ABSTRACT

To analyze the humoral immune response to melanoma, human-mouse hybridomas were generated by the fusions of regional lymph node lymphocytes of patients with the mouse myeloma cell line M5. Six stable hybridomas were cloned from six separate lymphocyte parents obtained from three patients. Ascites were obtained from nude mice after i.p. injection with cultured hybridoma cells. The monoclonal antibodies, four immunoglobulin Gs and two pentameric immunoglobulin Ms, were partially purified to remove mouse immunoglobulin and then conjugated to biotin for immunocytochemical and immunohistochimical studies. With the avidin:biotin:peroxidase complex method to detect and amplify binding by the biotin-conjugated human monoclonal antibodies, we found the six antibodies to be reactive against cytoplasmic determinants in five short-term melanoma cultures and formalin-fixed paraffin-embedded melanoma tumors from four patients. The antigenic target of the antibodies identified was not carcinomaembryonic antigen. Two antibodies, 2-139-1 and 6-26-3, were studied in more detail. Each stained 25 of 25 specimens of melanomas. Little or no reactivity was detected against fixed sections of normal skin, which included tissues such as epidermis, dermis, monocytes, lymphocytes, and vascular endothelium. More striking was the absence of binding to melanocytes in the basal layer of the skin or to pigmented nevus cells. Both antibodies showed cross-reactivity against other tumors, in particular colonic and prostatic carcinomas. In the normal colon, reactivity was restricted to the surface of the columnar epithelium; no reactivity was detected against normal prostatic epithelium. Reactivity was also not observed against liver and lung. However, the epithelia of the renal tubules, pancreatic ducts, and salivary ducts were all reactive. These human monoclonal antibodies identify cytoplasmic melanoma-associated tumor antigens that appear different from the membrane antigens defined by serological approaches and by most mouse monoclonal antibodies.

INTRODUCTION

Melanoma is one of the tumors that have been most intensively studied immunologically. Its antigenic complexity was first appreciated through the use of xenoantisera (1, 2), but more recently the availability of a number of murine monoclonal antibodies has permitted the identification of several important antigens with greater accuracy than was previously possible. In a number of instances these antigens have been characterized biochemically (3-5). With rare exceptions (6, 7), mouse monoclonal antibodies have identified components of the cell membrane and, in fact, have largely been selected for their reactivity with the cell surface. One of the critical issues that cannot easily be addressed with xenobodies is which of the melanoma-associated antigens is immunogenic to a human being. In addition, the repertoire of possible antibody responses to melanoma can be determined only by the study of human antibodies, such as those derived from hybridomas, rather than xenobodies. A related concern is that there may be antigens that are immunogenic only in the individual host or the species of origin, but not in unrelated species, and cannot be detected except with auto- or alloantibodies (8).

Human antibodies against 3 classes of surface antigen of cultured melanoma cells have been demonstrated by classical serology in autologous sera of melanoma patients (9, 10). "Class 1" antigens are those expressed only on autologous tumor cells; "Class 2" antigens are found on autologous and allogeneic tumors and on a restricted range of normal cells, while "Class 3" antigens are found on most cultured cells. Only the first 2 classes of surface antigen are important in the humoral immune response of patients to their tumor. However, antibodies to those classes comprise only a small minority of those in the serum, occurring in approximately 10% of the patients. This low incidence may in part reflect the limitations of the serological approach.

Our alternative approach to the question of tumor antigenicity has been through the production and characterization of human monoclonal antibodies. The early success of Schlom, et al. (11) with human-mouse hybridomas in generating human monoclonal antibodies to breast carcinoma encouraged us in this direction, even in the absence of an optimal human myeloma fusion partner. In order to search for antigens that most closely resemble those on the tumor in vivo, we have used only recently explanted tumor cells in culture or sections of biopsies to define the reactivities of the antibodies, avoiding long-term cell lines. This design has allowed us to examine our antibodies against a variety of normal and tumor tissues, many of which do not grow in tissue culture.

We describe here 6 human monoclonal antibodies against melanoma-associated antigens. These antibodies were generated from the fusions of regional lymph node cells of 3 melanoma patients with the mouse myeloma cell line, M5. The antibodies have a very different pattern of reactivity from most mouse monoclonal antibodies, particularly in their affinity towards cytoplasmic rather than cell surface antigens and their high degree of selectivity for malignant tissues.

MATERIALS AND METHODS

Cell Cultures. The mouse myeloma cell line M5, a subline of SP2/OAg14 adapted to grow in agammaglobulinemic horse serum, was grown in DMEM supplemented with 10% heat-inactivated horse serum (Biocell Laboratories, Carson, CA), 2 mM glutamine, penicillin (100 units/ml), and streptomycin (1 µg/ml). The cells were passaged for 1 wk each mo in medium containing 8-azaguanine (20 µg/ml) to ensure sensitivity to aminopterin. Growth was completely inhibited by 4 × 10⁻⁶ M aminopterin.

Human short-term tumor cultures were established from surgical specimens and cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum. The glioblastoma cell culture was a generous gift from Dr. Mark L. Rosenblum, University of California at San Francisco.

Production of Hybridomas. A single cell suspension from lymph nodes of melanoma patients was prepared by gentle dissociation of the
lymph nodes were washed 4 times in prewarmed serum-free medium before fusion. Exponentially growing M5 myeloma cells were similarly washed to remove serum proteins. Lymphocytes and myeloma cells were fused at a ratio of 2:5:1 in 1 ml of 34% (wt/vol) polyethylene glycol (M, 1500; Aldrich Chemical Co., Milwaukee, WI) for 1 min at 37°C. After washing, the cells were resuspended in complete DMEM at 2 x 10^6/ml. One-tenth ml was pipetted into each well of 96-well microtiter plates (Costar 3596; Costar, Cambridge, MA). Another 0.1 ml of complete DMEM containing 4 x 10^-5 M hypoxanthine, 8 x 10^-7 M aminopterin, and 6.4 x 10^-6 M thymidine was added to each well the day after fusion. Clones of hybrid cells were first noted 10 to 14 days after fusion.

Quantitation of Human Immunoglobulins in Spent Medium. Spent medium from wells containing growing hybrid cell clones was collected for immunoglobulin production by an ELISA. Wells of 96-well flexible plates (Falcon 3912) were coated with 50 μl of a 1:1000 dilution of a goat anti-human immunoglobulin antiserum (Cappel Laboratories, Cocke ranville, PA) in carbonate buffer (pH 9.6) at 4°C overnight. The plates were washed 3 times in PBS containing 0.05% Tween 20. Fifty μl of the test samples were added to the wells and incubated for 30 min at room temperature. The plates were washed as above before an equal volume of a peroxidase-conjugated goat anti-human IgG (Cappel), diluted 1:500 in PBS:Tween 20 containing 1% bovine serum albumin (radioimmunoassay grade), was added for a second incubation period of 30 min. Peroxidase activity was assayed with 50 μg of o-phenylenediamine (400 

Immunoperoxidase Staining. Paraffin blocks of tissues were selected from the files of the Department of Pathology at the University of Southern California. The procedures for removal of paraffin and fixation have been described (11). The sections were reacted with the biotin-conjugated monoclonal antibodies or an equivalent amount of a pooled human immunoglobulin control preparation (Sigma) for 1 h at room temperature. After further washing with PBS, an avidin:biotin:per oxidase complex prepared according to vendor's instruction (Vector Laboratories, Burlingame, CA) was added for 30 min as the indirect reagent. Peroxidase activity was determined by adding AEC (0.4 mg/ml; Sigma) in 0.1 M sodium acetate buffer (pH 5.2) containing 0.015% hydrogen peroxide for 30 min. The sections were briefly counterstained with Mayer's hematoxylin (Sigma) and mounted under a coverslip with Aquamount (Sigma). A red precipitate denoted binding by the human monoclonal antibody. AEC was preferable to dianisobenzidine for studies with melanoma tissues; the red precipitate was easily distinguishable from dark brown melanin granules associated with some melanoma cells.

A polyclonal rabbit anti-CEA antibody (Dako Immunoglobulin Ltd., Santa Barbara, CA) and a mouse monoclonal anti-CEA antibody, CEJ-326 (Hybritect, San Diego, CA), were used to identify CEA in the short-termed tumor cultures. The rabbit reagent was developed in 2 steps, first with peroxidase-conjugated swine anti-rabbit immunoglobulin and then with an amplification by peroxidase-conjugated rabbit antiperoxidase immunoglobulin. The mouse reagent was developed also in 2 steps with biotin-conjugated horse anti-mouse immunoglobulin and then with the avidin:biotin:peroxidase complex. The dilutions of all reagents were established to minimize background and optimize CEA detection in frozen sections of colon carcinoma. In our experiments, a 50-fold excess of the primary antibodies was used to increase the sensitivity of the tests.

RESULTS

Generation and Characterization of Human-Mouse Hybridomas. Lymphocytes from lymph nodes draining the primary tumor sites from 9 patients with malignant melanoma were fused with the M5 mouse myeloma line. Only grossly negative nodes, with minimal or absent microscopic metastases, were used. The percentage of wells plated that yielded viable hybrid cells varied with each experiment, ranging from 0 to 38% (average, 15.5%) at 2 wk after fusion (Table 1). Only cells that continued to secrete more than 1 μg of human immunoglobulin per ml by Day 30 were cloned. Some of these clones were relatively "stable," continuing to secrete immunoglobulin for 60 to 120 days. IgM hybridomas, in general, appeared to be more stable. By recloning the hybridoma every 2 to 3 mo, we have been able to carry some clones for over a year.

Six hybridoma cell lines from 3 different patients were ob-
tained from 6 separate hybrid cell cultures producing immunoglobulin for more than 30 days. Each clone was selected from a different well of the origin plating. Thus, the 6 clones were the progeny of 6 separate lymphocytes. Mouse immunoglobulin was not detectable in the supernatant fluids from wells containing the hybridoma, with an assay whose limit of sensitivity was 10 ng of mouse immunoglobulin per ml.

Four of the hybridomas secreted IgG at concentrations of approximately 1 µg/ml into the growth medium. All bound to 125I-Protein A. The other 2 antibodies (10-75-31 and 10-52-10) were pentameric IgMs, as determined by velocity sedimentation analysis with polyclonal human IgM (pentameric 19S) and IgG (7S) as markers, and were secreted at greater than 10 µg/ml. The IgG antibodies were further isoptyped. Three of them were IgG1, and one was IgG2 (Table 2). The light chain of 5 of the 6 was κ; only that of 6-26-3 was λ. Antibodies 2-139-1 and 6-26-3 fixed complement in a standard sheep erythrocyte hemolytic assay (performed by Dr. Hungyi Shau in our laboratory). Of the other antibodies, 10-3-44 (IgG1), 10-52-10 (IgM), and 10-75-31 (IgG) failed to fix complement, and 6-35-4 was not tested.

Immunoreactivity of Human Monoclonal Antibodies against Short-Term Cultures. We first tried to demonstrate reactivity of these human monoclonal antibodies against formaldehyde-fixed melanoma cell cultures using indirect immunofluorescence staining. The 6 antibodies all stained cytoplasmic determinants whereas an equivalent amount of (polyclonal) normal human immunoglobulin failed to stain the cells at all. However, the intensity of specific immunofluorescence was low, even when the fixed cells were treated with saponin to make the membrane more permeable to large molecules. This low level of reactivity made it difficult to measure the relative staining of several tumor targets by this technique. Photomicrographs were also difficult to obtain for the same reason.

To define their spectrum of the reactivity more precisely, we conjugated the partially purified antibodies from ascitic fluids directly to biotin. Binding was amplified by an avidin:biotin:horseradish peroxidase complex and visualized by the precipitation of red granules of the peroxidase substrate AEC. Normal human polyclonal IgG and IgM were also similarly conjugated with biotin, to be used as controls. As shown in Fig. 1A, reactivity was found with the antibody 2-139-1 in the cytoplasm of the melanoma cells indicated. In contrast, an equal concentration of polyclonal human IgG (2.5 µg/ml) failed to stain the cells (not shown). Antibody 2-139-1 at the same concentration had little reactivity against a glioma cell culture, indicated by the lack of staining in Fig. 1B.

Table 2 summarizes the reactivities of the 6 monoclonal antibodies to short-term cultures of 5 melanomas, a glioma, and a squamous cell carcinoma of the lung. All 6 antibodies were found to be reactive against cytoplasmic determinants in these melanoma cells. No binding was detected with the same concentration of normal polyclonal human immunoglobulin. Under these conditions, the antibodies had little or no reactivity against the 2 nonmelanoma tumor cultures tested. The specificity of binding, as studied with various tissues, will be addressed in more detail later. However, these data showed that our antibodies did not artifactually bind to substances such as constituents of the medium that were associated with the cells in culture. To avoid any possible loss of antigenic expression associated with prolonged cultures, short-term tumor cultures of fewer than 20 passages were used initially to screen for the reactivities of our antibodies. We have since found that these cytoplasmic antigens are expressed in melanoma cultures even after 100 passages and, in fact, were detected in the melanoma cell line M21.

Absence of Reactivity to the Cell Surface. While we saw no apparent staining of the cell membrane of fixed cultured melanoma cells by any of the 6 antibodies, we specifically investigated whether we could detect staining of viable, unfixed cells with 3 of the antibodies, 2-139-1, 6-26-3, and 10-75-31. As depicted for antibody 10-75-31 in Fig. 2A, there was no surface fluorescence with any of the 3 human antibodies, nor with the negative control, P3X63. Phase-contrast microscopy was required to ascertain that the melanoma cells were present and in proper focus in the microscopic field examined (Fig. 2B). In contrast, the positive antibody control, W6/32 shown in Fig. 2C, gave strong peripheral staining of 100% of the melanoma cells. We thus found no compelling evidence for any represen-
HUMAN MONOCLONAL ANTIBODIES

Fig. 2. Attempt at immunofluorescence staining of viable, unfixed M21 melanoma cells with human monoclonal antibody 10-75-31 and a control monoclonal antibody to HLA-A, B, C antigens (W6/32). A, failure of 10-75-31 to stain the membrane of viable melanoma cells; B, phase-contrast photomicrograph of the same field, showing the presence of melanoma cells; C, staining of viable melanoma cells in the same experiment by monoclonal antibody W6/32. x 800.

Table 3 Immunoreactivities of human monoclonal antibodies against fixed sections of melanomas

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Cases studied (stained/total)</th>
<th>Intensity of staining</th>
<th>% of tumor cells stained</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-139-1</td>
<td>4/4</td>
<td>3+</td>
<td>70</td>
</tr>
<tr>
<td>6-26-3</td>
<td>4/4</td>
<td>2+</td>
<td>60</td>
</tr>
<tr>
<td>10-3-44</td>
<td>4/4</td>
<td>2+</td>
<td>55</td>
</tr>
<tr>
<td>10-52-10</td>
<td>4/4</td>
<td>1-2+</td>
<td>60</td>
</tr>
<tr>
<td>10-75-31</td>
<td>4/4</td>
<td>3+</td>
<td>80</td>
</tr>
</tbody>
</table>
reactivity was against colonic (Fig. 3E) and prostatic carcinomas (Fig. 3C) (Table 4). Cross-reactivity was clearly not restricted to cells of neuroectodermal derivation, in contrast to the reactivities found with many mouse monoclonal antibodies to melanoma cells. The 2 specimens of mammary fibroadenoma, 3 of benign prostatic hypertrophy (Fig. 3D), and 1 specimen each of a small cell carcinoma and adenocarcinoma of the lung were not reactive.

No reactivity was observed against normal skin, liver, and lung (Table 5). The majority of the tissue components of colon, kidney, pancreas, and salivary gland were also not stained. However, binding was detected at the tip of the villi of normal colon, i.e., the surface epithelium (Fig. 3F). The collecting tubules in the kidney, which are non-specifically reactive with many mouse monoclonal antibodies,4 were also stained as were the ducts of the pancreas and salivary gland. Both antibodies reacted strongly with fetal colon (2 to 3+), but not against 2 specimens of fetal brain. One antibody, 2-139-1, stained a small proportion of fetal liver cells in 1 of 2 specimens, but both were generally not reactive with this tissue. These 2 antibodies also did not bind to pigmented cells in a variety of benign acquired nevi, reacting only with dysplastic nevi, as we have reported in detail elsewhere (14).

DISCUSSION

We have generated 6 human monoclonal antibodies that recognize determinants in the cytoplasm of short-term primary melanoma cultures and formaldehyde-fixed, paraffin-embedded tumor tissue sections. We chose to fuse human lymphocytes from regional lymph nodes with a mouse myeloma cell line because of the success reported in breast cancer with this approach (11, 15). From 9 successful fusions with the M5 mouse myeloma line, we generated 6 human monoclonal antibodies, 2 of which were IgMs and 4, IgGs, all of which were reactive against melanoma cells.

Differences in the properties of the lymphoid cell preparations or the use of a different line of mouse myeloma cell as

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4 C. R. Taylor, unpublished data.
fixation with formaldehyde, brief exposures to organic solvents, such as M21. The antigenicity is stable to treatments such as passages in culture, in the Dru culture, as well as in cell lines cells, they are conserved even after continuous growth and 100 unnecessary. Not only are the antigens present in uncultured to the use of short-term cultures of melanoma now appears appeared to be diffuse, although ultrastructural studies may ulti- plasmic determinants in the melanoma cells. The binding ap- reported by other investigators (17, 18).

frequency of anti-melanoma antibody-producing clones re- to human tumors. This might explain the apparently lower antigens, or relatively low affinity antibodies, both of which sensitive to detect reactivity against relatively sparsely represented structures. It is also possible that there are different antigens malignant tissues may simply be quantitative rather than ab- difference in binding that we observed between normal and for example, the endothelium of small blood vessels, a tissue that has shown intense reactivity with 10 different mouse monoclonal antibodies generated against melanoma cells.4 The difference in binding that we observed between normal and malignant tissues may simply be quantitative rather than absolute. It is also possible that there are different antigens containing the same epitopes in tumors and normal cells (20), where the antigen in the normal cells is more sensitive to degradation by the fixation procedure. Regardless of the explana- tion for our findings, the wide differences between peroxidase staining of melanoma cells and their normal counterparts suggest that our antibodies could be of some importance as diagnostic reagents in surgical pathology.

A major objective in our studies is to analyze the humoral response of melanoma patients to their tumor. The antigenic system defined by the human monoclonal antibodies appears to be distinct from the 3 classes of melanoma cell surface antigens defined by serological studies, in particular those experiments called “autologous typing” (21). The human antibodies identify molecules that are not Class 1 antigens, “private” antigens restricted to the autologous melanoma. Although they are shared by melanomas and related tumors such as astrocy- tomas, as are the Class 2 antigens defined by serology, these molecules are also found in embryologically unrelated tumors.

Table 4 Reactivity of 2-139-1 and 6-26-3 against fixed sections of human tumors

<table>
<thead>
<tr>
<th>Organ</th>
<th>Cases studied (stained/total)</th>
<th>Antibody 2-139-1</th>
<th>Antibody 6-26-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>0/3</td>
<td>2+</td>
<td>0/3</td>
</tr>
<tr>
<td>Colon</td>
<td>2/2</td>
<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>Liver</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Lung</td>
<td>0/2</td>
<td>1+</td>
<td>0/2</td>
</tr>
<tr>
<td>Kidney</td>
<td>6/6</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>Other structures</td>
<td>2/2</td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>1/1</td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td>Collecting ducts</td>
<td>0/1</td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td>Other structures</td>
<td>0/2</td>
<td>1+</td>
<td>1+</td>
</tr>
</tbody>
</table>

and periods of moderately elevated temperature, all of which are involved in the standard processing of pathology specimens. The antigenic target of human monoclonal antibody MBE6 (19) was similarly detectable in paraffin-fixed sections of breast carcinoma. In contrast, the target (surface) antigens recognized by most mouse antibodies are generally degraded by such a fixation, although a higher frequency of successful staining has recently been reported.

One of the most remarkable findings in these studies is the ability of our antibodies to discriminate between normal and malignant tissues, at least in fixed tissue sections. The melanoma-associated antigens appear to be absent, or at much reduced density, in the normal tissues examined. Of particular interest is the lack of reactivity of the antibodies with normal melanocytes in the dermal-epidermal junction and in intradermal nevi, or with fetal brain tissues. In contrast to many of the mouse monoclonal antibodies generated against human melanoma cells, the human antibodies appear not to recognize differentiation antigens associated with pigmented cells or those of neuroectodermal derivation. In addition, the human antibodies did not bind to the endothelium of small blood vessels, a tissue that has shown intense reactivity with 10 different mouse monoclonal antibodies generated against melanoma cells.4 The difference in binding that we observed between normal and malignant tissues may simply be quantitative rather than absolute. It is also possible that there are different antigens containing the same epitopes in tumors and normal cells (20), where the antigen in the normal cells is more sensitive to degradation by the fixation procedure. Regardless of the explana- tion for our findings, the wide differences between peroxidase staining of melanoma cells and their normal counterparts suggest that our antibodies could be of some importance as diagnostic reagents in surgical pathology.

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Table 5 Absence of reactivity of 2-139-1 and 6-26-3 with fixed sections of normal tissues

<table>
<thead>
<tr>
<th>Organ</th>
<th>Cases studied (stained/total)</th>
<th>Antibody 2-139-1</th>
<th>Antibody 6-26-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>0/3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Colon</td>
<td>2/2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Liver</td>
<td>0/1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lung</td>
<td>0/2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kidney</td>
<td>6/6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0/0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other structures</td>
<td>2/2</td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>1/1</td>
<td>1+</td>
<td>1+</td>
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<tr>
<td>Other structures</td>
<td>0/2</td>
<td>1+</td>
<td>1+</td>
</tr>
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</table>

fusión partner may account for our greater frequency of obtaining monoclonal antibodies with reactivity to melanoma. An- other pertinent consideration might be the procedures that were used to screen for the antibodies and to determine their range of reactivity. Our human anti-melanoma antibodies have a relatively low level of binding to cells, as Sikora, et al. (16) have reported with their monoclonals. We were, in fact, compelled to use an amplification system, avidin:biotin:peroxidase com-plex, to increase the sensitivity of detection of the antibodies in supernatants. Indirect immunofluorescence may be too insen- sitive to detect reactivity against relatively sparsely represented antigens, or relatively low affinity antibodies, both of which may conceivably be the case with human monoclonal antibodies to human tumors. This might explain the apparently lower frequency of anti-melanoma antibody-producing clones re-ported by other investigators (17, 18).

All 6 of our human monoclonal antibodies identified cyto- plasmic determinants in the melanoma cells. The binding ap- peared to be diffuse, although ultrastructural studies may ulti- mately reveal a more specific subcellular localization. Our antib-odies, unlike other human monoclonal antibodies (17, 18), do not appear to stain the cytoskeleton. Our original restriction to the use of short-term cultures of melanoma now appears unnecessary. Not only are the antigens present in uncultured cells, they are conserved even after continuous growth and 100 passages in culture, in the Dru culture, as well as in cell lines such as M21. The antigenicity is stable to treatments such as fixation with formaldehyde, brief exposures to organic solvents,
such as colon and prostate carcinomas. In contrast to Class 3 antigens, however, they are not detectable in normal cells or normal melanocytes. Autologous typing, like the use of mouse monoclonal xenon antibodies, preferentially studies antibodies to surface antigens, which may explain why human antibodies to cytoplasmic determinants such as ours have been relatively overlooked.

Our attempts to characterize the target antigens of our human monoclonal antibodies have thus far been fairly unsuccessful. We have tried to immunoprecipitate the antigens directly from lysates of melanoma cells labeled biosynthetically with [35S]-methionine for 24 h, but many cellular proteins have been coprecipitated, making it difficult to ascertain which was the specific target protein. The target cytoplasmic antigens may be present in a much lower concentration than the surface antigens identified by mouse monoclonal antibodies, judging from our need to amplify immunohistochemical assays to discern them. If this proved to be true, our difficulties with immunoprecipitation would be consistent with the experience of others in attempting to identify trace amounts of cellular antigens with monoclonal antibodies. Our one notable success has been with antibody 10-3-44. This antibody precipitated 2 proteins, M, 125,000 and M, 220,000, on sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis, that were strongly labeled with [32P]ATP. In other words, the target antigen of this antibody was either a self-phosphorylating kinase or was coprecipitated with such a kinase. We are now investigating whether the target of 10-3-44 was, in fact, an oncogene product as these preliminary results suggest. The other target antigens are being studied by several complementary approaches, including concentration of the melanoma antigens by affinity chromatography before performing immunoprecipitation, and the use of Western immunoblotting as an alternative procedure.

There are several theoretical reasons why human antibodies might react against antigens common to all melanomas while recognizing differences between normal and malignant cells. Most obviously, human beings should be able to discriminate between HLA-A or tissue antigens when reacting against their own melanoma. But also, more pessimistically, the human antibodies we have generated may represent the humoral immune responses of patients who require lymph node resection, i.e., those who have failed to control their disease. From such individuals it might not be possible to capture strong antibodies directed against highly specific or unique determinants associated with the melanoma, if antibodies, in fact, have a role in controlling the growth of the tumor. In vitro immunization of normal lymphocytes might be a more desirable, if difficult, means of obtaining useful antibodies. Nevertheless, the availability of the antibodies we have described and other human monoclonal antibodies with reactivity against melanoma cells will now make it possible to study the range of potential antibody responses of melanoma patients to their tumors and their cellular targets in far greater detail. Such information is particularly relevant to the design of an effective melanoma tumor vaccine, where the inclusion of antigens that are immunogenic to melanoma patients is of great importance.

ACKNOWLEDGMENTS

We gratefully acknowledge the help of Jay C. Owens, M.D., James T. Helsper, M.D., Peggy Fletcher, R.T., and Virginia Kortes, R.N., in the acquisition of lymph nodes for the fusions we have described here. We also thank Dr. Philip Fernsten for his collaboration and guidance in experiments on the staining of viable cells.

REFERENCES

Human Monoclonal Antibodies Directed against Melanoma Tumor-associated Antigens


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