Demonstration and Immunochemical Characterization of Carcinoembryonic Antigen in Human Pancreatic Juice 

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SUMMARY—Pancreatic juice collected from 10 patients without evidence of malignant disease of the pancreas or other organs was pooled, extracted, and fractionated by Sepharose 6-B and Sephadex G-200 gel filtration. The carcinoembryonic antigen (CEA) activity in the material was demonstrated and studied by: a) radioimmunoassay, b) competitive binding to antibodies against CEA, c) precipitin inhibition, and d) Ouchterlony analysis. The immunochemical identity of the active material to CEA purified from liver metastases of colon cancer was demonstrated.—J Natl Cancer Inst 56: 885-889, 1976.

Elevated levels of circulating carcinoembryonic antigen (CEA) are found in a high percentage of pancreatic cancer patients (1). The analysis of CEA in serum or plasma, however, is neither a specific nor early test for this disease; the high frequency of elevated levels is due partly to the fact that these patients usually present late in the course of their disease (2).

CEA activity has been reported in normal pancreatic juice as well as in that from patients with benign and malignant pancreatic disease (3, 4). This suggested that the quantitation of CEA activity in pancreatic juice may aid in the diagnosis of pancreatic disease (4). Banjo et al (5), comparing the properties of CEA isolated from gastric and colon tumors, suggested that CEA derived from tumors of different histologic sites may differ in composition and immunochemical properties. The present study investigates the immunochemical relationship of CEA-active material of human pancreatic juice to CEA purified from colon carcinoma.

MATERIALS AND METHODS

Collection of pancreatic juice.—Pancreatic juice from 10 patients clinically suspected of pancreatic disease (table 1) was collected by transduodenal cannulation of the pancreatic duct by one of us (J.A.G.). Output was stimulated by a single iv injection of 90 units of secretin. Samples were collected at 10-minute intervals, sealed, frozen immediately in Dry Ice, and stored at -20° C to minimize possible enzymatic destruction of the CEA activity. Three 10-minute collections were taken from most patients.

Radioimmunoassay of CEA activity.—Duplicate 0.5-ml aliquots of each 10-minute collection were diluted with 2.0 normal saline and assayed for CEA activity in the Hansen zirconyl phosphate gel (Z-gel) radioimmunoassay (RIA) (6) with the use of reagents from Hoffman-LaRoche Inc., Nutley, N.J. All samples were centrifuged (15,000×g for 20 min at 4° C) before analysis in order to remove mucoid aggregates and materials with high molecular weight that were potentially capable of interfering in the assay. The RIA standard inhibition curve was prepared with 0.01 M ammonium acetate (pH 6.5) rather than the Versene buffer used to simulate normal human plasma in the Z-gel plasma assay (7). Samples greater than 20 ng/ml were diluted with normal saline and reassayed.

Pancroitic juice extraction and concentration.—Only collections of pancreatic juice free of visible contamination by biliary secretion were studied. The need for microgram quantities of reactive material required the pooling of collections. The pool was diluted with four volumes of normal saline and extracted with an equal volume of 1.2 M perchloric acid (PCA). Following centrifugation (2,000×g for 20 min), the supernatant was dialyzed exhaustively versus deionized water, and the retentate was concentrated by ultrafiltration to 1.5 ml. No loss of CEA activity was detected.

Isolation of pancreatic juice material (PJ M).—The concentrate was partially purified by Sepharose 6-B and Sephadex G-200 gel filtration. The RIA-active material eluted from the Sephadex G-200 column as a single peak and was designated PJ M. After the PJ M was used in the RIA inhibition study, it was concentrated by ultrafiltration to 3.9 ng/μl and used in the other immunochemical studies.

Table 1.—Patient diagnoses, volumes, and activities of pancreatic juice samples

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Volume (ml)</th>
<th>CEA activity (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pancreatitis</td>
<td>5.2</td>
<td>39.1</td>
</tr>
<tr>
<td>2</td>
<td>Pancreatitis</td>
<td>15.6</td>
<td>117.5</td>
</tr>
<tr>
<td>3</td>
<td>Diagnosis not made</td>
<td>7.0</td>
<td>15.7</td>
</tr>
<tr>
<td>4</td>
<td>Diagnosis not made</td>
<td>7.7</td>
<td>40.4</td>
</tr>
<tr>
<td>5</td>
<td>Chronic cholecystitis</td>
<td>17.3</td>
<td>122.8</td>
</tr>
<tr>
<td>6</td>
<td>Cirrhosis</td>
<td>11.2</td>
<td>184.8</td>
</tr>
<tr>
<td>7</td>
<td>Common bile duct stone</td>
<td>2.8</td>
<td>29.7</td>
</tr>
<tr>
<td>8</td>
<td>Gastric ulcer</td>
<td>15.7</td>
<td>190.9</td>
</tr>
<tr>
<td>9</td>
<td>Diagnosis not made</td>
<td>22.2</td>
<td>190.4</td>
</tr>
<tr>
<td>10</td>
<td>Chronic alcoholic hepatitis</td>
<td>80.6</td>
<td>377.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>185.3</td>
<td>1,447.6</td>
</tr>
</tbody>
</table>

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2 Supported by Public Health Service grants CA04486-15 and CA16750-01 from the National Cancer Institute (NCI); contract N01 CP35264 from the Division of Cancer Cause and Prevention, NCI; and by grant IM-18C from the American Cancer Society.
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8 We thank Drs. Ronald Herberman and Hans Hansen for reviewing this manuscript; Dr. Phil Gold for providing antiserum against carcinoembryonic antigen; and Roche Diagnostics, Nutley, N.J. for providing the radioimmunoassay reagents.
Immunoelectrophoresis.—Purified CEA obtained from Dr. Hans Hansen, Nutley, N.J. (740 ng/10 µl) (BP-102) and PJM (27 ng/µl) were added to the antigen wells of a standard immunoelectrophoresis (IEP) pattern. Small quantities of ¹³¹I-CEA [15,000 counts per minute (cpm)] and ¹³¹I-PJM (3,000 cpm) (see below) were also added to the appropriate wells for subsequent autoradiographic visualization. Electrophoresis was performed for 90 minutes (20mA, 50V, 20° C, 0.05 M sodium barbital buffer at pH 8.4). Goat antiserum against CEA was obtained from Dr. Phil Gold (M₈, 1:50 dilution, 50 µl) and was added to the trough separating the two wells. Following 48 hours’ incubation in a humid chamber, the IEP pattern was washed, dried, stained, and developed with X-ray film as described below. The electrophoretic mobility of the PJM was compared with that of the purified CEA.

Because only small quantities of PJM were available, the immunochromic behavior of CEA and PJM were evaluated by four methods, each requiring only nanogram quantities of reactive material.

Inhibition in RIA.—RIA inhibition curves prepared from 50–500 µl PJM or 0–125 ng purified CEA in 5.0 ml of 0.01 M ammonium acetate were analyzed. The occurrence of a PJM inhibition curve parallel to that prepared from the CEA standard would indicate identical immunochromic behavior in the RIA.

Competitive binding.—PJM (100 µl, 390 ng) and CEA (5 µl, 3.6 µg) were radiolabeled with 1 mCi [¹³¹I]Na and [¹²⁵I]Na, respectively, by means of the chloramine-T method of Hunter (8) (100 mg chloramine-T, 250 mg Na₂S₂O₅, reaction; 3.6 µg) were radiolabeled with 1 mCi [¹³¹I]Na and [¹²⁵I]Na, respectively, by means of the chloramine-T method of Hunter (8) (100 mg chloramine-T, 250 mg Na₂S₂O₅, 90-second reaction; 359 j-μl, total vol). The ¹³¹I-PJM and the ¹²⁵I-CEA were separated from the unreacted iodide by Sephadex G-100 gel filtration. Peak fractions were dialyzed versus three changes (greater than 100 volumes each) of 0.05 M Na₂B₄O₇, at pH 8.4.

To a constant amount of the ¹³¹I-CEA (5 µl, 33,000 cpm) were added increasing amounts of ¹²⁵I-PJM (1–10 µl, 3,000–30,000 cpm). These mixtures were incubated (15°C for 30 min) in 5.0 ml of 0.01 M ammonium acetate with sufficient antibody to bind only 40% of the ¹³¹I-CEA. After precipitation of the antigen–antibody complexes with Z-gel, the ratio of ¹³¹I-CEA to ¹²⁵I-PJM in the precipitate was compared with that of the initial mixtures. An identical ratio in both the original mixture and the precipitate would indicate an inability of the antibody against CEA to distinguish between the two antigens.

Absorption studies.—Increasing quantities of PJM (0–38 ng) and purified CEA (0–22 ng) were added to aliquots of goat antiserum against CEA (M₈; adjusted to a final antibody dilution of 1:80) in order to gradually absorb out all antibody activity against CEA. The mixtures were incubated at 4°C overnight and centrifuged at 15,000×g (20 min at 4°C). To test for residual anti-CEA activity by counterimmunoelectrophoresis (CIEP) against CEA, 10 µl of each mixture was placed in the anodal wells and current was applied at 30 mA and 300 V (4°C) for 20 minutes. After this “prerun,” 5 µl purified CEA (10 ng) was added to the cathodal wells, and electrophoresis was continued for an additional 70 minutes at 15 mA and 150 V at 4°C. The electrophoresis buffer, the gel buffer, and dilution medium consisted of 0.05 M barbital sodium, pH 8.4. The plate was washed for 18 hours in normal saline and for 30 minutes in deionized water, dried, and stained with 0.1% amido black.

To aid precipitin band detection, 5 µl ¹³¹I-CEA (1,500 cpm, 0.25 ng) was added to each antigen well at the time of antigen addition. The dried and stained plate was placed in a cassette with a sheet of Kodak No-Screen X-ray film. After 48 hours, the film was developed. The complete absorption of the anti-CEA activity by the PJM indicated the probable identity of the two antigens.

Ouchterlony analysis.—Ouchterlony double diffusion was performed by means of the immunoelectrophoresis technique (9) in preformed gel (Abbott Laboratories). Six peripheral wells (6 mm in diameter) were arranged 4 mm from the central well (3 mm in diameter). The central well received 10 µl M₈, antigen (diluted 1:10 with 0.05 M borate buffer, pH 8.2) and 40-µl samples to be tested were placed in the peripheral wells. After incubation for 48 hours at 4°C, the proteins were precipitated with 0.5% phosphotungstic acid, the gel washed in saline and deionized H₂O (containing 1% glycerine), dried, and stained with 0.1% amido black.

RESULTS

Electrophoretic Mobility

Comparison of the IEP mobilities of the PJM (plus ¹²⁵I-PJM) and purified CEA (plus ¹³¹I-CEA) (fig. 1) showed a diffuse staining pattern for both preparations. The PJM had a more restricted range than did the purified CEA. In view of the broad range of mobilities reported for CEA and due to the differences in concentration (740 ng CEA vs. 27 ng PJM), the restricted length of the precipitin arc of the PJM could not be interpreted to indicate a difference between the two preparations.

RIA Inhibition Curves

Increasing amounts of PJM were assayed in the CEA RIA and compared to an inhibition curve obtained with purified CEA (text-fig. 1). The resulting inhibition curves were parallel, with no significant difference between the slopes of the two curves.

Competitive Binding

Purified CEA radiolabeled with ¹³¹I was mixed with increasing amounts of ¹²⁵I-PJM. Binding to anti-CEA

Text-figure 1.—Purified CEA inhibition curve compared with that obtained from aliquots of PJM. Both X coordinates are drawn on a log scale; (●) (purified CEA) and (●) (PJ-2) represent means of duplicate trials. Slopes were calculated and regression lines drawn by the method of least squares.
CARCINOEMBRYONIC ANTIGEN IN HUMAN PANCREATIC JUICE

The findings demonstrated that the CEA-active component in pancreatic juice of patients without pancreatic cancer, apparently free of biliary, duodenal, and gastric substances, 1) was immunochemically identical to CEA purified from liver metastases of colon cancer, 2) was soluble in 0.6 M PCA, and 3) had an electrophoretic mobility within the range observed with CEA.

Because two antigens cannot be identified completely by any single immunochemical method, the immunochemical activity of PJM and purified CEA was compared by four methods. The parallel inhibition curves and the competitive binding study demonstrated identity of immunochemical behavior in the RIA, as well as an inability of the antibody against CEA to distinguish between the two antigens when present in the same system. Furthermore, specific absorption and Ouchterlony analysis showed identity between purified CEA and PJM in different systems with the use of a goat antibody against CEA different from that used in the RIA. The CEA activity of PJM was also immunochemically identical to that extracted from pancreatic cancer tissues, as shown by the Ouchterlony reaction.

The pancreatic juice pool was obtained from patients with no evidence of malignant disease. The activity in this pool was immunochemically identical to that of CEA, thus confirming and expanding findings of previous workers. Go et al. (10) reported high levels of CEA-like material in perfusates of the normal gastrointestinal tract and noted increased amounts in the duodenum following stimulation of pancreatico-biliary secretion by cholecystokinin—pancreozymin. Sharma et al. (4) detected CEA activity in normal pancreatic juice and material obtained from patients with pancreatitis and pancreatic cancer. This study showed that the CEA activity of pancreatic juice from patients with nonmalignant disease of the pancreas was immunochemically identical to that of CEA derived from colon carcinoma. Analysis of this activity may be significant in the diagnosis of pancreatic cancer (11).

REFERENCES

(9) van Oss CI, Birenson PM: Immunoenerphoresis. Immunochemistry 5:775-778, 1969
FIGURE 1.—IEP pattern of PJM and purified CEA. Purified CEA (740 ng) plus 125I-CEA (15,000 cpm) was placed in upper well. PJM (27 ng) plus 125I-PJM (5,000 cpm) was placed in lower well. The trough contained 50 µL antibody Mab against CEA (1:30 dilution). Overlay of X-ray film on IEP gel.

FIGURE 2.—Autoradiograph of precipitin-inhibition reactions with the use of CEA and PJM to absorb antibody against CEA. Figure shows a standard CIEP pattern consisting of three rows of vertical pairs of wells parallel to the plane of electrophoresis. The anode is at top of figure. Antiserum against CEA (10 µL, 1:80 dilution) absorbed with 0-22 ng CEA (increasing increments of 2.75 ng) was added in duplicate to the anodal well of each pair in the first row and first half of the second row. Antiscrum against CEA, similarly absorbed with 0-33 ng PJM (increasing increments of 4.12 ng), was added in duplicate to the anodal wells of each pair in the second half of the second row and the third row of the pattern. Residual anti-CEA activity was determined by electrophoresis against CEA from the lower well of each pair (10 µL, 10 ng plus 125I-CEA at 1,500 cpm; <1 ng). The gradual decrease in intensity of the precipitin band formed indicates the gradual absorption of anti-CEA activity by the CEA and PJM. Diagram (lower half) shows precipitin bands drawn in for clarity.

FIGURE 3.—Direct Ouchterlony reactions between absorbed antibody against CEA (AB) and LM2 [liver metastases of colon cancer (20 µg)], PJM2 [pancreatic juice material (400 ng)], P1 [primary pancreatic cancer (10 µg)], PJM1 [pancreatic juice material (160 ng)], purified CEA (200 ng), and LM1 [liver metastases of pancreatic cancer (24 µg)]. The P1, LM1, and LM2 are concentrates of the CEA-active fractions from Sepharose 6-B gel filtration of PCA extracts.