Radioimmunodetection of Gastrointestinal Neoplasms with Antibodies to Carcinoembryonic Antigen


Department of Immunology, University of Birmingham [K. R. H., A. R. B., P. W. D.], and Department of Nuclear Medicine, Queen Elizabeth Hospital [T. A. R., Z. D.], Edgbaston, Birmingham 15, England

Abstract

Primary and secondary gastrointestinal tumors have been identified using sheep immunoglobulin G antibody to carcinoembryonic antigen radiolabeled with 131I. 99mTc-pertechnetate and 99mTc-human serum albumin were used to identify tissue spaces and blood pool and to facilitate external subtraction imaging.

In 13 patients with tumors, 4 of 5 primary sites and 8 of 11 secondary sites were successfully demonstrated. Two patients with benign disease had negative scans. Comparison with conventional methods of scanning showed good correlation.

Introduction

The research of Paul Ehrlich (1) in the early years of this century first suggested that the antigenic expression of tumors differed from that of normal cells. This encouraged work to raise antibodies which would selectively localize to neoplasms, but their development was hampered by the absence of antigens truly specific to tumors (11, 12). In 1965, Gold and Freedman (3, 4) reported the discovery of a component of human gastrointestinal carcinomas which was present in the fetal digestive system but was absent from normal gut and cancers of other tissues. This was termed CEA. Subsequently, CEA has been found to be present in a wide variety of malignant and normal tissues (7, 8, 17), but there is no doubt that it is quantitatively increased in the majority of gastrointestinal cancers (9).

In animal experiments, localization of antibodies to CEA was achieved in transplanted tumor deposits (6, 10, 13, 14), but the animals used did not have circulating CEA. An initial attempt to identify human neoplasia with anti-CEA was not successful even though the serum CEA was cleared by a prior loading dose of antibody (15). Recently, using a subtraction scanning technique, Goldenberg et al. (5) were able to demonstrate all 6 of 38 tumor deposits in 18 patients with a variety of cancers. The only patient in which no tumor was demonstrable despite the existence of primary and secondary deposits had a lymphocytic lymphoma (which is usually devoid of CEA).

In this study, we have attempted to identify primary and secondary deposits of various gastrointestinal cancers using radiiodinated anti-CEA, and to compare the γ camera images thus obtained with conventional methods of scanning for tumor.

Materials and Methods

Antibody. Antibodies to CEA were produced in sheep by immunization with CEA purified from hepatic metastases of colorectal cancer. The hyperimmune sheep serum was adsorbed with normal colon, lung, liver, and spleen, the process being monitored by using the serum for staining tissue sections by the immunoperoxidase method.

Adsortion was considered to be complete when there was staining of colon carcinoma cells but not of normal colon. The adsorbed serum was then fractionated on a DEAE 52 cellulose column, and the IgG fraction was affinity purified using CEA linked to Sepharose 4B. The purified antibody was tested for acute toxicity and pyrogenicity in rabbits, and all tests were satisfactory. Radiiodination was performed under sterile conditions using the chloramine-T technique, and free iodide was removed on a Dowex column. The labeled antibody was diluted in 1% albumin in 0.9% NaCl solution and then centrifuged overnight at 20,000 x g before its injection into patients through a 0.22-μm filter.

Each patient received 10 to 15 ml of antibody solution equivalent to between 100 and 200 μg of sheep IgG. This was administered i.v. over 15 min after first testing for anaphylactic hypersensitivity. The total amount of 131I given varied between 300 and 1000 μCi (usually 600 to 700 μCi). Prior to each scan, the patients also received 200 μCi 99mTc-pertechnetate and 500 μCi 99mTc-human serum albumin to outline tissue spaces and blood pool and to facilitate subtraction scanning.

Imaging. Images of the chest and abdomen were taken with a γ camera (Searle LFOV with medium energy collimator) at 0.5, 4, 24, and 48 hr after injection of the antibody. The camera was linked to a DEC pdp 11/40 computer with dual isotope facility and a gray-scale visual display unit.

The data were stored and displayed in a 64 x 64 matrix. After normalizing over the heart, subtraction of the technetium components from the iodine components was performed to highlight the areas of selective uptake of antibody by CEA-producing tissue.

Patients. Fifteen patients with gastrointestinal cancer were studied, in 5 of whom the primary tumor was unresected. Three of the 5 had rectal cancers. In one patient, the neoplasm proved to be completely resectable at laparotomy 7 days after the antibody scans; in the second, extensive peritoneal deposits were demonstrated by laparoscopy, and in the third, the tumor was advanced locally but there were no demonstrable distant deposits. Of the other 2 patients with primary tumor in situ, one had a pancreatic carcinoma with several metastases in only the left lobe of the liver demonstrated at operation, and the other had a widely disseminated, poorly differentiated adenocarcinoma from an undetermined primary site. Eight of

---

1 Presentated at the UICC Workshop on Radioimmunodetection of Cancer, July 19 to 21, 1979, Leomington, Ky. The work was supported by a grant from the Medical Research Council.

2 The abbreviation used is: CEA, carcinoembryonic antigen.
the 15 patients had developed recurrent disease after resection of colorectal carcinomas. Seven of these had liver metastases confirmed by conventional isotope scanning, and in the other patient there was clinical evidence of pelvic recurrence. The remaining 2 patients were found to have large bowel strictures on barium enema, and it was thought probable that these were neoplastic. At operation, however, both individuals were found to have benign disease, diverticular disease in one and chronic ulcerative colitis in the other.

Three patients received affinity-purified antibody; in the remainder, this step in the preparation was omitted. In 14 patients, regular blood samples were taken during the first 5 hr and again at 24 hr. Thyroid uptake of radioiodine was blocked by the administration of potassium iodide (180 mg/day, p.o.), starting at least 24 hr before the injection of antibody and continuing for 3 weeks. All patients gave their informed consent to the procedure.

Results

Demonstration of tumor has been achieved in 4 of the 5 patients with unresected primary neoplasms (Table 1). This includes the patient with a pancreatic carcinoma in whom liver metastases confined to the left lobe had been demonstrated at laparotomy (Patient 12). The antibody scan agreed very closely with the operative findings, and there was no selective uptake of radioiodine into the right hepatic lobe (Fig. 1). The primary neoplasm was missed in Patient 13 who had disseminated adenocarcinoma and in whom only one scan (at 4 hr) was undertaken. Eleven patients had metastatic disease; in 8, this was demonstrated on the antibody scans. The 3 exceptions included Patient 3, who had a large (25 x 20 cm), solitary deposit in the liver, and Patient 13. In the 2 patients (Patients 14 and 15) with benign disease of the colon, all scans were negative.

Where possible, the antibody images have been compared with computerized tomography and colloidal technetium scans, and the distribution of demonstrable tumor has correlated well (Figs. 2 to 4). In 6 patients, metastases were demonstrated at laparotomy, and in 5 of these, positive scans were obtained.

Serial measurements of serum CEA after injection of the antibody have shown no consistent trend. Three patients showed changes of less than 10% (assay variation), 5 showed an immediate fall of 10 to 25%, and 6 fell by 25 to 50%. The pattern of CEA change did not appear to correlate with either the absolute preinjection value of CEA, the chances of achieving a positive scan, or the time at which the scan became positive. Preinjection CEA levels of up to 16,000 µg/liter did not prevent the demonstration of hepatic metastases.

No serious complications have been observed after administration of the antibody. One patient developed a pyrexia of 38.1 ° which was maximal 8 hr after injection and disappeared 9 hr later. Hourly temperature and pulse recordings were normal in all other patients.

Discussion

These results appear to confirm the report of Goldenberg et al. (5). The demonstration of tumor deposits has been clear and has correlated well with established techniques of scanning. One advantage of this technique is that it gives positive information about both primary and secondary tumor simultaneously; this may well be of value in the preoperative assessment of a patient. In 5 patients with primary tumor, this has been demonstrated on the antibody scans in 4 patients. Two of these patients had metastases, and a third had local extension of the primary; these features were clearly evident on the antibody images.

Theoretically, there is more than sufficient CEA in the serum to fully occupy the binding sites on the small amounts of injected sheep IgG, but in this study the presence of circulating antigen does not appear to have prevented the demonstration of tumor. Furthermore, there has been no consistent trend in the changes in serum CEA after injection of the antibody. It is well established that CEA is heterogeneous (16), and this may be the explanation of the findings. The antibody that we have used has been raised against CEA extracted from hepatic metastases and it could well be that it has greater affinity for tissue CEA than for serum CEA. This suggestion awaits further investigation.

It is therefore encouraging that sheep IgG antibody to CEA can be successfully used for the radioimmunodetection of

Table 1
Results of subtraction scans after injection of radiolabeled anti-CEA in 15 patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Site</th>
<th>Identified</th>
<th>Site</th>
<th>Identified</th>
<th>+0.5</th>
<th>+4</th>
<th>+24</th>
<th>+48</th>
<th>Serum CEA (g/liter)</th>
<th>Immediate % of change in CEA after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colon</td>
<td>0</td>
<td>Liver</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>210</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Rectum</td>
<td>0</td>
<td>Liver</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>195</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Colon</td>
<td>0</td>
<td>Liver</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Rectum</td>
<td>0</td>
<td>Liver</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>410</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>Rectum</td>
<td>0</td>
<td>Liver</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>900</td>
<td>+6</td>
</tr>
<tr>
<td>6</td>
<td>Colon</td>
<td>0</td>
<td>Liver</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>225</td>
<td>-22</td>
</tr>
<tr>
<td>7</td>
<td>Rectum</td>
<td>0</td>
<td>Pelvis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>95</td>
<td>-21</td>
</tr>
<tr>
<td>8</td>
<td>Colon</td>
<td>0</td>
<td>Liver</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>750</td>
<td>-13</td>
</tr>
<tr>
<td>9</td>
<td>Rectum</td>
<td>+</td>
<td>Peritoneum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>50</td>
<td>-38</td>
</tr>
<tr>
<td>10</td>
<td>Rectum</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14</td>
<td>-36</td>
</tr>
<tr>
<td>11</td>
<td>Rectum</td>
<td>+</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>12</td>
<td>-13</td>
</tr>
<tr>
<td>12</td>
<td>Pancreas</td>
<td>+</td>
<td>Liver</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>300</td>
<td>-50</td>
</tr>
<tr>
<td>13</td>
<td>Undetermined</td>
<td>-</td>
<td>Peritoneum and liver</td>
<td>+</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>65</td>
<td>-12</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25</td>
<td>-28</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13</td>
<td>-35</td>
</tr>
</tbody>
</table>

*The abbreviations used are: 0, tumor absent or resected; +, tumor identified; -, tumor missed; N, scan not performed.
of cell-damaging agents linked to the antibody is theoretically possible but depends on the selective concentration and distribution of antibody in tumor tissue. The discovery of more specific tumor antigens such as those derived from breast (18) and bronchus (2) may allow for the preparation of antibodies with greater selective uptake into neoplastic tissue.

Acknowledgments

We thank Professor A. M. Neville and Dr. J. Westwood, Institute of Cancer Research, Royal Cancer Hospital, Sutton, Surrey, U. K., who supplied purified CEA, Dr. J. McCartney, Department of Pathology, University of Birmingham, who monitored the antiserum adsorption by immunochemical staining; and Dr. F. Burrows, Department of Radiology, Queen Elizabeth Hospital, Birmingham, who gave assistance with isotope and computerized tomography scans.

References

Fig. 3. Rectilinear scan after injection of colloidal technetium in Patient 4 showing areas of decreased uptake consistent with metastases.

Fig. 4. View of the liver by computerized tomography in Patient 4 showing areas of decreased density consistent with metastases.
Radioimmunodetection of Gastrointestinal Neoplasms with Antibodies to Carcinoembryonic Antigen


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/40/8_Part_2/2993

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/40/8_Part_2/2993.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.