The Isolation and Characterization of Tumor-specific Antigens of Rodent and Human Tumors

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Summary

Putative tumor-specific transplantation antigens (TSTA) from both a carcinogen-induced rodent tumor (MC-1) and 2 human tumors were purified. The antigens were solubilized from the tumor cell membranes by limited papain digestion in a manner similar to that described for the isolation of normal histocompatibility antigens. The antitumor immune response of the tumor-bearing host was used to monitor the purification of the putative TSTA in both the rodent and human tumor systems. In the case of the rodent tumor, a major step in the purification of the TSTA involved affinity chromatography on Sepharose beads coupled to autologous antitumor antiserum. A comparable procedure was utilized in the purification of the TSTA from human tumors by using affinity chromatography on anti-human \( \beta_2 \)-microglobulin antiserum coupled to a solid phase. The data obtained indicate that the TSTA of human tumors contains a \( \beta_2 \)-microglobulin chain that is immunochemically identical with, and very similar in size to, that found in normal human histocompatibility antigens. A subunit of similar size was also identified in the carcinogen-induced rodent tumor. These results suggest that the TSTA in both humans and rodents may well be altered histocompatibility antigens.

Introduction

Investigations based on the rejection of experimentally induced tumors into previously immunized syngeneic recipients have established the existence of TSTA in most animal tumor systems studied (12, 23). Whereas the TSTA of tumors induced by the same virus are common, those induced by chemical carcinogens are most usually unique to the individual tumor (23). In both cases, however, the TSTA sites of major functional importance, as concerns immunological interaction, would appear to be on the surface membrane of the tumor cell (2). In murine tumor systems, at least, a good deal of evidence has accumulated that suggests the TSTA bears immunochemical and structural similarities to normal histocompatibility antigens (4, 7, 8, 16, 24).

Although the rejection of transplantable tumors among syngeneic animals has been used profitably in the study of animal tumors, this type of experimental design has been precluded in humans by both ethical considerations and the lack of a syngeneic donor-host relationship. Thus, a number of in vitro assays of the host's cellular and humoral immune responses against putative TSTA have been developed which are applicable to both animal and human tumor systems (1, 13, 14). Because the TSTA appear to be only weakly immunogenic, the antitumor immune responses evoked on the part of the tumor host are correspondingly weak (12, 23). Nevertheless, these immune responses are apparently far more tumor specific than can be evoked by the immunization of xenogeneic animals.

On the basis of the foregoing observations, the experiments to be described were designed with the following objectives: (a) to use the host's antitumor immune response in order to monitor the purification of the putative TSTA of rodent and human tumor tissues; (b) to determine whether any structural and/or immunochemical similarities exist between human and rodent TSTA; and (c) to determine whether there are any immunochemical and/or structural relationships between the TSTA and normal histocompatibility antigens in either the rat or human.

Materials and Methods

Tumor Tissues

In the experiments involving rodents, the methylcholanthrene-induced sarcomas, MC-1 and MC-11, 2 TSTA-distinct tumors serially passaged in syngeneic male hooded rats, were used (31). Early generations of MC-1 and MC-11 were stored in liquid nitrogen and removed at intervals for passage in syngeneic hooded rats. Human malignant melanoma tissue and breast cancer tissue were obtained either at surgery or at autopsy and stored at —40° until processed.

Purification of the Putative TSTA of Rodent and Human Tumors

Preparation of Tumor Cell Membranes. The tumor tissue was thawed and finely minced with stainless steel scissors. The mince was suspended in 4 volumes of 0.25 m sucrose containing 0.05 m Tris, 2 m\( \text{mM CaCl}_2 \), and 2 m\( \text{mM MgCl}_2 \), pH 7.5, at 4°. The tumor tissue was homogenized with an Ultra-
The soluble tumor membrane components were sub-jected to chromatography on a DEAE-Sephadex A-50 col-
umn equilibrated with the buffer described above. Ion-ex-
change chromatography on DEAE-Sephadex removes con-
taminants of highly negative charge, and there appears to
be a separation of protein from nucleic acid components
(22). The unbound fraction, which contained the TSTA ac-
tivity, was then dialyzed against a 0.1 M Tris-0.3 M glycine-
0.2 M NaCl buffer, pH 8.0, containing 0.001 M disodium
EDTA, and was then concentrated by ultrafiltration in an
Amicon chamber as described above. The concentrate was
centrifuged at 120,000 × g for 1 hr, and the supernatant was
then subjected to molecular sieve chromatography on a
 calibrated Sephadex G-150 column equilibrated with the
same Tris-glycine-NaCl buffer described above. The frac-
tions obtained were concentrated by ultrafiltration and ass-
ayed for tumor antigenic activity.

**Affinity Chromatography of the Tumor Antigenic Prepara-
tions.** The γ-globulin fraction from the sera of syngeneic
hooded rats, hyperimmunized to the MC-1 sarcoma as pre-
viously described (30, 31), was precipitated by 50% satu-
rated ammonium sulfate. This material was covalently cou-
pled to cyanogen bromide-activated Sepharose 4B in 0.2 M
citrate buffer, pH 6.5 (6, 31).

Antiserum to human β,m was raised in horses, using puri-
fied β,m kindly provided by Dr. M. D. Poulik of the
William Beaumont Hospital, Royal Oak, Mich. Serial bleed-
ings were obtained from these animals, and their serum
antibody activity against β,m was measured by radioimmu-
noassay as previously described (28). At the earliest evi-
dence that these animals were producing anti-β,m anti-
body, presumably of relatively low affinity, the γ-globulin
fraction of the horse serum was obtained by batch chroma-
tography on Whatman DE-52 DEAE-cellulose equilibrated
with 0.01 M phosphate buffer at pH 7.6. This material was
then coupled to Sepharose 4B using cyanogen bromide as
described above (6, 31).

The TSTA-containing fractions obtained from the Sepha-
dex G-150 columns described above were subjected to af-
finity chromatography on an appropriate antibody-bound
column. After the column effluent had been thoroughly
washed with 0.01 M Tris-0.14 M NaCl buffer, pH 7.4, the
antibody-bound fraction was then eluted with 2.5 M MgCl₂,
pH 6.8, and 3 M KCNS for the rodent tumor antigens and the
human tumor antigens, respectively. The MC-1 TSTA was
trace-labeled with ¹²⁵I before application to the affinity col-
mum. The material that did not adhere to the affinity column
was designated as the unbound fraction, while the material
that was bound to the column and then eluted with the
chaotropic agent was designated as the bound fraction.

The dialyzed eluate was centrifuged for 1 hr at 75,000 × g
and concentrated by ultrafiltration in an Amicon chamber
fitted with PM-10 membrane. The concentrated eluate was
again centrifuged at 100,000 × g for 1 hr. This final cen-
trifugation step removed any Sepharose particles that may have
escaped from the column and/or any denatured and aggre-
gated proteins that had been eluted, both of which may
non-specifically interfere with the LAI assay for tumor anti-
genicity to be described below. Table 1 summarizes the
steps in the isolation of the putative rodent and human
tumor antigens.

**Papain Solubilization of Membrane Protein.** The purified
membranes were suspended in PBS, pH 7.3, at a protein
concentration of approximately 10 to 12 mg/ml, as deter-
mined by the procedure of Lowry et al. (20). The suspension
was incubated at 37°C in a water bath, and twice-crystallized
papain (Worthington Biochemical Corp., Freehold, N. J.)
preactivated in cysteine was then added to a final concen-
tration of 0.5 unit per mg of membrane protein. The incuba-
tion was continued for 1 hr at 37°C, during which period the
suspension was stirred continuously. Proteolysis was ar-
rested by the addition of iodoacetamide to a final concen-
tration of 0.1 M. The suspension was then centrifuged for
1 hr at 75,000 × g in a fixed-angle rotor in a MSE
Superspeed-65 centrifuge (19). The supernatant fluid, which
constituted two-thirds of the volume in each bottle, was de-
canted, and the pellet was resuspended in an equal volume
of the same buffer. Both the homogenization and centrifu-
gation steps were repeated as described above, and the
decanted supernatants were pooled. An aliquot of 3 M lith-
ium chloride in distilled water was gradually added to the
total supernatant to a final concentration of 0.1 M. The
membranes in the supernatant were sedimented by centri-
fugation for 1 hr at 75,000 × g in a fixed-angle rotor in a MSE
Superspeed-65 centrifuge (19). The supernatant fluid was
discarded, and the crude membrane preparation was sus-
pended in 0.5 M Tris buffer, pH 7.5, containing 0.1 M lithium
chloride, to a volume equal to about one-half of the original
pooled supernatant. The pellets were then homogenized to
a smooth suspension with the Ultraturrax homogenizer. The
resuspended crude membranes were again sedimented at
75,000 × g for 1 hr. The supernatant was discarded and the
membrane pellet was suspended in 0.01 M Tris-HCl, pH 7.5,
containing 1 unit of penicillin and 1 μg of streptomycin per
ml. The volume was adjusted to approximately 200 ml and
stirred overnight at 4°C. The membranes were sedimented at
120,000 × g for 1 hr, and this fraction was then separated by
centrifugation through a dextran-polyethylene glycol
aqueous 2-phase system adapted from that described by
Brunette and Till (3). On completion of this centrifugation
step, the membranes were found at the interface of the 2-
phase system. The membrane fraction was collected, di-
luted with approximately 4 times its volume of cold distilled
water, and sedimented by centrifugation at 75,000 × g for 1
hr. The membrane pellet was resuspended in cold distilled
water and the centrifugation step was repeated. The pellets
were then resuspended in cold PBS, pH 7.3, and stored at
-40°C.

**Chromatographic Procedures in the Purification of
TSTA.** The soluble tumor membrane components were sub-
Assays for TSTA Activity. Indirect Membrane Immunofluorescence. Indirect membrane immunofluorescence was performed on washed viable single tumor cell suspensions prepared from finely minced solid tumor digested in 0.04% trypsin and 0.05% collagenase in the presence of a small amount of DNase (30). The presence of antigenic activity in the papain-soluble tumor digest of MC-1 membranes was detected and quantitated by the capacity to inhibit MC-1 tumor-immune antiserum from binding to cell surface antigens on viable MC-1 tumor cells. The fluorescence index was computed and expressed as previously described (30).

Tube LAI Assay. Isolated membranes, cell sap, and papain-solubilized membrane fractions obtained upon gel chromatography were assayed for human tumor antigen activity by determining their capacity to inhibit the adherence of leukocytes in test tubes. The assay was performed, and the NAI was calculated and expressed as previously described (10, 21). The NAI represents the difference in reactivity to the specific and nonspecific tumor extracts. The higher the NAI, the greater is the differential in reactivity between the tumor and control tissue extracts, whereas a low NAI indicates minimal or no difference in reactivity. A NAI of 30 or more is indicative of a positive response, i.e., a significant inhibition of leukocyte adherence to glass based on large series of breast cancer, melanoma, and control subjects (10, 21). Leukocytes from patients with limited cancer (Stage I or II) that reacted in the tube LAI assay to the appropriate PBS extracts of either breast cancer or melanoma were used to determine whether the isolated tumor materials contained tumor-specific activity. Control subjects had either benign surgical diseases or unrelated cancers.

Electrophoretic Analysis of Tumor Antigens

The papain-solubilized membrane preparations, obtained as described above, and those isolated at each step of purification were subjected to assays for tumor antigen. In addition, those fractions containing tumor antigenic activity were radiolabeled with $^{125}$I by the chloramine-T method (9) and analyzed by SDS-polyacrylamide gel electrophoresis in 7.5 and 10% gels (32), and in 12.5% 8.0 M urea gels (29). The gel was then cut into individual 1-mm sections and counted in a Nuclear-Chicago γ-sciillation spectrometer. These procedures provided information concerning both the degree of purity of the preparations in question and the molecular weights of the molecules, or their subunits, involved.

Results

Purification of the Papain-solubilized Tumor Antigen of the MC-1 Rat Sarcoma. Table 2 reveals that the TSTA activity of the MC-1 tumor is present on the tumor cell membranes rather than in the cell sap. Moreover, this surface-associated tumor antigen was solubilized by limited papain digestion and was partially purified by ion-exchange and molecular sieve chromatography. From the data obtained from molecular sieve chromatography on Sephadex G-150, the maximum TSTA activity appeared to reside in the molecular weight range of 40,000 to 60,000 daltons (Table 2; Chart 1). After affinity chromatography on the anti-MC-1 affinity

<table>
<thead>
<tr>
<th>Extract used for inhibition of syngeneic anti-MC-1 immune serum</th>
<th>Fluorescence index with MC-1 sarcoma cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>9.6</td>
</tr>
<tr>
<td>Normal tissue extract</td>
<td>9.0</td>
</tr>
<tr>
<td>Purified membranes</td>
<td></td>
</tr>
<tr>
<td>MC-1</td>
<td>2.0</td>
</tr>
<tr>
<td>MC-11</td>
<td>8.4</td>
</tr>
<tr>
<td>Papain-soluble MC-1-1' Sephadex G-150</td>
<td></td>
</tr>
<tr>
<td>Fraction 1</td>
<td>8.0</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>6.6</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>4.0</td>
</tr>
<tr>
<td>Fraction 4</td>
<td>7.1</td>
</tr>
<tr>
<td>Papain-soluble MC-11' Sephadex G-150</td>
<td></td>
</tr>
<tr>
<td>Fraction 1</td>
<td>8.5</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>8.9</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>8.3</td>
</tr>
<tr>
<td>Fraction 4</td>
<td>8.8</td>
</tr>
<tr>
<td>Affinity chromatography</td>
<td></td>
</tr>
<tr>
<td>MC-1 TSA</td>
<td></td>
</tr>
<tr>
<td>Eluate, bound</td>
<td>4.6</td>
</tr>
<tr>
<td>Effluent, unbound</td>
<td>8.9</td>
</tr>
<tr>
<td>MC-11 TSA</td>
<td></td>
</tr>
<tr>
<td>Eluate, bound</td>
<td>9.1</td>
</tr>
<tr>
<td>Effluent, unbound</td>
<td>9.0</td>
</tr>
</tbody>
</table>

* Anti-MC-1, immune serum was used at a final dilution of 1:10 with tissue extracts.

| Protein contents of fractions were approximately 8 mg/ml. |
| Effluent, unbound contained a protein content of approximately 500 μg/ml. The effluent contained a protein content of 8 mg/ml. |
Characterization of Tumor Antigens

Column chromatography. Papain-soluble membrane antigen from DEAE-Sephadex chromatography was applied to the column of Sephadex G-150 (5 x 87 cm) equilibrated with 0.3 M glycine-0.2 M NaCl-0.001 M disodium EDTA and 0.1 M Tris-HCl, pH 8.0; and 10-ml fractions were collected and assayed for MC-1 TSA activity by inhibition of membrane immunofluorescence. Maximum MC-1 TSA activity was in the area shown by the bar.

Column, it was found that only the bound fraction contained tumor antigenic activity (Table 2; Chart 2). Hence, a good deal of the papain-solubilized membrane preparation that had copurified with the TSTA to the point of molecular sieve chromatography failed to bind to the specific antitumor antibody, appeared in the column effluent, and lacked tumor antigenic activity.

Papain-soluble membrane material from the unrelated and non-cross-reacting MC-11 tumor applied to the MC-1 affinity column showed no specific binding, since the material in the eluate did not inhibit in the membrane immunofluorescence assay (Table 2).

SDS-Gel Electrophoresis of Purified MC-1 TSA. The 125I-labeled MC-1 TSA eluted from the anti-MC-1 affinity column was applied to a Sephadex G-200 column and eluted with a single major peak with molecular weights of approximately 45,000 to 60,000. However, an analysis of the 125I-labeled MC-1 TSA by SDS-polyacrylamide gel electrophoresis revealed that, in the presence of a reducing agent, 125I-labeled MC-1 TSA appeared to be composed of 3 polypeptide chains with apparent molecular weights of 12,000, 18,000, and 25,000 daltons (Chart 3). The major peak had an apparent molecular weight of 12,000 daltons, and this peak had a mobility identical to that of 125I-labeled human β2m.

Isolation of Tumor Antigenic Material from Human Breast Cancer and Malignant Melanoma. The development of the LAI assay in test tubes for the detection of antitumor immunity in patients with breast cancer (10) and malignant melanoma (21) has provided an immunological technique for monitoring the purification of the tumor antigens from melanoma and breast cancer preparations. The results shown in Tables 3 and 4 reveal that the tumor antigenic activity of these tumors, as monitored by LAI, was found in the purified tumor cell membrane preparation and not in the cell sap. The material containing this activity eluted maximally in the early fractions from the Sephadex G-150 column, in the apparent molecular weight range of 70,000 to 150,000 daltons (Fraction 2) (Tables 3 and 4). However, when the LAI-active fractions were chromatographed on anti-human β2m affinity columns, only the bound fraction retained this activity (Tables 5 and 6). No LAI-active material was found in the unbound fractions at any time. Moreover, there was no evidence of cross-reactivity of the tumor antigenic activity between the breast and melanoma systems.

SDS-Polyacrylamide Gel Electrophoresis of Isolated Melanoma and Breast Tumor Antigens. The papain-soluble breast cancer and melanoma antigens in the 70,000- to 150,000-molecular-weight fraction of a Sephadex G-150 column were labeled with 125I by the chloramine-T method and analyzed by SDS-polyacrylamide gel electrophoresis in the presence of reducing agents. Both tumor antigens had
Antigenic activity determined by tube LAI assay of papain-solubilized malignant melanoma antigen

<table>
<thead>
<tr>
<th>Leukocytes from</th>
<th>PBS extract*</th>
<th>Purified membranes</th>
<th>Cell sap</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma Patient 1</td>
<td>88</td>
<td>125</td>
<td>85</td>
<td>115</td>
<td>30</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Melanoma Patient 2</td>
<td>60</td>
<td>65</td>
<td>75</td>
<td>60</td>
<td>28</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Control subject</td>
<td>12</td>
<td>3</td>
<td>15</td>
<td>12</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Control subject</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

No. of nonadherent cells in the presence of specific tumor extract

\[ \text{NAI}^* = \frac{\text{No. of nonadherent cells in the presence of specific tumor extract}}{\text{No. of nonadherent cells in the presence of nonspecific tumor extract}} \times 100 \]

A NAI of 30 or greater indicates significant inhibition of adherence.

* A PBS extract of breast cancer was the nonspecific extract.

Chromatographic fractions were concentrated to approximately 6 mg/ml and tested at a 1:4 dilution in Medium 199.

Antigenic activity determined by tube LAI assay of papain-solubilized breast cancer antigen

<table>
<thead>
<tr>
<th>Leukocytes from</th>
<th>PBS extract*</th>
<th>Purified membranes</th>
<th>Cell sap</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer Patient 1</td>
<td>92</td>
<td>58</td>
<td>8</td>
<td>48</td>
<td>110</td>
<td>31</td>
<td>6</td>
</tr>
<tr>
<td>Breast cancer Patient 2</td>
<td>70</td>
<td>41</td>
<td>10</td>
<td>78</td>
<td>115</td>
<td>25</td>
<td>9</td>
</tr>
<tr>
<td>Control subject</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>13</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Control subject</td>
<td>1</td>
<td>3</td>
<td>12</td>
<td>15</td>
<td>22</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

* A NAI of 30 or greater is positive.

* A PBS extract of the melanoma antigen was used as the nonspecific control extract.

Chromatographic fractions were concentrated to approximately 5 to 8 mg/ml protein and tested in tube LAI assay at a 1:4 dilution in Medium 199.

Antigenic activity of TSA of malignant melanoma isolated by affinity chromatography with anti-human βm

<table>
<thead>
<tr>
<th>Leukocytes from</th>
<th>NAI* to PBS extract of malignant melanoma</th>
<th>NAI* affinity chromatography</th>
<th>Unbound*</th>
<th>Bound*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma Patient 3</td>
<td>89</td>
<td>11</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Melanoma Patient 4</td>
<td>84</td>
<td>17</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Control subject</td>
<td>5</td>
<td>-16</td>
<td>-6</td>
<td></td>
</tr>
</tbody>
</table>

* NAI was calculated with PBS extract of ovarian tumor as a nonspecific control antigen. Human βm isolated identically from a hepatoma was also used as a nonspecific control, and the results were unchanged

Unbound and bound fractions were concentrated to approximately 2 mg/ml and tested in the tube LAI assay at a protein concentration of 75 to 150 μg.

similar profiles on SDS electrophoresis (Charts 4 and 5). There were 2 major peaks; the peak with the fastest mobility moved identically with that for 125I-labeled human βm. The minor intermediate peak had an electrophoretic mobility of approximately 25,000 daltons. The slowest component had an approximate molecular weight of 40,000 (Charts 4 and 5).

The material that bound to the anti-human βm affinity column and that had demonstrated LAI activity was also labeled with 125I. The radiolabeled material, when analyzed by SDS gel electrophoresis, showed the same 3 peaks of 12,000, 25,000, and 40,000 daltons (Chart 6).

Approximately 70% of the material applied with TSTA activity was bound to the anti-human βm column. After application of the chaotropic agent, 70 to 90% of the bound fraction was recovered as determined by measurements of human βm or total protein. The unbound fraction contained no, or minimal amounts, of human βm as determined by radioimmunoassay for human βm. In contrast, the bound fraction was considerably enriched in βm.
Characterization of Tumor Antigens

Since the papain-solubilized TSA of the MC-1 tumor retains its antigenicity, as determined by its ability to neutralize MC-1 tumor-immune serum in the membrane immunofluorescence assay, the technique of affinity chromatography was chosen to achieve selective isolation and purification of the MC-1 tumor-specific antigen. The Sepharose-anti-MC-1 antibody column, unlike conventional techniques, could selectively isolate the tumor-specific moiety from a variety of other membrane components. The isolated TSA of the MC-1 rat sarcoma was approximately 50,000 to

Discussion

The results of the present investigation indicate that the tumor antigens of both a carcinogen-induced rodent tumor and those human tumors that were studied are present in the tumor cell membrane and can be solubilized by limited papain digestion in a manner similar to that used for the purification of histocompatibility antigens from various species. Moreover, it was possible to use the host's antitumor immune response as a method of monitoring the purification of the tumor antigenic preparation. The data obtained suggest that, in the case of the human tumor studied, at least, the surface tumor antigens involved contain a $\beta_2m$ chain that is immunochemically identical with and is very similar in size to that found in normal human histocompatibility antigens. A subunit of similar size was also identified in the carcinogen-induced rodent cancer.
60,000 daltons as determined by Sephadex G-200 chromatography. On SDS-gels, when reduced, this material consisted of 3 subunits with molecular weights of approximately 11,000, 18,000, and 25,000 daltons. The 11,000-molecular weight subunit had a mobility identical with that of human $\beta_m$.

The isolated MC-1 TSA of the chemically induced tumor thus appears to be composed of subunits with molecular weights similar to those described for the rat Ag-B histocompatibility antigens (17, 18).

Halliday and Miller (11) have described a simple in vitro technique of detecting hostantitumor immunity called LAI. Holan et al. (15) described a modified version of this assay in rats that was performed in test tubes. In our laboratory this technique was adapted and modified for the detection of sensitization to human breast cancer and malignant melanoma tumor antigens (10, 21). With this assay, leukocytes sensitized to the tumor antigen respond by not adhering to glass. It appeared feasible, therefore, to use the same responding cells to monitor the purification of the sensitizing antigen, since, as long as some form of the sensitizing antigen was added to the test tubes, the sensitized cells should demonstrate the response of nonadherence to glass.

The results of the present study indicate that the tube LAI assay can be used to monitor the isolation of tumor antigens and that tumor antigens isolated from human breast cancer and melanoma tissue have similar physicochemical properties. Both tumor antigens appear to be composed of subunits of similar size. SDS-polyacrylamide gel electrophoretic studies revealed that both human breast and melanoma tumor antigens consisted of 3 molecular species weighing about 11,000, 25,000, and 40,000 daltons. Although the HLA molecules are now thought to be composed of 2 distinct subunits ($\beta_m$ and a heavier alloantigenic chain), similar structural components have been obtained for the HLA antigens isolated from human lymphoid cells after papain digestion (5, 25, 26). Furthermore, $\beta_m$, the 11,000-dalton polypeptide chain found in all histocompatibility antigens, appears to be an integral part of the breast and melanoma tumor antigens.

It appears that rodent and human TSTA have structural similarities and, at least in man, the 11,000-dalton subunit is apparently $\beta_m$. Thus, the TSTA in rodents and humans is very similar to the AG-B and HLA species-specific histocompatibility antigens. The tumor-specific polypeptide chain may, in fact, represent a modified histocompatibility antigen. By analogy to the histocompatibility antigens, the larger subunits therefore appear to carry the distinctive tumor antigenic determinant. The biological significance of these observations is compatible with the hypothesis (27) that histocompatibility molecules serve as adapters that combine with non-self-components that are recognized by cytotoxic T-lymphocytes. Current work in our laboratory is designed to establish a similarity, at the level of primary structure, between the histocompatibility antigens and tumor antigens.

**References**


14. Holan, V., Hasek, M., Bubenik, J., and Chutna, J. Antigen-Mediated Immunization. D. M. P. Thomson et al. 60,000 daltons as determined by Sephadex G-200 chromatography. On SDS-gels, when reduced, this material consisted of 3 subunits with molecular weights of approximately 11,000, 18,000, and 25,000 daltons. The 11,000-molecular weight subunit had a mobility identical with that of human $\beta_m$.

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Halliday and Miller (11) have described a simple in vitro technique of detecting hostantitumor immunity called LAI. Holan et al. (15) described a modified version of this assay in rats that was performed in test tubes. In our laboratory this technique was adapted and modified for the detection of sensitization to human breast cancer and malignant melanoma tumor antigens (10, 21). With this assay, leukocytes sensitized to the tumor antigen respond by not adhering to glass. It appeared feasible, therefore, to use the same responding cells to monitor the purification of the sensitizing antigen, since, as long as some form of the sensitizing antigen was added to the test tubes, the sensitized cells should demonstrate the response of nonadherence to glass.

The results of the present study indicate that the tube LAI assay can be used to monitor the isolation of tumor antigens and that tumor antigens isolated from human breast cancer and melanoma tissue have similar physicochemical properties. Both tumor antigens appear to be composed of subunits of similar size. SDS-polyacrylamide gel electrophoretic studies revealed that both human breast and melanoma tumor antigens consisted of 3 molecular species weighing about 11,000, 25,000, and 40,000 daltons. Although the HLA molecules are now thought to be composed of 2 distinct subunits ($\beta_m$ and a heavier alloantigenic chain), similar structural components have been obtained for the HLA antigens isolated from human lymphoid cells after papain digestion (5, 25, 26). Furthermore, $\beta_m$, the 11,000-dalton polypeptide chain found in all histocompatibility antigens, appears to be an integral part of the breast and melanoma tumor antigens.

It appears that rodent and human TSTA have structural similarities and, at least in man, the 11,000-dalton subunit is apparently $\beta_m$. Thus, the TSTA in rodents and humans is very similar to the AG-B and HLA species-specific histocompatibility antigens. The tumor-specific polypeptide chain may, in fact, represent a modified histocompatibility antigen. By analogy to the histocompatibility antigens, the larger subunits therefore appear to carry the distinctive tumor antigenic determinant. The biological significance of these observations is compatible with the hypothesis (27) that histocompatibility molecules serve as adapters that combine with non-self-components that are recognized by cytotoxic T-lymphocytes. Current work in our laboratory is designed to establish a similarity, at the level of primary structure, between the histocompatibility antigens and tumor antigens.

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