A Preliminary Evaluation of Tissue Polypeptide Antigen in Serum or Urine (or Both) of Patients with Cancer or Benign Neoplasms

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We evaluated assays of Tissue Polypeptide Antigen in serum and urine, as an index to the presence of cancer. In the assay, serum, which is first absorbed with human albumin-labeled sheep erythrocytes, or untreated urine (diluted with an equal volume of TPA-free serum) is incubated with antibody specific to Tissue Polypeptide Antigen and then reacted with sheep erythrocytes labeled with Tissue Polypeptide Antigen. We found an increased concentration of Tissue Polypeptide Antigen in the serum of 378 of 513 (74%) and in the urine of 49 of 77 (64%) patients with cancer, as compared with 40/112 (36%) and 7/29 (24%), respectively, for individuals with benign neoplasms. Normal individuals were defined as those with less than 0.09 unit of the antigen per milliliter of specimen. Concentrations exceeding this were found in 2/67 (3%) sera and 6/56 (11%) urines from supposedly normal persons. Tissue Polypeptide Antigen was found in above-normal concentrations in patients with cancer, regardless of neoplasm type and extension, with a higher proportion of abnormal values in patients with distal metastases.

Additional Keyphrases: cancer • diagnosis and monitoring treatment • hemagglutination inhibition • tumor-related antigens • diagnostic aids

During the past several years there has been much interest in identifying, characterizing, and making clinical use of tumor-related antigens (1-4). Several of these have been measured in plasma and other biological fluids in an attempt to use them as diagnostic aids and in following the course of malignant disease. Björklund and his associates described an antigen that is present in a wide variety of anaplastic tumors (5-13), a polypeptide that can be isolated from human cancer tissue and human placenta. They have called it “Tissue Polypeptide Antigen” (TPA).3

Lüning et al. (13) reported that the TPA polypeptide is composed of more than 200 amino acid residues and that its relative molecular mass is from 2.2 to 2.5 x 104. Arginine and tyrosine are essential for TPA reactivity with anti-TPA antibody. TPA contains only traces of cysteine and no carbohydrates. They synthesized a 26-residue peptide showing TPA activity, which had a symmetry of charges around the central positively charged arginine residues.

A TPA assay has been described for clinical use (11). It is based on a classical hemagglutination inhibition reaction (10), and less than nanogram quantities of TPA can be measured in serum, urine, or body fluids. We describe here our experiences in a preliminary evaluation of the results of TPA assays in serum and urine of patients with cancer, non-malignant disease, and of normal individuals.

Materials and Methods

Apparatus

Specimens were stored at -20 °C. For centrifugation we used a Model IEC PR 6000 centrifuge (Demon/IEC, Needham Heights, Mass. 02194).

The hemagglutination-inhibition reaction was performed at 0-4 °C in a Micrortit U Plate (Cooke Engineering Co., Alexandria, Va. 22314), placed in a refrigerator made vibration free by relocating its motor to a position outside the box.

Sheep erythrocytes were counted in the Coulter Counter Model ZF after being diluted 50,000-fold with Isotone by use of a dilutor (all from Coulter Electronics, Inc., Hialeah, Fla. 33010).

Routinely, photographs of the Micrortit U Plates were taken with the Immunodiffusion camera (Cordis Labs., Miami, Fla. 33137) with use of type 180 Polacolor film (Polaroid Corp., Cambridge, Mass. 02139).

Reagents and Solutions

Serum, plasma, and urine specimens: These were randomly obtained from specimens submitted for diagnostic purposes to our department. The disease type and extent of cancer was established by careful review of the patients’ medical history and confirmed by the histopathology of surgical or autopsy specimens.

Sheep erythrocytes: These, in Alsever’s solution, were obtained from the Hemoline Co., Denver, Colo. 80206.

Sodium and potassium phosphate buffer, 13 mmol/liter, pH 6.8 or pH 7.5, containing 0.15 mol of NaCl per liter.

Human albumin, tannic acid, TPA antigen, and TPA antibody: These were prepared by Dr. Bårtel Björklund, National Bacteriological Laboratory, S-105 21, Stockholm, Sweden, and provided to us by the Damen Corp. Björklund defined 0.09 unit of TPA per milliliter to correspond to 0.09 μg of TPA per milliliter.

Carcinoembryonic antigen (CEA): Reagents for CEA were from Hoffmann-La Roche Inc., Nutley, N.J. 07110.
Procedure

Sample preparation: Urine specimens were stored in a freezer at -20 °C within 1 h after collection. Sera were first warmed for 30 min in a water bath set at 56 °C, then frozen. Specimens were kept for as long as a month until processed.

Before the hemagglutination inhibition reaction, the urines were diluted with an equal volume of pooled human serum that on previous analysis showed no detectable TPA antigen (equal or less than 0.02 units per milliliter) or antibody. The sera were absorbed with an equal volume of packed erythrocytes (20 × 10⁹ erythrocytes per milliliter) overnight, at 0-4 °C. The following morning the sera were centrifuged for 30 min and the supernatant fluid was stored at -20 °C until assayed.

To check on the stability of the frozen samples, we did six experiments during one month, with use of a standard containing 0.1 unit of TPA per milliliter, a negative control (0.03 unit/milliliter) obtained from a pool of serum from patients with benign neoplasms, and a positive control (0.5 unit/ml) obtained from a pool of serum from patients with cancer. The stability showed a variation of ±0.02 unit/milliliter.

Preparation of the Sheep Erythrocytes

At the beginning of each week the sheep erythrocytes in Alsever's medium are washed twice in 13 mmol/liter phosphate buffer, pH 6.8, containing 150 mmol of NaCl per liter. The erythrocytes are then diluted with buffer to contain 1 × 10⁹ erythrocytes per milliliter. A portion of these cells is saved for use as controls and the remainder of them are “tanned” by diluting them with an equal volume of tannic acid solution (20-30 mg of tannic acid per milliliter of the buffer). The tanned cells are gently stirred for 10 min at 4 °C, washed once with a 13 mmol/liter phosphate buffer, pH 7.5, in 15 mmol/liter NaCl, and rediluted with buffer to contain 1 × 10⁹ erythrocytes per milliliter. A portion of the tanned cells is saved for TPA labeling; the rest are used for albumin absorption of patients' samples.

The tanned erythrocytes are labeled with TPA by mixing equal volumes of cells and TPA reagent. After being stirred for 10 min at 4 °C, the cells are washed, first with the pH 7.5 buffer and then with an aliquot of a pool of human serum with a low titer of TPA. The erythrocytes are diluted with the serum to contain 1.6 × 10⁸ cells per milliliter. The remaining tanned erythrocytes are diluted with an equal volume of a human serum albumin solution (120 mg/ml of the pH 7.5 phosphate-NaCl buffer). After being stirred for 10 min at 4 °C, the cells are washed with the buffer. An aliquot of the albumin-tagged cells is diluted with human serum pool to contain 1.6 × 10⁶ cells per milliliter (control cells) and the remainder of the packed cells are used to absorb patients' serum.

Selection of the Antibody Dilution

The evening before the assay, a standard titration plate is prepared by pipetting 12 serial dilutions of antibody into the wells. The dilutions, made with low-titer human serum pool, range from 2000- to 4800-fold. TPA labeled cells are added to all the wells and, after mixing, the plates are stored in the vibrationless refrigerator at 0-4 °C overnight.

The following morning the antibody titer to be used for the hemagglutination inhibition assay is chosen, the dilution chosen being that which permits obvious visual hemagglutination inhibition at 0.09 unit of antigen but not in the next lower dilution of antigen.

Hemagglutination Inhibition Assay for TPA Detection

The hemagglutination inhibition assay is done in a Microtiter U Plate containing eight rows of 12 wells each. Each plate includes a titration of TPA antigen from 1.5 to 0.02 unit of TPA per milliliter. This is used to quantitate the TPA concentrations in the patient's sample. An empirically determined normal–abnormal cutoff value of greater than 0.09 unit of TPA per milliliter was used. A typical plate is illustrated in Figure 1. Each titration well contained 25 μl of the standard or the patient's sample. To this was added 25 μl of the selected antibody dilution. Then the plate was gently shaken, incubated at room temperature for 30 min to allow the antigen and antibody to react, then refrigerated (0-4 °C) for 30 min, whereupon 50 μl of the TPA-labeled erythrocytes was added to each well. The plate was covered with cellophane tape, the well contents were thoroughly mixed, and the plate was refrigerated (0-4 °C) in the vibration-free refrigerator for a minimum of 4 h, or overnight.

The plate was read with the aid of an angled mirror, and color photographs were taken of each plate. A negative reaction (those with agglutination) shows a complete dispersion of the erythrocytes in the bottom of the well and a positive reaction appears as a doughnut-shaped formation.

CEA Determinations

Plasma samples were obtained from the same patients as those from which serum was obtained for TPA analysis. CEA was determined by the zirconyl phosphate gel procedure described by Hansen et al. (14), and an empirically determined normal–abnormal cutoff value of ≥25 μg/liter was used (15).
Table 1. Tissue Polypeptide Antigen in Patients with Cancer

<table>
<thead>
<tr>
<th>Site of cancer</th>
<th>Serum No. with increase/total</th>
<th>Urine No. with increase/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>67/94</td>
<td>12/25</td>
</tr>
<tr>
<td>Lung</td>
<td>28/35</td>
<td>1/4</td>
</tr>
<tr>
<td>Stomach</td>
<td>23/31</td>
<td>2/3</td>
</tr>
<tr>
<td>Colon/rectal</td>
<td>49/60</td>
<td>3/3</td>
</tr>
<tr>
<td>Pancreas</td>
<td>12/15</td>
<td>—</td>
</tr>
<tr>
<td>Bladder</td>
<td>18/24</td>
<td>5/5</td>
</tr>
<tr>
<td>Cervix-uterus</td>
<td>22/28</td>
<td>3/3</td>
</tr>
<tr>
<td>Prostate</td>
<td>9/17</td>
<td>1/1</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>16/23</td>
<td>0/1</td>
</tr>
<tr>
<td>Melanoma</td>
<td>19/29</td>
<td>5/6</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>22/26</td>
<td>4/6</td>
</tr>
<tr>
<td>Leukemia</td>
<td>23/31</td>
<td>2/5</td>
</tr>
<tr>
<td>Other cancers</td>
<td>70/100</td>
<td>11/15</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>378/513</strong></td>
<td><strong>49/77</strong></td>
</tr>
</tbody>
</table>

Results

TPA in Patients with Cancer

Table 1 summarizes our preliminary studies. Supranormal concentrations were observed in the serum of 378 of 513 patients (74%) and in the urine of 49 of 77 patients (64%) with various carcinomas. The carcinomas were divided into different groups according to sites of origin and the percentage of TPA elevations was reported. The diagnosis of disease was obtained from the patients' medical history and confirmed by histopathology.

Table 2 shows the relation between extent of disease and results for serum and urine of patients with cancer. The patients were categorized by disease and then divided into four groups: those with previous cancer but with no evidence of disease at the time the specimen was obtained, those with no known metastases, those with regional metastases, and those with distal metastases. TPA was found to be increased irrespective of the kind of cancer, with a higher percentage of increased values in patients with distal metastases.

Table 3 summarizes the relation of TPA concentrations in serum to the extent of disease. TPA concentrations in the range 0.13 to greater than 1.0 unit/milliliter were found in 71% of the patients with distal metastases, whereas such increases were found in 42% of patients who had no evidence of disease, 41% of those who had no known metastases, and 49% of those who had regional metastases.

TPA in Normal Persons and Patients with Non-Malignant Tumors

Serum and urinary TPA values were increased in a variety of non-malignant diseases (Table 4). Seven of 29 patients (24%) had TPA concentrations in their urine exceeding 0.09 unit/milliliter, as did 40 of 112 (36%) sera from patients, two of 67 (3%) sera from normal individuals, and six of 56 (11%) urines from the same normal persons.

Table 5 shows the relationship of TPA concentration in patients with benign neoplasms. Most (99%) of these patients were found to have values in the range of <0.09 to 0.25 units of antigen per milliliter of serum.

Comparison of TPA and CEA

In Table 6, results for TPA and CEA are compared in 118
patients with cancer and 53 patients with benign neoplasms. Of the patients with cancer, TPA was above normal in 69% and CEA in 42%. The concordance data for the cancer patients showed a correlation of 63%. Both assays were performed in 14 normal individuals; only one (7%) was found to have increased values for both TPA and CEA.

Discussion

TPA, a peptide in serum, has been suggested by Björklund as an index for identifying patients with cancer. Our data indicate that the antigen is present in abnormal concentrations in a relatively large proportion of patients with cancer, but also in a large proportion (36%) of patients with benign neoplasms. These data are similar to those originally reported by Björklund et al., who found increased values for about 25% of patients with non-malignant diseases. We emphasize that our study only includes a single specimen from each patient. Björklund has stated that it is necessary to assay several sequentially collected specimens (16), because infection and other inflammatory processes also may lead to increased values. A patient who has values that remain increased for several weeks is, according to Björklund, a “true” positive. TPA increases in benign neoplasm probably preclude its use as a single test for specific cancer-screening purposes.

Our studies indicate that TPA may be useful in the evaluation of the cancer patient, particularly if determined in association with other markers such as CEA, but a complete assessment of the value of TPA awaits the results of other studies. Prospective studies of a large group of cancer patients, as well as of normal individuals and persons with noncancerous disease, are required to establish TPA’s role in screening and to determine its usefulness in monitoring the course of the disease. In addition, further studies must be conducted to determine if TPA in association with the assay of other tumor-associated antigens or enzymes can yield specific information concerning the type and extent of disease.

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References


