that Ag 165 and Ag 376 may be differentiation antigens since they are expressed by neuroectodermally derived neoplasms. However, they were not detectable in adult or fetal brain by absorption analysis. This does not exclude the possibility that they are expressed by a subpopulation of normal cells derived from neuroectoderm or that they are expressed for a limited time during normal development. Alternatively, these antigens may be related to the neoplastic transformation process rather than to normal differentiation.

Antibodies 165 and 376 clearly defined qualitative and quantitative antigenic heterogeneity for each class of tumor reacting with them. These results suggest that monoclonal antibodies may provide useful reagents for assessing the biological and clinical significance of the expression of a given type of antigenic determinant. The relative lack of expression of these determinants by normal cells and tissues compared to neoplasms also suggests potential clinical applications. For ex-

ample, the antibodies may be useful for radioimaging, for immunohistological diagnosis of tumors, and for immunotherapy.

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