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

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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 131I-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 xenonantisera 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 IgG1, k, and Ab 376.80 is IgG2a, k.

131I-SPA6 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 [125I]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 131I-SPA. Each incubation step was 45 min at 37°C, and plates were washed by immersion in D-PBS between steps. After the plates were dried, individual wells were counted for 125I and 131I simultaneously in a dual-channel γ counter. The cpm of 131I 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). Absorvents were prepared by homogenizing normal or tumor tissues in D-PBS and then by washing them twice in D-PBS by centrifugation (15 min, 6 The abbreviations used are: 131I-SPA, 125I-labeled staphylococcal Protein A; D-PBS, Dulbecco’s complete phosphate-buffered saline; ED50, estimated dose of absorbent necessary to remove 50% of monoclonal antibody activity.
was mixed with 0.5 ml of diluted hybridoma supernatant me
tissue homogenate, and 5 doses (1 to 150 mg) were weighed
48,000 x g). The last centrifugation served to pack the whole-
cells as targets.

Appropriate dilutions of hybridoma supernatants were estab-
lished 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 131I-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%.

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 131I-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 anti-
genic determinants 165 and 376 are expressed by tumors in
vivo, each antibody was absorbed with homogenates of surgi-
cally 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 sar-
comas 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 sarco-
mas, 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 indi-
cates 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

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Monoclonal Antibodies to Human Tumor Antigens

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 131I-SPA test. Five doses of each
homogenate between 1 and 150 mg were used. Tissues used were brain (B),
lung (L), liver (R), kidney (K), colon (C), adrenal (A), and muscle (M). 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.
Table 1

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<tr>
<th>Target cell</th>
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Data are the average of 2 experiments in which the binding of each antibody to different tumor cell lines was assessed with the $^{125}$I-SPA-binding test, as described in Materials and Methods. The cell line binding the most antibody, as indicated by the highest cpm of $^{125}$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 $^{125}$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.

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.

ACKNOWLEDGMENTS

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REFERENCES


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


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