Cytotoxic Antibody Reactivity in Sera of Melanoma Patients against Allogeneic and Autologous Cultured Tumor Cells and Fibroblasts


ABSTRACT

Using a complement-dependent microcytotoxicity assay, sera from melanoma patients were analyzed for antibody reactivity with cultured melanoma and normal adult skin fibroblasts. Sera from Stage I tumor-bearing patients prior to surgical excision, and poor-prognosis Stage I and Stage II patients after tumor excision and lymphadenectomy but prior to adjuvant therapy, and normal individuals with a similar age and sex distribution were tested against a melanoma-fibroblast pair from an allogeneic donor. The groups displayed a wide range of cytotoxicity against both cell types, and no serum possessed melanoma-specific reactivity. Mean cytotoxicity of the Stage I tumor-bearing group was not significantly different (p > 0.05) from that of the normal group for either target cell, and patients whose tumors went on to recur were not different from non-recurrent patients. The Stage I and II postlymphadenectomy patients were not different from the normals in fibroblast reactivity. However, melanoma reactivity was significantly higher in the postlymphadenectomy patients than the normals (p < 0.02). This was the result of an elevated reactivity in the patient population who remained disease free compared to patients whose tumors went on to recur (p < 0.01), although a large overlap existed between these two groups. Cytotoxicity against autologous melanoma and fibroblasts was observed with sera obtained throughout the clinical course of four Stage II patients, and no melanoma-specific reactivity was detected. Absorption with cultured fetal fibroblasts of sera from Stage II patients both before and after immunotherapy with Bacillus Calmette-Guérin and autologous melanoma removed reactivity against autologous and adult melanoma and adult fibroblasts. Therefore, the predominant antibody reactivity detected in these patients was directed against common fetal fibroblast-associated antigens, and no evidence was obtained for the presence of antibodies reactive with unique or shared tumor-specific antigens.

INTRODUCTION

Numerous investigators have examined sera from melanoma patients for the presence of antibodies directed against tumor-associated antigens. Many assay systems have been utilized, including those which measure the ability of antibody bound to the tumor cell surface to fix complement and result in cell damage. The studies involving detection of complement-dependent cytotoxic antibodies have yielded contradictory results, and in most instances conclusions concerning tumor specificity have been based on insufficient testing of normal tissues or sera of normal individuals. It has been reported that the cytotoxicity of sera from melanoma patients was generally restricted to autologous tumor cells, indicating that the predominant antibody response was against individually unique tumor antigens (1, 15, 19). In contrast, several studies have found no evidence for unique antigens but have detected an antibody response to shared antigens that may be specific only to melanomas (4) or broadly expressed on a variety of cancers (8).

Previously, we have analyzed the cytotoxic antibody response to human osteogenic sarcoma. A majority of human sera were found to possess antibodies against both allogeneic and autologous sarcoma and normal skin fibroblasts in tissue culture (6, 23). This natural antibody reactivity was directed against fetal antigens expressed on these cells in tissue culture (32, 33). Following absorption with fetal cells, sera from osteosarcoma patients but not normal individuals were demonstrated to contain additional reactivity against common antigens present on most osteosarcomas (24, 25, 33).

Because of the confusion surrounding the serological studies of melanoma with complement-dependent cytotoxicity techniques, we have attempted an analysis using an approach similar to that used in our studies of osteogenic sarcoma. Sera were obtained from melanoma patients and normal individuals and tested for reactivity against both allogeneic and autologous melanoma and normal skin fibroblasts in tissue culture. Absorption of fetal fibroblasts was performed to determine if residual antibodies directed against tumor-specific antigens were present in sera of melanoma patients. Patients and normal individuals were found to be similar in their reactivity to both cell types, and significant cytotoxicity was observed against autologous as well as allogeneic cells. However, increased levels of tumor reactivity were detected in a group of patients with advanced disease and were associated with a more favorable prognosis. The predominant reactivity appeared to be directed against common fetal fibroblast-associated antigens, and no evidence for antibodies to unique or shared tumor-specific antigens was obtained with any sera.

MATERIALS AND METHODS

Tissue Culture. Melanoma and normal skin tissues, obtained from patients at the time of surgery, and human first-trimester whole fetal tissue (Pre-Term Clinic, Washington, D. C.) were established in tissue culture by explant techniques described previously (23, 32, 33). Once established, cell lines were routinely cultured in Eagle’s minimum essential medium supplemented with 10 to 20% heat-inactivated and...
Cytotoxic Antibodies in Sera of Melanoma Patients

Filtered (0.45 μm) FBS, 2 100 units penicillin per ml, 100 μg streptomycin (Grand Island Biological Co., Grand Island, N. Y.) per ml, and 1 μg Fungizone (Flow Laboratories, Inc., Rockville, Md.) per ml. Confluent cultures were passaged by treatment with 0.25% trypsin and 0.5 mm EDTA in phosphate-buffered saline (10 mM P, and 0.15 M NaCl, pH 7.4). Melanoma cell line SK-MEL-13 and autologous normal skin fibroblasts (designated 86 and 86A, respectively) were generously provided by Dr. Lloyd Old, Memorial Sloan-Kettering Cancer Center, New York, N. Y., and cultured as described above. Cultured melanoma cells used in these studies were identified as malignant by the presence of premelanosomes. Electron microscopy was kindly performed by Dr. Timothy Triche, Laboratory of Pathology, National Cancer Institute, Bethesda, Md. Except for melanoma line 86, all cells used in these studies were in the first 10 tissue culture transfer generations. Line 86 was used in generations 28 to 34.

Sera. Blood was collected from melanoma patients and from normal healthy individuals with no history of cancer. Sera were obtained by centrifugation, heat inactivated at 56° for 30 min, and stored at -20° prior to use in the microcytotoxicity assay. Twenty patients with Stage I, Level III-V, melanoma (no involved lymph nodes) were entered into a surgical trial conducted by the Surgery Branch of the National Cancer Institute. Sera were obtained from these patients after biopsy of the primary tumor but prior to surgical excision. Twenty-five patients with poor-prognosis Stage I, Level IV-V, disease (no involved lymph nodes) and 134 patients with Stage II disease (regional lymph node involvement) were entered into an adjuvant treatment trial conducted by the Surgery, Immunology, and Medicine Branches of the National Cancer Institute. Sera were obtained after excision of the primary tumor and within 1 week to 4 months following regional lymphadenectomy but prior to initiation of adjuvant treatment. Multiple sera were obtained throughout the clinical course of 4 Stage II patients entered into the adjuvant treatment trial. One patient received weekly (up to 12 weeks, then bimonthly) injections of viable Trudeau strain BCG using the heat gun technique. Three patients received BCG, as above, plus intradermal injections of viable cultured autologous melanoma cells pooled from 3 patients and treated with neumaminidase.

Postlabeling Microcytotoxicity Assay. Sera were evaluated for complement-dependent cytotoxicity against adherent tissue cultured target cells as described previously (2, 3). In brief, target cells were obtained by trypsinization of confluent cultures, and 300 cells in 10 μl of complete medium were seeded into wells of microtiter plates (No. 3034; Falcon Plastics, Oxnard, Calif.). Complete medium consisted of Roswell Park Memorial Institute Tissue Culture Medium 1640 (Grand Island Biological Co.) supplemented with 20% heat-inactivated FBS, 100 units penicillin per ml, and 100 μg streptomycin per ml. After overnight incubation at 37° with 5% CO2 and high humidity, the plates were humidified once with 10 ml HBSS, and the wells were aspirated gently. Four μl of test sera, either undiluted or serially diluted in complete medium, were added to each well, and after incubation for 2 hr at 37° the plates were washed 3 times with HBSS. Ten μl of newborn rabbit serum as a source of complement, diluted 1:6 in complete medium, were then added to each well. After 1 hr of incubation, the plates were washed twice with HBSS. The wells were then pulsed with 1 μCl [3H]leucine (110 Ci/mmol; New England Nuclear, Boston, Mass.) in 10 μl leucine-free complete medium with 1% FBS. After a 2- to 4-hr incubation, the wells were washed 4 times with phosphate-buffered saline and allowed to dry. Pairs of wells were removed with a bandsaw, and residual radioactivity was analyzed by liquid scintillation counting.

Serum dilutions were tested in triplicate pairs of wells. Each assay plate contained complete medium controls, complement controls, and serum controls, also in triplicate. The lytic capability was calculated as follows:

\[
\% \text{ of cytotoxicity} = \left(1 - \frac{\text{cpm in test wells}}{\text{cpm in control wells}}\right) \times 100
\]

The abbreviations used are: FBS, fetal bovine serum; BCG, Bacillus Calmette-Guerin; HBSS, Hanks' balanced salt solution; OIA, oncopetal antigen.

For the serum plus complement wells, the controls were wells exposed to complement alone. When the toxicity of the serum or complement controls was evaluated, the controls were wells exposed to complete medium alone. Complement control toxicity was less than 20% in all experiments, and serum control toxicity was less than 30% with all sera tested. Unless otherwise noted, sera were tested undiluted. Results are presented as mean percentage of cytotoxicity ± S.E.

Absorptions. Sera were absorbed with tissue-cultured human fetal fibroblast line HF-3 as described previously (25). In brief, cells were harvested by trypsinization of confluent cultures and washed with complete medium, and appropriate numbers of cells were pelleted in centrifuge tubes. The pellets were then mixed with test sera and incubated for 2 hr at 4° with intermittent mixing. Recovered sera were stored at -20° before use in the microcytotoxicity assay.

Immunoglobulin Determinations. IgG, IgA, and IgM levels in patient sera were analyzed by an automated immune precipitation technique (Clinical Pathology Department, NIH, Bethesda, Md.).

Statistical Analysis. Results were analyzed by 2-tailed t tests for unpaired measurements and by linear regression analysis (31). Differences were considered significant if p was less than 0.05.

RESULTS

Reactivity of Sera from Stage I Tumor-bearing Patients. Sera from 20 tumor-bearing Stage I melanoma patients entered in the surgery trial and 19 normal donors with a similar age and sex distribution were tested for cytotoxicity simultaneously against an allogeneic melanoma tissue culture cell line (line 86) and its autologous normal skin fibroblasts (line 86A). The results are shown in Chart 1. The groups displayed a wide range of cytotoxicity against both cell types. Serum reactivity was generally higher against fibroblasts than against melanoma. With the possible exception of one patient serum showing 20.5 ± 6.3 and -3.4 ± 2.7% cytotoxicity against melanoma and fibroblasts, respectively, no serum displayed unique reactivity against the cultured melanoma line. When the mean values of the 2 groups were compared, patient serum cytotoxicity against melanoma (23.3 ± 5.3%) was not significantly

<chart image> Chart 1. Serum reactivity of 20 Stage I tumor-bearing melanoma patients and 19 normal controls against autologous melanoma (line 86) and paired skin fibroblasts (line 86A). Patients are divided into those whose tumors recurred and those who remained disease free.
different from the normal controls (31.4 ± 6.1%). Similarly, patient serum reactivity against fibroblasts (68.5 ± 6.0%) was not significantly different from the normal controls (72.6 ± 7.3%). In addition, the patients were divided into those whose tumors went on to recur and those who remained disease free after excision of the primary melanoma (follow-up minimum, 2.5 years; median, 3.8 years). Melanoma reactivity in the recurrent patients (17.9 ± 7.0%), although lower than that of the nonrecurrent patients (31.5 ± 7.7%), was not significantly different at the p < 0.05 level. Fibroblast reactivity in the recurrent patients (69.8 ± 6.7%) also was not significantly different from that of the nonrecurrent patients (66.5 ± 11.9%).

Reactivity of Sera from Stage I and II Postlymphadenectomy Patients. Sera from 5 Stage I and 35 Stage II patients in the adjuvant treatment trial were obtained shortly after lymphadenectomy but prior to initiation of treatment. These sera, along with 20 sera from normal individuals with a similar age and sex distribution, were tested against the melanoma-fibroblast pair 86 and 86A (data not shown). Again, a wide range of cytotoxicity was observed against both cell types, and no serum displayed melanoma-specific reactivity. Mean cytotoxicity of the patient group was not significantly different from that of the normals for either target cell. However, when the patients were divided into those whose tumors would recur and those who would remain disease free, melanoma reactivity but not fibroblast reactivity was significantly higher in the nonrecurrent patients.

As a result of this finding, a further experiment was performed with sera from 25 Stage I and 134 Stage II postlymphadenectomy patients and 50 normals. The sera were tested only with the melanoma cell line 86. The results are shown in Chart 2 and Table 1. Patients were divided into those whose tumors went on to recur and those who remained disease free after lymphadenectomy (follow-up minimum 1 year; median, 2.5 years). As summarized in Table 1, sera from melanoma patients had significantly higher levels of cytotoxicity than the normal controls (p < 0.02). Patients who remained disease free had significantly higher levels than either the controls (p < 0.001) or the patients whose tumors recurred (p < 0.01). When the data were stratified for sex, the trends remained in both males and females, although differences were more pronounced in the male sera. There was, however, considerable overlap in all of these populations, and the cytotoxicity of any individual patient serum was not of strong predictive value.

Serum control toxicity in the absence of complement was similarly analyzed. Toxicity values for all patients (−2.9 ± 1.1%), disease-free patients (−4.6 ± 1.7%), recurrent patients (−1.4 ± 1.4%), and normal controls (−6.7 ± 2.3%) were not significantly different from each other. This indicated that the significant cytotoxicity differences among the groups in the presence of complement were not due to nonspecific serum toxicity.

A linear regression analysis was performed to determine whether cytotoxicity values correlated with age at the time of serum collection. A weak but significant negative correlation was observed in the patient group (r = 0.23; p < 0.01), indicating that older patients tended to have lower cytotoxicity values. The normal controls did not show a significant correlation with age (r = 0.15; p > 0.05). Mean age (years) ± S.E. for all patients (42.1 ± 1.1), disease-free patients (42.1 ± 1.6), recurrent patients (42.0 ± 1.5), and normal controls (39.8 ± 1.9) did not differ significantly. Thus, although a significant negative correlation was demonstrated between cytotoxicity and age, it was unlikely that this effect accounted for the observed cytotoxicity difference among the various groups.

Immunoglobulin levels were determined on sera from 106 patients. Sera were obtained within 1 month of those used in the cytotoxicity study. A linear regression analysis indicated no

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**Table 1**

Summary of serum reactivity of Stage I and II postlymphadenectomy patients against allogeneic melanoma (line 86). Data were stratified for sex and clinical course.

<table>
<thead>
<tr>
<th>Sera</th>
<th>Both sexes</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. All patients</td>
<td>25.2 ± 2.6 (159)</td>
<td>28.6 ± 3.4 (109)</td>
<td>17.8 ± 3.8 (50)</td>
</tr>
<tr>
<td>2. Nonrecurrent</td>
<td>32.9 ± 4.1 (75)</td>
<td>37.1 ± 5.4 (49)</td>
<td>25.1 ± 5.6 (28)</td>
</tr>
<tr>
<td>3. Recurrent</td>
<td>18.2 ± 3.3 (84)</td>
<td>21.6 ± 4.1 (60)</td>
<td>10.0 ± 4.8 (24)</td>
</tr>
<tr>
<td>4. Normals</td>
<td>12.9 ± 3.3 (50)</td>
<td>14.0 ± 4.3 (35)</td>
<td>10.6 ± 4.7 (15)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>p values</th>
<th>1 vs. 4</th>
<th>2 vs. 4</th>
<th>3 vs. 4</th>
<th>2 vs. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p &lt; 0.02</td>
<td>p &lt; 0.001</td>
<td>NS</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.02</td>
<td>p &lt; 0.01</td>
<td>NS</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.01</td>
<td></td>
<td>NS</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

4 Mean ± S.E.
5 Numbers in parentheses, sample size.
6 NS, not significant (p > 0.05).
significant correlation between cytotoxicity and levels of IgG ($r = 0.05$), IgA ($r = 0.12$), or IgM ($r = 0.14$). In addition, immunoglobulin levels in the patients who went on to recur were not significantly different from those who remained disease free. It is unlikely, therefore, that the cytotoxicity results were a simple reflection of total immunoglobulin levels.

Reactivity of Sera from 4 Stage II Patients to Autologous Cells. Paired melanoma and normal skin fibroblasts from 4 Stage II recurrent patients in the adjuvant treatment trial were established in tissue culture. Multiple sera obtained throughout the clinical course of these patients were tested against their autologous cells. The results are shown in Chart 3. Reactivity against both tumor and normal cells was observed with all 4 patients, and in general, higher reactivity was obtained against fibroblasts than against melanoma. No unique tumor reactivity was displayed with any sera. Patient M. H. showed a rapid rise in melanoma and fibroblast reactivity within 2 months after start of therapy with BCG and allogeneic melanoma. Patient V. G., also receiving BCG and allogeneic melanoma, displayed a more gradual increase in tumor reactivity after start of therapy. Because fibroblast cytotoxicity was nearly 100% prior to therapy, an increase in posttreatment reactivity could not be determined in this case. Patient T. H., treated with BCG and allogeneic melanoma, and Patient H. E., treated with BCG only, displayed less clearly defined changes in serum reactivities over the clinical course.

Absorption with Fetal Fibroblasts. Reactive sera from 6 Stage II postlymphadenectomy patients were absorbed with cultured human fetal fibroblasts and tested against the allogeneic melanoma-fibroblast pair 86-86A (Table 2). Although the single fetal cell absorption only partially removed fibroblast reactivity, essentially all melanoma reactivity was removed from the 6 sera. Thus, no evidence was obtained in any patient for antibodies specifically reactive with allogeneic melanoma.

A fetal absorption analysis was also performed on sera obtained from 2 Stage II patients during the course of therapy with BCG and allogeneic melanoma. Because of possible interference due to anti-HLA reactivity generated during the course of allogeneic immunization, the sera were tested against autologous as well as allogeneic cells. The results are shown in Table 3. Sera from Patient T. H. displayed essentially complete loss of reactivity against both autologous and allogeneic cells after absorption. Although all autologous reactivity in sera of Patient V. G. was removed, reactivity against both allogeneic melanoma and fibroblasts remained essentially unchanged after absorption, possibly due to high-titered anti-HLA antibodies. The results gave no evidence for an autologous or allogeneic tumor-specific reactivity generated after immunotherapy with BCG and allogeneic melanoma.

**DISCUSSION**

The studies reported here indicate that sera of melanoma patients and normal individuals contain various levels of antibodies reactive with both cultured melanoma and normal skin fibroblasts. The assay used was a complement-dependent

### Chart 3

Reactivity against autologous melanoma and skin fibroblasts of multiple sera from 4 recurrent Stage II patients. Patient H. E. received BCG therapy. Patients V. G., M. H., and T. H. were treated with BCG and allogeneic melanoma cell vaccine (BCG + Vac).

### Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Absorbed with a</th>
<th>Melanoma</th>
<th>Fibroblasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:1 b</td>
<td>1:2</td>
<td>1:1</td>
</tr>
<tr>
<td>P. L.</td>
<td>63.5 ± 4.2 c</td>
<td>31.7 ± 2.9</td>
<td>73.8 ± 1.0</td>
</tr>
<tr>
<td>A. S.</td>
<td>7.7 ± 7.3</td>
<td>9.6 ± 9.5</td>
<td>47.7 ± 5.8</td>
</tr>
<tr>
<td>M. M.</td>
<td>39.8 ± 2.6</td>
<td>27.7 ± 2.3</td>
<td>94.0 ± 0.7</td>
</tr>
<tr>
<td>D. T.</td>
<td>8.6 ± 6.8</td>
<td>8.7 ± 7.6</td>
<td>42.6 ± 9.4</td>
</tr>
<tr>
<td>T. F.</td>
<td>5.3 ± 9.4</td>
<td>46.7 ± 12.1</td>
<td>98.5 ± 0.3</td>
</tr>
<tr>
<td>E. M.</td>
<td>0.0 ± 9.4</td>
<td>4.2 ± 5.8</td>
<td>53.5 ± 5.7</td>
</tr>
<tr>
<td>H. F.</td>
<td>39.8 ± 4.2</td>
<td>0.0 ± 2.6</td>
<td>48.6 ± 1.4</td>
</tr>
</tbody>
</table>

a Undiluted sera were absorbed with 8.4 x 10^7 HF-3 cells/ml.
b Serum dilution.
c Mean ± S.E.
of both cytolytic and cytostatic (2). Cell damage is detected as a decrease in the number of adherent cells and an inhibition of protein synthesis in the remaining adherent cells. A minority of sera from both patients and normals yielded negative cytotoxicity values, probably as a result of serum factors which stimulate protein synthesis in cultured cells. Inasmuch as the average serum control values in the absence of complement in the Stage I and II postlymphadenectomy study were very close to zero, the overall contribution of the stimulatory effect to the cytotoxicity results was probably minimal.

Positive cytotoxicity was observed in sera from patients and normals against both cell types of an allogeneic melanoma-fibroblast pair and in sera of patients against both autologous tumor cells and fibroblasts. In addition, with the possible exception of one serum from a Stage I tumor-bearing patient, no melanoma-specific reactivity was detected. These results are in contrast to the findings of Lewis et al. (15), also using a complement-dependent cytotoxicity assay, who reported that reactivity in sera of melanoma patients was limited to autologous cultured tumors and that no sera, from either patients or normals, reacted with allogeneic melanomas. The reason for the greater extent of reactivity obtained in the present study is not known but may be related to different degrees of sensitivity of the 2 cytotoxicity assay systems used.

Our results indicate that sera of poor-prognosis Stage I and Stage II melanoma patients, after primary lymphadenectomy but prior to adjuvant therapy, show a small but statistically significant increase in reactivity to an allogeneic melanoma tissue-cultured line but not to paired fibroblasts when compared to normal donors. The increased reactivity was not associated with patient age or serum immunoglobulin levels. Using complement-dependent cytotoxicity assays, other workers have also demonstrated increased reactivity to melanoma lines in patient sera. Thus, Canevari et al. (4) found that 31% of sera from melanoma patients gave positive reactions, while only 7% of normals and nonmelanoma cancer patients were positive. Ferrone and Pellegrino (8) demonstrated a 38% incidence of reactivity to melanoma lines in melanoma patients, while only 21% of normal donors reacted. Similar findings have also been described in studies utilizing assays involving immunofluorescence (18, 20, 22, 35) and immune adherence (7).

In contrast to the results with sera from Stage I and II postlymphadenectomy patients, tumor-bearing sera of Stage I patients were not significantly different from the normal control sera in reactivity against either the allogeneic melanoma cells or the paired fibroblasts. It should be noted that the 2 patient groups, along with their normal serum controls, were tested in different experiments. Due to day-to-day assay variability, cytotoxicity values between experiments cannot be compared directly. Several explanations may account for the increased reactivity observed in the poor-prognosis Stage I and II postlymphadenectomy patients but not in the Stage I tumor-bearing patients. The numbers of Stage I tumor-bearing patients may have been too small to detect a significant difference. Increased melanoma reactivity could be associated with regional disease, rather than with localized or widespread disease, as reported by several groups (4, 15, 35). Alternatively, surgical removal of tumor may be followed by increased immune responses to cross-reactive antigens expressed on both melanoma and normal repair tissue. The increased reactivity of the Stage I and II postlymphadenectomy patients versus the normals in our study was a result of increased reactivity in those patients who would remain disease free. Thus, melanoma reactivity was found to be significantly greater in those patients who remained disease free after lymphadenectomy when compared to normals, while the relapse patients were not significantly different from the normals. Although this indicated that higher antibody levels may
be associated with a more favorable prognosis, the overlap in cytotoxicity values with all populations studied precludes the use of this measurement in making predictions concerning any individual patient. The increased levels of cytotoxic antibodies in nonrecurrent patients suggests that the antibodies may be active in tumor cell destruction in vivo. However, the observed differences in antibody levels may not be directly related to tumor cell destruction but instead may be a result of other mechanisms such as absorption of antibody by residual tumor or immunosuppression in patients who recur. The association of increased survival with high antibody titers in melanoma patients has been described by Bodurtha et al. (1), also using a complement-dependent assay, and by Jones et al. (13), using an immunofluorescence assay. We have recently completed a study of serum reactivity to the melanoma-fibroblast pair 86-86A using an immune adherence assay (27). Paralleling our findings here, sera from Stage II but not Stage I patients were significantly more reactive than normal sera. However, no correlation was observed between levels of reactivity and clinical course. The reason for this discrepancy is not clear but may be related to the different levels of sensitivity in the 2 systems used.

Absorption with cultured fetal fibroblasts of sera from 6 Stage II postlymphadenectomy patients removed all reactivity against an alloimmune melanoma line. In addition, sera from 2 patients during the course of immunization with allogeneic melanoma lost reactivity to autologous and allogeneic melanoma after fetal absorption. These results suggest that the cytotoxic antibodies detected in sera of melanoma patients are directed against common fetal fibroblast antigens expressed on cultured melanoma cells. In light of the fact that sera may contain alloreactive antibodies, especially as a result of pregnancy or transfusion, one cannot entirely rule out the possibility that the antibodies are detecting histocompatibility antigens. The finding of positive reactivity with autologous sera and target cells suggests that alloantibodies to histocompatibility antigens, including la-like antigens found on some melanomas (34), are not responsible for the observed reactivity. However, alien histocompatibility antigens have been described in several tumor systems (21), and the presence of such antigens could result in reactivity under strictly autologous conditions.

Fetal fibroblast absorption also removed reactivity from sera of melanoma patients when tested against normal skin fibroblasts, which indicates that these normal cells in culture are also expressing common fetal fibroblast antigens. However, several lines of evidence suggest that reactivity to melanoma and fibroblasts may be mediated at least in part by different antibodies. Generally higher levels of reactivity were observed against fibroblasts than against the paired melanoma cells. The association of high melanoma cell reactivity with favorable prognosis in the postlymphadenectomy patients was not paralleled by significant differences in fibroblast reactivity. Also, with some of the sera, fetal fibroblast absorption only partially removed reactivity to fibroblasts. Alternatively, the higher fibroblast reactivity could be due to a greater susceptibility to complement-dependent lysis. As a consequence, the high cytotoxicity values might obscure the differences among disease-free and recurrent patients, and a single fetal cell absorption could be insufficient to remove all detectable reactivity. Further studies involving absorption with melanoma and fibroblasts will be required to make definitive conclusions concerning similarity of antigens detected on the 2 cell types.

Our previous studies of cytotoxic antibodies in sarcoma patients and normal individuals indicated that a majority of human sera were able to lyse both allogeneic and autologous sarcomas and skin fibroblasts in culture (23). Cytotoxicity levels were inversely correlated with age, and the antibodies were not directed against antigens acquired from the serum used in the culture medium (23, 32). Absorption studies with fresh fetal tissues and cultured fetal fibroblasts indicated that the natural antibodies were directed against cross-reactive fetal antigens expressed on both tumor and normal cells in tissue culture (25, 32, 33). These studies support our present findings and suggest that the reactivity detected in sera from melanoma patients is also due to antibodies directed against common fetal antigens expressed on both melanoma and normal fibroblasts in culture. Alternatively, the antibodies may be directed against fibroblast-associated antigens. To clarify this point, further absorption analyses involving fresh fetal and adult tissues will be required.

Irie et al. (12) and Seibert et al. (26), using immune adherence assays, also detected common fetal antigens on cultured melanoma cells with human sera. The OFA described by Irie et al. (12) was demonstrated on fetal brain, fresh and cultured melanomas and other tumors, and cultured normal cells. The similarity of OFA with the antigens detected in the present study is further supported by the finding that elevated serum anti-OFA levels are associated with a favorable prognosis in Stage II melanoma patients (13). Although similar anti-OFA levels were found in melanoma patients and normals (17), immunotherapy with allogeneic melanoma cells bearing OFA produced increased anti-OFA antibody levels in the patients tested (10). In our study, because only 3 Stage II patients receiving therapy with BCG plus allogeneic melanoma were tested over the clinical course and with variable results, we cannot draw any conclusions regarding the immunogenicity of the fetal antigens.

In previous analyses of osteosarcoma, absorption of patient sera with fetal cells permitted the identification of residual antibodies in 9 of 11 patient sera directed against tumor-specific antigens (24, 25, 33). In our present study, fetal cell absorption of 10 sera from Stage II melanoma patients, either postlymphadenectomy or during immunotherapy, did not reveal the presence of residual antibodies directed against allogeneic or autologous tumor-specific antigens. A number of studies have demonstrated the existence of antibodies in melanoma patient sera with specificity for antigens expressed only on the surface of tumor cells. In addition to the findings of Lewis et al. (15) using a complement-dependent assay, several studies using antibody-binding assays have demonstrated serological reactivity against shared melanoma-specific or tumor-specific antigens (7, 9, 11). Carey et al. (5) and Shiku et al. (28–30), in a series of melanoma studies utilizing several different serological tests, identified 3 sera reactive with autologous tumor, 2 sera reactive with a common melanoma antigen, and 17 sera reactive with antigens present on a variety of normal and malignant cells. Siebert et al. (26) were unable to detect any unique or common melanoma-specific antigens with an immune adherence assay. However, Leong et al. (14) and Liao et al. (16), using membrane immunofluorescence and mixed hemadsorption assays, demonstrated the development of a common melanoma-specific reactivity in previously unreactive pa-
tients after receiving immunotherapy with melanoma cells.

The inability to demonstrate tumor-specific reactivity in our study may derive from the complement-dependent cytotoxicity assay used which could be relatively insensitive to such antibodies. Alternatively, as indicated by Carey et al. (5) and Shiku et al. (28–30), the incidence of tumor-specific reactivity in the melanoma patient population may be low and will require screening larger numbers of patients and cells for detection. Finally, our results may indicate that the sera of melanoma patients do not contain specific anti-melanoma antibodies or that tumor-specific antigens are not expressed on melanoma cell lines in tissue culture.

REFERENCES


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