Incidence of Serum Antibody Reactivity to Autologous Head and Neck Cancer Cell Lines and Augmentation of Antibody Reactivity following Acid Dissociation and Ultrafiltration

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ABSTRACT

Serum antibody reactivity to squamous cell carcinoma of the head and neck (SCCHN) was evaluated in 41 autologous tumor-serum line cell combinations using the protein A hemadsorption assay. Autologous antibody reactivity (median titer of 1:4) was detected in sera from 24 of the patients tested. In 10 cases autologous antibody reactivity could be detected only in undiluted serum preceding further analysis. Analysis of higher titer sera from one patient revealed antibodies that define an antigen expressed on autologous tumor cells cultured from both the primary tumor (UM-SCC-17A) and from a metastasis (UM-SCC-17B). Absorption analysis showed that this antigen was also expressed on 6 of 10 allogeneic SCCHN cell lines but not on autologous fibroblasts or on allogeneic melanoma cell lines. Due to the low titer of autologous antibody reactivity in most sera, we sought to determine if dissociation of immune complexes through acidification and ultrafiltration of serum might enhance detectable antibody reactivity as has been done in previous studies in melanoma. Twelve serum samples from eight patients were subjected to acid dissociation and ultrafiltration (AD-U). Only six of the untreated sera had detectable antibody activity against the autologous SCCHN cell line whereas following AD-U all 12 sera had enhanced IgG reactivity against autologous SCCHN. Specificity analysis of one serum sample after dissociation revealed that the antibody detected an antigen common to SCCHN cell lines as well as melanoma, glioma, renal, and colon carcinoma cell lines. Circulating immune complexes may provide a reservoir of antibody with potential diagnostic and therapeutic applications.

INTRODUCTION

A central premise of tumor immunology is that tumor cells may express antigens which distinguish them from normal cells. Through the identification of such antigens it should be possible to develop a better understanding of the nature of the host-tumor interaction and thus to develop new immunodiagnostic and therapeutic methods. Whereas monoclonal antibodies directed against squamous cell carcinoma have been developed (1-5), none have absolute specificity for tumor cells. However, very specific antibodies have been detected in autologous patient sera in a number of tumor types (6-12). A potential advantage to studying autologous antibody reactivity is that the autologous response may detect antigens not readily identified by heteroantiserum or monoclonal antibodies. In addition, antigens detected by the host may be physiologically relevant to the host-tumor interaction. Therefore, we used this approach as a means to identify antigens with potential clinical relevance in head and neck cancer.

MATERIALS AND METHODS

Human Squamous Carcinoma Cell Lines. The methods used for the establishment and characterization of the UM-SCC cell lines have been reported previously (3, 15, 16). Tumor specimens were washed in Earle’s balanced salt solution containing penicillin (100 IU/ml), streptomycin (100 μg/ml), and amphotericin B (10 μg/ml), minced and placed in culture with minimal essential medium plus 1% nonessential amino acids, 2 mM L-glutamine, 15% fetal bovine serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin. Fibroblasts were removed by brief treatments with trypsin (314 μg/ml) and 0.4% EDTA in Earle’s balanced salt solution.

Non-SCCHN Cells. Acquisition and maintenance of non-SCCHN cell lines has been reported (6, 14). For specificity testing the following additional cell lines were used: glioma cell lines LN-18, GLL-19, and CL-105, neuroblastoma cell lines JMC-32 and SK-N-MC and fetal fibroblast cell lines P-FF 86:100 and P-FF 86:110 supplied by Dr. Theresa Whiteside (University of Pittsburgh); renal cell carcinoma cell lines 5106W, 630D, 531W, and IGR3 supplied by Dr. Bryan Ballou (University of Pittsburgh School of Medicine); bladder carcinoma cell line HTB4 was kindly provided by Dr. Craig McCune (University of Rochester, School of Medicine, Rochester, NY). MeWo was obtained from Dr. Michael Bean (Virginia Mason Cancer Research Institute, Seattle, WA).

The antigens detected by autologous antibody responses previously reported in malignant melanoma (6-9), leukemia (10), brain tumors (11), and renal carcinomas (12) could be grouped into three classes: Class I, tumor antigens restricted to tumor cells of a single individual; Class II, antigens shared among tumors of a similar histotype; and Class III, antigens found on both neoplastic and nonneoplastic cells. Autologous antibody to melanoma in native serum specimens was found in about one-third of patients studied (13), however, the antibodies were frequently of low titer which hampered further investigation in all but a few cases (6-9). The failure to detect antibodies to autologous tumor cells more frequently may be due to circulating antigens that form immune complexes. In melanoma, Kirkwood and Vlock (14) demonstrated that dissociation of immune complexes by acidification and ultrafiltration results in the augmentation of autologous antibody reactivity in the majority of cases studied.

In this report, we extend our studies to a more common malignant disease, squamous cell carcinoma. To date, 41 autologous systems from the UM-SCC cell line series have been evaluated. The frequency of direct autologous antibody reactivity in this group of patients is reported. One example of a Class II SCCHN antigen defined by autologous antibody in native serum is described and the initial results of efforts to reveal occult antibody responses by the dissociation of circulating immune complexes are presented.

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* The abbreviations used are: SCCHN, squamous cell carcinoma of the head and neck; PBS, phosphate buffered saline; PBS, fetal bovine serum; AD-U, acid dissociation and ultrafiltration.

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Protein A Hemadsorption (PA). The protein A hemadsorption assay was performed after the method described by Pfreundschuh et al. (11). Indicator cells were prepared by conjugating Staphylococcal protein A (Pharmacia Fine Chemicals) to the surface of human blood group O-Rh-positive red blood cells with 0.01% CrCl3. The cells were washed in PBS4 containing 1% γ-globulin-free FBS (GIBCO, Grand Island, NY) and diluted for use in this medium. Target cell cultures in 96- or 72-well assay plates were incubated at room temperature or 37°C with serial dilutions of autologous sera. After incubation, the cells were washed three times with PBS containing 2% FBS and indicator cells were added. Plates were washed two to four times after 45 min and using a microscope-positive cells were scored as follows: (+) cells are those with a >50% erythrocyte rosette. The endpoint of the assay was the last well with 10% of target cells (+).

The last well with 10% of target cells (+).

Cells were washed three times and mixed 1:1 v/v (1 x 10⁶ cells/ml PBS, pH 7.4).

Absorption Analysis. Absorptions were performed using fresh, cryopreserved, and cultured heterologous, allogeneic, and autologous cells. Cells were washed three times and mixed 1:1 v/v (1 x 10⁶ cells/ml minimum) with the serum to be tested (0.1 ml, at a serum dilution two doublings above the 50% endpoint of the titration curve for that serum). Absorptions were carried out for 30 min at 37°C then 30 min at 37°C. Specimens were centrifuged at 2000 rpm x 20 min at 4°C. Absorbed serum was tested, together with an aliquot of the same dilution of unabsorbed serum, on autologous tumor cells.

RESULTS

Reactivity of Native Serum. Antibody reactivity in native serum against autologous SCCHN cell lines was evaluated in 41 systems by protein A hemadsorption. The results are summarized in Table 1. Autologous antibody reactivity was found in sera from 24 patients with a median titer of 1:4. In many cases (10 of 24) autologous antibody reactivity could only be detected in undiluted serum. The highest antibody titer observed in native serum was found in the autologous UM-SCC-17A and -17B systems. Antibody was present in sera taken early in the patient's course, but declined to undetectable levels approximately 6 months after surgery (2). The antibody was strongly reactive with both UM-SCC-17A and -17B but did not bind to skin fibroblasts or Epstein-Barr virus-transformed lymphoblasts cultured from Patient UM-17.

Specificity Analysis of UM-SCC-17 Serum Antibodies. To determine whether the UM-17 autologous antigen was expressed by other cells, serum 1046 known to contain a high antibody titer (1:512) was absorbed with packed cells and re-tested for residual reactivity with UM-SCC-17A and -17B. The UM-17 antigen was found to be shared by some, but not all, other SCC lines. Six of ten UM-SCC lines including UM-SCC-17A and -17B absorbed the autologous antibody activity although for three lines (UM-SCC-18, -21A, and -22B) the level of expression was much lower than on UM-SCC-17A and -17B since it was necessary to absorb twice to get complete absorption (Table 2). Four other SCC cell lines (UM-SCC-1, -2, -10A, and -19), two melanoma cell lines and autologous fibroblasts did not reduce the autologous reactivity even if the absorption was repeated (Table 2).

Acid Dissociation and Ultrafiltration of Sera. Serum samples from eight patients were subjected to AD-U. Native sera samples from five of these patients (UM-9, -17, -21, -24, and -30) had reactivity against autologous SCCHN cells by protein A hemadsorption. After acid dissociation and ultrafiltration 12 of 12 sera from all eight patients demonstrated enhanced IgG reactivity against SCCHN. These results are shown in Table 3. Sera from eight normal individuals showed no reactivity against SCCHN cells either before or after acid dissociation and ultrafiltration.

AD-U Analysis of System UM-SCC-23. Prior to acid dissociation and ultrafiltration, sera from patient UM-23 showed no reactivity against the autologous UM-SCC-23 cell line. After acid dissociation and ultrafiltration, antibody reactivity was noted in sera number 1095 and 1522 with titers of 1:64 and 1:32, respectively (Table 3). Direct antibody binding and absorption specificity tests of serum 1522 were performed using autologous and allogeneic cells. The results are summarized in Table 4. Absorptions indicated that the antibody in sample 1522 detects an antigen that is also present on other SCCHN (UM-SCC-17A and UM-SCC-35), as well as on three of four melanoma, three of three glioma, one of three renal cell, and one of one colon carcinoma cell lines. The antigen was not detected on neuroblastoma, breast, and bladder carcinoma cell lines or on fibroblasts, autologous lymphoblasts, allogeneic lymphocytes, red blood cells, and platelets.
Complete absorption, squamous cell carcinomas
UM-SCC-17A (larynx)
UM-SCC-17B (metastasis)
UM-SCC-8 (alveolar ridge)

Partial absorption, squamous cell carcinomas
UM-SCC-18 (base of tongue)
UM-SCC-21A (skin)
UM-SCC-22B (metastasis from hypopharynx)

No absorption
Squamous cell carcinomas
UM-SCC-1 (floor of mouth)
UM-SCC-2 (alveolus)
UM-SCC-10A (larynx)
UM-SCC-19 (base of tongue)

Autologous fibroblasts
UM-FB-17

Malignant melanomas
MeWo
SK-MEL-28

* Cells listed under this heading required two absorptions to remove all autologous antibody reactivity with UM-SCC-17A and UM-SCC-17B cells.

Table 3 Autologous antibody reactivity in native and acid-dissociated sera

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Serum sample no.</th>
<th>Native</th>
<th>AD-U</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-SCC-9</td>
<td>818</td>
<td>1:16</td>
<td>1:64</td>
</tr>
<tr>
<td>UM-SCC-17A</td>
<td>1100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>±&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1:1024</td>
</tr>
<tr>
<td>UM-SCC-17B</td>
<td>1100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>1:64</td>
</tr>
<tr>
<td>UM-SCC-21A</td>
<td>1986&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1:512</td>
<td>2:2048</td>
</tr>
<tr>
<td>UM-SCC-23</td>
<td>1782</td>
<td>1:4</td>
<td>1:128</td>
</tr>
<tr>
<td>UM-SCC-24</td>
<td>1522</td>
<td>0</td>
<td>1:32</td>
</tr>
<tr>
<td>UM-SCC-30</td>
<td>1694</td>
<td>1:32</td>
<td>1:512</td>
</tr>
<tr>
<td>UM-SCC-35</td>
<td>1873</td>
<td>0</td>
<td>1:4</td>
</tr>
<tr>
<td>UM-SCC-36</td>
<td>1794</td>
<td>0</td>
<td>1:8</td>
</tr>
<tr>
<td></td>
<td>2042</td>
<td>0</td>
<td>1:16</td>
</tr>
</tbody>
</table>

* Corresponding autologous serum.
<sup>a</sup> Two tumor specimens and cell lines from the same patient.
<sup>b</sup> <10% positive cells at a 1:4 dilution but some reactivity still noted.

DISCUSSION

By focusing upon the autologous immune response it may be possible to detect antigens that are immunogenic to the host. In native sera only a small proportion of sera possess antibody reactivity of sufficient titer to allow further analysis. Of the 47 autologous SCCHN systems analyzed in this study, only four demonstrated titers of greater than 1:8. Indeed, in many cases autologous antibody reactivity could only be detected in undiluted sera.

We selected two cases, patients UM-17 and UM-23, for further study. Both are female patients with laryngeal carcinoma and both have remained alive and free of disease for more than 5 years after surgery. The donor of the UM-SCC-17A and -17B cell lines had detectable antibody in several serum specimens, whereas we found no antibody in native sera from the donor of UM-SCC-23. In both cases, autologous antibody was detectable in serum specimens after acid dissociation and ultrafiltration, including some sera from donor UM-17 that did not contain detectable antibody activity in the native state. Sera from donor UM-23 became positive and those from donor UM-17 either became positive or had a higher titer after treatment.

To determine the distribution of the antigens detected by the autologous antibodies in these two systems we used absorption analysis. Antibodies in both systems appear to define antigens that are shared by some but not all other tumors. The antibody in UM-17 serum 1046 defines an antigen that so far has been found on both UM-SCC-17A and -17B as well as on four other SCCHN lines. No expression was detected on normal fibroblasts from the UM-17 donor nor on four other SCCHN lines or two melanoma lines. There seems to be no site-specific distribution for antigen-positive lines since lines from the alveolar ridge (UM-SCC-8), base of tongue (UM-SCC-18), skin (UM-SCC-21A), and hypopharynx (UM-SCC-22B) were positive, while UM-SCC-1, UM-SCC-2, and UM-SCC-10A from the floor of the mouth, alveolar ridge, and larynx, respectively, were negative. The remaining serum from this time point is limited in quantity and is reserved for experiments designed to identify the biochemical nature of the autologous antigen. For this reason, the full distribution of the antigen is not yet known, nor do we expect to carry out AD-U analysis of this serum. At the time of this writing, partial specificity analysis of AD-U treated serum 1986 from the UM-17 donor is being carried out. Both UM-SCC-17A and UM-SCC-8 (positive with 1046), as well as several other SCC lines (UM-SCC-9, -30, and -35), but not allogeneic lymphocytes, red cells or fibroblasts, exhibit reactivity with the antibodies revealed by AD-U. However, two melanoma lines (Y-Mel-84:420, Y-Mel-84:710) not tested with 1046 also bind these antibodies. Nevertheless, seven other
nonsquamous malignant lines do not. Thus it is too early to determine whether the antibody in native serum 1046 is the same as that revealed in 1986 by AD-U treatment.

The autologous antibody detected in UM-23 sera after acid dissociation also defines a broadly distributed antigen expressed by two other SCCHN lines, three melanoma lines, four glioma lines, one renal cell carcinoma line, and one colon carcinoma line. Thus both of the antigens defined by these autologous serum-tumor combinations have Class II distribution (18). The basis for Class II antigens is not known. However, one possible explanation is that the positive lines share a common etiological factor such as a virus. At present, no common viral etiologies are known for the range of tumors that express the UM-23 antigen. However, recent evidence from our laboratory and others implicates human papilloma virus as a possible common factor in some SCC of the head and neck (19, 20) while zur Hausen and coworkers have found a new human papilloma virus genotype in malignant melanoma (21).

The relatively low frequency and titer of antibody to tumor cells in patients' sera has raised questions regarding the relevance of the host's humoral immune response to cancer. We postulated that, as in melanoma, the low titer and incidence of autologous antibody reactivity may be secondary to circulating antigen forming immune complexes. Elevated levels of immune complexes have been reported in SCCHN patients as well as those with other malignant diseases. Maxim et al. (22) and Veltri et al. (23, 24) reported circulating immune complexes in 15 patients with SCCHN using two different Raji cell assays. Elevated levels of immune complexes were noted in 75% of patients studied. Furthermore, (24) they showed a correlation between a rise in immune complexes and a drop in cell-mediated immunity as measured by leukocyte migration-inhibition. The authors postulated that elevated levels of immune complexes may be involved in modulating the host's immune response. However, the nature of the antigen and antibody forming the immune complexes was never determined.

One potential objection to the use of acid dissociation and ultrafiltration is that it limits the examination of the autologous immune response to those antigens of less than M, 100,000. While it is certainly possible that there are antigens of greater than M, 100,000 that may be immunogenic to the host, this does not diminish the potential significance of the antigens detected in this study. The range of tumors carrying this antigen suggests the existence of shared etiological factors or alternately may indicate common pathways of gene activation that results in expression of a shared immunogenic membrane antigen on tumors from different sites and donors. The significance of these antigens to the host's response to cancer and to possible immunological interventions is yet unknown. However, previous studies involving melanoma have demonstrated correlations between autologous antibody, clinical course, and prognosis (25, 26). Whether similar conclusions can be drawn with SCCHN may be determined by serial studies of autologous antibody reactivity currently underway.

The role that the autologous immune response plays in cancer remains to be determined. It may be that circulating immune complexes, tumor-associated antigen, and antibody are somehow involved in immunosuppression (24, 27–29). At this stage of our analysis it is not possible to say with assurance that the serological reactivity revealed by AD-U is the result of the separation of antigen-antibody complexes. Prior analyses of AD-U-treated serum from melanoma patients has led to the identification of antigenic molecules present in both spent media and the ultrafiltrate (30). Future analyses of squamous cancer systems may yield similar results. Characterization of the tumor-associated antigens recognized by the host may increase our understanding of the host-tumor interaction and may lead to an ability to augment the autologous immune response in patients with cancer.

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