Novel Antigenic Markers of Human Tumor Regression


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Summary: The development of tumor-specific antibodies was studied in a group of cancer patients undergoing active specific immunotherapy with irradiated human allogeneic and autochthonous (autologous) tumor cells injected by the intralymphatic route. Immunoblotting studies on extracts of various established tumor cell cultures and fresh tumor biopsies were performed using sera from these patients. Evaluable tumor regressions were associated with detection of antibodies against human tumor cell antigens of 22,000 daltons (22 kd), 38,000 daltons (38 kd), 43,000 daltons (43 kd), and 70,000 daltons (70 kd). Similar antigens of ~22, 43, and 70 kd have also been detected in fresh extracts of certain human tumor tissues when tested with antisera from patients responding to immunotherapy. Production of antibodies to these antigens may play a role in tumor regression with active specific immunotherapy. These human regression-associated antigens may, therefore, represent novel agents for cancer immunotherapy. Key Words: Tumor regression—Regression-associated antigens—Immunotherapy.

Specific antibodies directed against certain tumor cell-associated antigens may mediate tumor destruction directly or indirectly (1). There have been attempts in a limited number of clinical investigations to evaluate the efficacy of whole tumor cells as active immunogens. Various preparations of suspensions of autologous tumor cells, allogeneic tumor cell lines, or partially fractionated tumor cell extracts have been used in experimental active specific immunotherapy protocols (1–6). The cellular preparations are generally treated by irradiation, mechanical disruption, or freeze–thaw cycles to render the tumor cells nonviable. They are

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then used as immunogens with or without an adjuvant and administered by different routes (i.e., as intradermal, subcutaneous, intramuscular, or intralymphatic) to immunize cancer patients. These studies have revealed relatively little toxicity while providing encouraging clinical responses in advanced cancer patients.

Results from a number of animal models lend support to the use of tumor cell components in active specific immunization to induce tumor regression (7–10). One of the best studied systems has been the immunization of cats with preparations containing FOCMA (feline oncornavirus-associated cell membrane antigen) to protect from subsequent tumor development following challenge with feline sarcoma virus (8). Neoplasms induced in mice by polycyclic aromatic hydrocarbons such as 3-methylcholanthrene express individually distinct tumor-associated transplantation antigens (9). These antigens are immunogenic in their syngeneic hosts and provide specific transplantation immunity only against their respective tumors and not against tumors induced by the same or a different carcinogen or against virally induced tumors. Transplantation immunity can be elicited in mice by prior growth and removal of tumor transplants or by immunization with irradiated tumor cells, tumor cell membranes, or solubilized antigen preparations. In a murine melanoma model, immunization with a purified melanoma-specific 65-kd glycoprotein antigen elicited tumor rejection and protection against experimental metastases (10). Other workers have identified additional murine antigens associated with tumor rejection (11,12). We describe the identification of tumor cell-associated antigens using antibodies developed in patients immunized by the intralymphatic route with irradiated human tumor cells.

MATERIALS AND METHODS

Human Cell Lines and Culture Conditions

The following human tumor cell lines were obtained from the American Type Culture Collection (ATCC) and propagated according to ATCC recommendations: LoVo colonic adenocarcinoma cells (ATCC No. CCL 229), A549 lung adenocarcinoma cells (ATCC No. CCR 185), and SW480 colon carcinoma cells (ATCC No. CCL 228). A375 melanoma cells (ATCC No. CRL 1619) were cultured continuously over a 2-year period prior to their use in these studies. M-14 is a melanoma cell line described previously (13).

Human tumor cell lines established here are designated RO 82-2W (squamous cell carcinoma of the esophagus), ING-69-1, ING-69-11, CAL-2, RTH351 and RO 84-1F (ovarian adenocarcinomas), RTH1513 and RO 81-5C (melanomas), RO 81-1R and RO 81-1B (renal cell carcinomas), RO 84-1H and RO 84-1S (prostatic carcinomas), RO 83-1M and RO 81-4U (adenocystic carcinoma), and RO 81-1A and RO 81-1D (thyroid carcinomas). In each case, fresh tissue specimens from the histologically confirmed tumors were minced and sieved through fine-mesh gauze prior to plating at 37°C in 75-cm² falcon tissue culture flasks containing RPMI 1640 media supplemented with 10% fetal bovine serum. When confluent cultures were obtained from the primary cell cultures, several subcultures were
subsequently required to establish morphologically uniform populations of cells capable of growth for \( \geq 50 \) passages without observable cytologic changes.

**Immunotherapy Protocol**

Intralymphatic infusions of irradiated tumor cell suspensions were performed as previously described (2,13,14).

**Analysis of Patient Sera by Immunoblotting**

Normal or neoplastic cells, either cultured or fresh specimens, were rinsed twice with phosphate-buffered saline and then extracted using a mixture of ionic and nonionic detergents [25 mM Tris-HCl, pH 7.4, 1% Nonidet P-40, 0.5% sodium deoxycholic acid, 0.1% sodium dodecyl sulfate (SDS), 1 M sodium chloride, 1% Aprotinin, 2 mM sodium azide] or solely in a nonionic detergent buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 2.5 mM EGTA, 10 mM NaMoO_4, 12 mM monothioglycerol, 10% glycerol, 40 \( \mu \)g/ml leupeptin, and 0.2% Triton X-100). Insoluble materials were removed by centrifugation at 10,000 g, and the supernatant solutions were retained. Subcellular fractionation of A375 and RO 82-2W cells were performed using published procedures (15). Aliquots of the supernatants containing 10–20 \( \mu \)g cell protein were subjected to reducing SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Assays for membrane protein glycosylation consisted of incubating an enzyme excess of endoglycosidase F (Bethesda Research Laboratories, Bethesda, MD, U.S.A.) with 20-\( \mu \)g protein aliquots of cell membranes according to the manufacturer’s instructions. Proteins fractionated by gel electrophoresis were transferred to nitrocellulose filter paper and, after blocking in 3% bovine serum albumin, the filters were incubated with appropriate test sera dilutions, washed, finally incubated with \( ^{125} \)I-labeled Protein A of *Staphylococcus aureus* and analyzed by autoradiography (16, New England Nuclear instruction manual No. NEF-972). Efficient transfer of equivalent amounts of fractionated extract proteins was confirmed by Coomassie blue staining of the electroblotted gel and the nitrocellulose filter (after completion of the autoradiography procedure). We have detected variable antibody responses with calf serum proteins of 68, 120, and 180 kd in patients immunized with different human tumor cell cultures (propagated in media supplemented with fetal calf serum). As expected, the addition of a large excess of bovine serum in the blocking and antibody incubation buffers for the immunoblots effectively competed with such reactivities but did not interfere with the detection of 22, 38, 43, and 70 kd antigens described in the Results sections.

**RESULTS**

**Therapy and Clinical Evaluation**

Patients with metastatic cancers as assessed by conventional clinical procedures, including radiologic and direct physical monitoring, received intralymphatic infusions as sole treatment (2,13,14) of irradiated tumor cells derived from
the corresponding recipient patient or from established allogeneic cell lines. We present twelve of these patients falling into three distinct clinical response groups in detail. As indicated in Table 1, clinical responses were characterized by (a) complete regression (Table 1, patients 1–3); (b) partial regression and stabilization as indicated by significant reduction of tumor mass or no objective change for at least 3 months (patients 4–7); and (c) tumor progression (patients 10–12).

**TABLE 1. Summary of patients and therapies**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Previous treatment</th>
<th>Cell infused</th>
<th>No. ASILI</th>
<th>Skin test</th>
<th>Tumor response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>69</td>
<td>Melanoma</td>
<td>S</td>
<td>M14, A375, RO 81-5C</td>
<td>43</td>
<td>++</td>
<td>CR</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>71</td>
<td>Melanoma</td>
<td>None</td>
<td>RO 82-2W A375</td>
<td>17</td>
<td>+</td>
<td>CR</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>79</td>
<td>Melanoma</td>
<td>S-RT</td>
<td>RO 81-5C, RO 82-2W M14</td>
<td>23</td>
<td>+</td>
<td>CR</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>64</td>
<td>Ovarian cancer</td>
<td>S-RT-CX</td>
<td>ING 69-1 (Auto) RO 82-2W CAL-2</td>
<td>11</td>
<td>–</td>
<td>ST</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>68</td>
<td>Prostatic cancer</td>
<td>RT</td>
<td>RO 84-1 H RO 84-1 S</td>
<td>18</td>
<td>+</td>
<td>ST</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>56</td>
<td>Renal cancer</td>
<td>Nephrectomy + 10 intradermal injections of irradiated autochthonous TC + CP CX interferon</td>
<td>RO 81-1 R RO 81-1 B</td>
<td>21</td>
<td>+</td>
<td>ST</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>59</td>
<td>Recurrent adenocystic cancer (nasopharynx)</td>
<td>RT</td>
<td>RO 83-1M RO 81-4U A375</td>
<td>24</td>
<td>++</td>
<td>ST</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>49</td>
<td>Squamous cell cancer (esophagus)</td>
<td>RT S</td>
<td>RO 82-2 W (Auto)</td>
<td>6</td>
<td>+</td>
<td>NE</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>38</td>
<td>Ovarian cancer</td>
<td>S-CX</td>
<td>RTH 351 (Auto) M14</td>
<td>9</td>
<td>+</td>
<td>NE</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>34</td>
<td>Melanoma</td>
<td>S</td>
<td>RO 81-5C RO 82-1W RO 81-1A RO 81-1D</td>
<td>3</td>
<td>+</td>
<td>P</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>30</td>
<td>Thyroid cancer</td>
<td>S-RT-CX</td>
<td>RO 81-5C M14 RTH 1513 (Auto)</td>
<td>4</td>
<td>–</td>
<td>P</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>57</td>
<td>Melanoma</td>
<td>S-RT</td>
<td>M14 RTH 1513 (Auto)</td>
<td>5</td>
<td>–</td>
<td>P</td>
</tr>
</tbody>
</table>

S, surgery; CX, chemotherapy; RT, radiotherapy; TC, tumor cells; CP, Corynebacterium parvum; ST, stabilization; P, progression; CR, complete response; NE, not evaluable for tumor response; Auto, autochthonous in this patient; Cell infused, irradiated tumor cell infused; No. ASILI, number of active specific intralymphatic infusions.

Skin tests: 24-h reactivity following 10⁶ irradiated TC intradermal injection: (−) negative; (+) 5–10 mm in diameter; (+++) >10 mm in diameter.
Patients 8 and 9 were nonevaluable clinically for tumor response, but are included since they were treated only with their own tumor cells.

Analysis of Antibody Response to Immunotherapy

Serum samples obtained before, during the course of therapy, and after termination of immunotherapy were analyzed for their ability to detect specific antigens in various cultured human tumor cells, including those used for treatment. Immunoblots were performed using preimmune sera and postimmune sera from patients 4, 8, and 9, who received intralymphatic immunotherapy using cell lines established from their own tumors and, in the case of patient 4, additional tumor cell lines established from other patients. Postimmune sera diluted 1:500 and taken 6–8 weeks after the first immunotherapy from these three patients readily detected antigens of ∼43 and 70 kd in extracts from malignant cells of ovarian origin; no significant immunoreactivity was observed with preimmune sera from the same patients (Fig. 1).

Patient 5 (metastatic prostatic cancer) received intralymphatic infusions of two irradiated prostatic cancer cell lines, RO84-1S and RO84-1H, and developed antibodies reactive with 38- to 43-kd antigenic species in the RO-84-1H tumor extract (Fig. 2) and in RO-84-1S cells (data not shown). Associated with this patient’s

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**FIG. 1.** Immunoblot analyses using sera obtained before or after immunotherapy. Proteins in aliquots of reduced and boiled detergent extracts of normal tissues and human tumor cells were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose filter paper by electroblotting (16). Sera from patients 4 (ovarian cancer), 8 (squamous cell carcinoma), and 9 (ovarian cancer) were tested for antibodies binding to proteins extracted from the following sources: lane 1, normal human breast; lane 2, normal human ovary; lane 3, ovarian tumor cell line ING 69-1; lane 4, ovarian tumor cell line ING 69-11; and lane 5, metastatic ovarian adenocarcinoma. Preimmune sera taken before the onset of immunotherapy were incubated with filters A, C, and E for patients 4, 8, and 9, respectively, and sera taken after four immunizations were incubated with identical filters B, D, and F for patients 4, 8, and 9, respectively. All serum dilutions were 1:500. The positions of mol-wt markers run in lanes adjacent to the extracts are indicated (right) (bovine serum albumin 68 kd, and ovalbumin 43 kd).

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immunotherapy were significant declines in serum acid phosphatase levels determined within the first 2 weeks following immunizations and a substantial reduction in required pain medication for bone pain due to metastatic tumor involvement. Subsequently, the patient received local radiotherapy to the lumbar spine, with further decrease of serum acid phosphatase levels. None of the patients characterized by tumor progression during immunotherapy (data not shown) developed new antibody reactivities with tumor cell antigens in immunoblots (patients 10–12 in Table 1).

Postimmune sera (samples taken after at least three intralymphatic immunizations) from the patients who responded to immunotherapy and developed antibodies against specific antigens in tumors did not reveal new antibody reactivities toward antigenic species in various normal human tissue. This is illustrated with sera from patients 4, 8, and 9, which failed to react with extracts of normal human breast and ovary shown in lanes 1 and 2 for the immunoblots of Fig. 1. A survey of certain normal human cell lines including human skin and lung fibroblasts revealed no reactivities on immunoblots with these and all other postimmune sera which react with the 43- and 70-kd species.

Tests for Antigens Detected by Postimmune Sera in Different Tissues

Postimmune sera and ascites fluid from patient 4 (ovarian cancer) shown in Fig. 1 yielded comparable antibody reactivities with 70- and 43-kd proteins (visible as two bands of 38–40 and 43–45 kd) present in several different ovarian and non-ovarian tumor cell lines (Fig. 3). These antigen reactivities were readily detected even with 1:10,000 dilutions of this patient's sera. In contrast, indirect immunofluorescence studies using the same serum sample revealed a specific detectable signal only at serum dilutions <1:250 (data not shown). Fig. 1B shows that the antigenic species in the range of 43 kd detected with this patient's sera consisted of at least two distinct entities. These sera were then used to screen a variety of human tumor cell lines from the American Type Culture Collection. Comparable antigenic reactivities to those illustrated in Figs. 1 and 2 have been detected in extracts of two colon carcinoma cell lines (LoVo and Sw480) and a lung carcinoma (A549).

![Image of immunoblot analysis](image-url)

**FIG. 2.** Immunoblot analysis of extracts of prostatic cancer cells, RO 84-1H and RO 84-1S, using preimmune (A) and postimmune (B) sera from patient 5 diluted 1:1000.
FIG. 3. Immunoblot analysis of antigens in extracts of tumor cell lines using ascites fluid or serum from patient 4 (ovarian cancer) as the sources of posttherapy antibody preparations. Ascites fluid and serum were obtained after immunotherapy of patient 4 and were diluted 1:1000 for incubations with filters containing: lanes 1 and 6, RO-82-1F; lanes 2 and 7, ING 69-1; lanes 3 and 8, CAL-2; lanes 4 and 9, A375 melanoma cells; and lanes 5 and 10, RO 82-2W.

Preliminary subcellular fractionation studies were performed to assess the localization of these antigens in tumor cells. A375 melanoma cells were homogenized in hypotonic buffer and the homogenate was subjected to differential centrifugation to separate the soluble (cytoplasmic), particulate (membrane), and nuclear fractions (15). Antigens from these subcellular fractions were resolved by SDS-PAGE, and immunoblotting was performed with post-immune sera from responding patients 2, 3, and 7. Immunoblot analyses employing sera from patients 2 and 7 shown in Fig. 4 and with sera from patient 3 (data not shown) detected the 38-, 43-, and 70-kd antigens in the particulate fraction and not in the nuclear or soluble fractions. At higher antibody concentrations or with longer exposures of the autoradiographs shown, a 22-kd band is also detected in the particulate fraction (data not shown). Identical immunoblot results have been obtained using comparable fractions derived from the squamous cell carcinoma cell line RO 82-2W (data not shown). Recently, monoclonal antibodies have been raised in mice immunized with this particulate fraction from A375 cells. Our preliminary immunohistochemical testing has shown these antibodies to bind to fresh human tumors (Ewing’s sarcoma and biliary carcinoma) as well as a large number of human tumor cell lines with negligible reactivity with peripheral blood cells and human fibroblasts. Additional characterization of these antibodies is underway.

These antigenic species appear to be significantly different from the major histocompatibility antigens (HLA-A,B,C) in that their mobility in reducing SDS-
PAGE is unaffected by treatment with endoglycosidases H and F under conditions in which the carbohydrate moiety of HLA-A,B,C is degraded by these enzymes. The results of endoglycosidase F treatment of A375 membranes are shown in Fig. 5. Immunoblot probing of the enzyme-treated membranes with sera from patient 4 revealed no change in migration of the 38-, 43-, and 70-kd antigens, whereas probing with a monoclonal antibody specific for HLA-A,B,C molecules showed the glycosylated 45-kd HLA molecule shifting to a 39-kd species as a consequence of endoglycosidase F action. Furthermore, the 38-, 43-, and 70-kd antigens are distinguishable from HLA-A,B,C in two-dimensional gel electrophoretic immunoblot analyses using polyclonal and monoclonal anti-HLA-A,B,C antibodies (data not shown). Additional monoclonal antibody immunoblot analyses revealed that antibodies specific for carcinoembryonic antigen, α-fetoprotein and human chorionicadotropin were nonreactive with 38-, 43-, and 70-kd antigens.

We assayed postimmune sera from a group of metastatic melanoma patients who received a mixture of two allogeneic melanoma cell lysates along with the experimental adjuvant DETOXm (Ribi Immunochem, Hamilton, MT, U.S.A.) in an active specific immunization study directed by Dr. Malcolm Mitchell at the University of Southern California School of Medicine. Figure 6 shows that dilutions >1:500 of postimmune sera from three of these patients who received the same melanoma cell lysate vaccine detect numerous antigens in the A375 mem-

**FIG. 5.** Endoglycosidase treatment of regression-associated antigens and HLA antigens. A375 membranes [20 µg/lane were incubated alone or with an excess of endoglycosidase F (Bethesda Research Laboratory)] according to the manufacturer’s instructions for 1 h at 37°C and then subjected to immunoblot analysis, as described in the Materials and Methods section, using either postimmune sera from patient 1 or murine monoclonal anti-human HLA-ABC (Bethesda Research Laboratory) as antibody probes. Results are depicted schematically. Lanes identified as + endo F contained the enzyme-treated A375 membranes.
FIG. 6. Immunoblot detection of antibodies to regression-associated antigens using sera from melanoma patients immunized subcutaneously. Sera from three patients identified as USC-B, USC-S, and USC-C were used at dilutions of 1:500 to probe immunoblots of A375 membranes or fractionated melanoma vaccine lysate, Mac 3. The Mac 3 lysate was prepared by Dr. Malcolm Mitchell (University of Southern California) and consisted of two allogeneic melanoma cell lines that had been sonicated prior to their use in patients. The lysate was subjected to centrifugation at 10,000 g for 15 min at 4°C, and supernatant (sup.), and pellet fractions were taken for immunoblot testing.

brane extract, including antigens of 24, 38, 43, and 70 kd. Preimmune sera from these patients when immunoblotted at the same dilutions as postimmune sera failed to detect these antigens. These sera reacted very weakly with supernatant and pellet fractions from another cell lysate vaccine termed Mac-3, a batch of the melanoma cells used to immunize other patients in this study. Postimmune sera from these patients, however, reacted strongly with 38-, 43-, and 70-kd antigens present in the Mac-I vaccine lysate used for their immunizations (Fig. 7). The details of this study together with immunologic evaluations of the patients is the subject of a report in preparation by Dr. Mitchell, who kindly provided us with these sera and lysate samples.

FIG. 7. Demonstration of regression-associated antigens in the melanoma lysate particulate material used in subcutaneous immunizations. Sera from patient USC-B was used to probe the fractionated (as described in the legend to Fig. 6) Mac I vaccine lysate used for his immunization therapy. Pre- and postimmune sera were diluted 1:1000.
Identification of Antigenic Reactivities in Fresh Tumor Extracts

With the identification of postimmune sera reactive with tumor cell lines of different tumor origin, we screened a wide variety of fresh human tumor extracts of the same or different tissue origins as that of the immunized patient. Figures 8 and 9 illustrate the detection of 43- and 70-kd antigen species in fresh tumor extracts using the postimmune sera from patients 1 and 6. In Fig. 8, sera from patient 6, who had metastatic renal cell carcinoma, was examined before (lanes 1 and 2) and after (lanes 3 and 4) intralymphatic immunizations with a mixture of allogeneic renal cancer cell lines. The test extracts used were from a non-Hodgkin's lymphoma (lanes 1 and 3) and a breast carcinoma (lanes 2 and 4). Preimmune serum from this patient showed negligible reactivity in the 43-kd antigen region with these two extracts, whereas postimmune serum revealed a specific band (arrow in Fig. 8) in the lymphoma extract (the apparent reactivity at 68 kd with the preimmune serum in lanes 1 and 2 is due to a fivefold longer exposure time for this autoradiograph as compared with the postimmune blot for lanes 3 and 4). This postimmune serum also reacted with specific antigens of 65 and 70 kd present in a Kaposi's sarcoma extract (Fig. 9, lane 1). In contrast, the postimmune serum from patient 1 (melanoma) immunized with a single human melanoma cell line detected only a 70-kd protein (Fig. 9, lane 2). The preimmune sera from both these two patients were nonreactive in immunoblots with the same Kaposi's sarcoma extract. Three separate Kaposi's sarcoma tumor biopsies, two from acquired immune deficiency syndrome (AIDS) patients and one classic Ka-

FIG. 8. Immunoblot detection of 43-kd antigen in a lymphoma tumor extract using antibodies from an immunized patient. Sera from patient 6 before (lanes 1 and 2) or after (lanes 3 and 4) immunotherapy were reacted at dilutions of 1:500 with extracts of a non-Hodgkin's lymphoma (lanes 1 and 3) and of an adenocarcinoma of the breast (lanes 2 and 4). Autoradiography was performed for 30 h for lanes 1 and 2 and for 6 h for lanes 3 and 4. Arrow indicates the major antigenic reactivity after immunotherapy.

FIG. 9. Immunoblot detection of 65- and 70-kd antigens in a Kaposi's sarcoma extract. Lanes 1 and 2 contain 5-μg protein aliquots from a Kaposi's sarcoma specimen obtained from a patient with AIDS. These filter strips were incubated with postimmune sera diluted 1:500 from patient 6 (lane 1) and patient 1 (lane 2).
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posi's sarcoma from a non-AIDS patient, have been tested; the above described reactivities were identified in all. With sera from other patients who responded to immunotherapy and formed antibodies to the 22-, 38-, 43-, and 70-kd antigens specific antigenic species including antigens of ~22 kd were detected in a variety of other fresh human tumors including melanoma, gastric carcinoma, adenocarcinomas of the breast and ovary, lymphoma, and squamous cell carcinoma.

DISCUSSION

Sera from patients immunized by intralymphatic infusions of irradiated tumor cell suspensions and characterized by significant tumor regression have been used to search for specific antibodies associated with tumor regression. Varying degrees of antibody responses and clinical regression of tumors were observed when different tumor-derived cell lines (autologous and allogeneic) were used for immunizations. The development of antibodies to antigenic reactivities associated with proteins of 22, 38, 43, and 70 kd shows a significant correlation with a positive clinical outcome of the immunotherapy. These antigens appear to be potent immunogens that may provoke antibody responses in patients showing tumor regression after immunotherapy. As expected, varying levels of these antigens are associated with different tumor-derived cell lines. Further characterization of these antigens, including their comparison to those obtained from the cell line sources, will be needed to elucidate their significance and possible involvement in tumor regression.

The results we describe differ from previous serologic studies carried out on actively immunized cancer patients (5,17) in which adsorption assays were utilized to evaluate the serologic responses and relatively rare responses to tumor-specific antigens were noted. These serologic differences may in part be attributed to different tumor cell infusions (immunogens), patient populations, and immunotherapy techniques, as well as to the differences in immunoassay methodology. The immunoblotting technique we employed, in addition to identifying specific molecular species of antigen molecules, appears to be much more sensitive than adsorption assays, since antigen reactivities were detected at >1:10,000 dilutions of sera from some of the responding patients. Preliminary results with cells containing these regression-associated antigens bound to test wells suggest that comparable antibody titers to those obtained by immunoblotting may be obtained using an ELISA format.

A rapidly growing list of tumor-specific antigens have been identified with murine monoclonal antibodies. Many of these antibodies show remarkable specificity with regard to tumor type and histologic origin for reactivity (18); in contrast, a growing number of antibodies which react across a broad histologic range of tumor types exists (19,20). The sizes, cross-reactivities, and biochemical properties of the regression-associated antigens are different from those of the tumor antigens already reported (18–21); however, they appear to share the property of moderately widespread tumor distribution observed with selected monoclonal antibodies. The basis for selection of monoclonal antibodies against human tumor
antigens is not necessarily relevant to tumor regression. In contrast, the selection of human–human hybridomas producing tumor cell-reactive monoclonal antibodies from actively immunized cancer patients (22) or the production of human monoclonal antibodies from fusions with draining lymph node cells for particular malignancies (23) may yield antibodies associated with tumor regression.

A knowledge of the antibody responses associated with human tumor regression following active specific immunotherapy and identification of subcellular components involved in eliciting such specific antibodies should lead to the development of improved immunogens for immunotherapy. The development of such preparations enriched in regression-associated antigens and of specific, high-titer antibodies which can standardize different immunogen preparations by quantitating regression-associated antigens are desirable steps toward advancing active specific immunotherapy.

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REFERENCES


