Radioimmunodetection of Primary and Metastatic Ovarian Cancer Using Radiolabeled Antibodies to Carcinoembryonic Antigen

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ABSTRACT

$^{131}$I-Labeled goat immunoglobulin G (IgG) prepared against carcinoembryonic antigen (CEA) was administered at an average dose of 1.0 mCi (180 to 250 μg IgG protein) to patients with ovarian tumors in order to evaluate this method of tumor detection and localization, termed the radioimmunodetection of cancer. All primary cancers in 13 patients could be localized, whereas the metastases in six of nine cases could be imaged by external scintigraphy. However, only two of these cases showed metastatic spread by more conventional diagnostic techniques, including computer-assisted tomography, ultrasonography, and angiography. Successful tumor radiolocalization appeared to depend on tumor size, with lesions smaller than 2 cm in diameter not being detected. Tumors containing a CEA concentration above 115 ng/g, including a benign neoplasm, could be localized with radioactive anti-CEA antibodies. Administration of radiiodinated normal goat IgG to four patients with malignant or benign ovarian tumors failed to show tumor radioimmunodetection. One of these cases subsequently demonstrated a 4- × 4-cm tumor after receiving specific radiolabeled anti-CEA IgG. This study shows that ovarian neoplasms containing CEA can be detected and localized by external photoscanning after the application of radiiodinated antibodies to CEA and that, in this small series of patients, primary and secondary tumors could be detected in 100 and 67% of the cases, respectively.

INTRODUCTION

The continued high mortality of ovarian cancer is generally attributed to the lack of a reliable method for its early detection. Consequently, there has been much effort directed toward the development of a sensitive immunodagnostic method for detecting an ovarian cancer-associated “marker” released from ovarian cancer cells into the blood. However, no such immunological test for early ovarian cancer has been accomplished as yet, although a number of promising ovarian tumor-associated antigens have been described (see Ref. 3). CEA (4) has been identified in high concentrations in the plasma and tumors of patients with ovarian as well as other cancers (5, 16). Moreover, previous studies from this institution have indicated that ovarian cancer CEA is immunologically similar to colonic cancer CEA (5, 16).

With the advent of radioimmunodetection (7), which permits the external imaging of tumors showing an increase in radioactivity because of the selective tumor accretion of a radiolabeled antitumor antibody administered to the patient, we chose to study the detection and localization of ovarian tumors with radiolabeled antibody to CEA. Our initial reports of CEA-tumor radioimmunodetection already indicated in a few cases that ovarian carcinomas could be demonstrated by this method (7, 8, 10).

MATERIALS AND METHODS

Subjects for this investigation included 21 patients with ovarian tumors who were admitted to the Gynecologic Oncology Division of the University of Kentucky Hospital from July 1977 to December 1978. All patients had palpable pelvic masses, and selected patients with ovarian carcinoma had extrapelvic disease. Preoperative evaluation included i.v. pyelography and ultrasonography, as well as CEA radioimmunodetection. In addition, computer-assisted tomography and angiography were utilized to identify sites of metastatic disease. Thirteen patients were shown to have histologically confirmed ovarian cancer, of which 9 were found to have metastatic spread. A second group of 5 patients included in the study had benign ovarian tumors. Both groups received radiiodinated antibody to CEA. A third group of 4 patients, 2 with malignant and the other 2 with benign ovarian tumors, received radiiodinated normal goat IgG. The patients usually were operated upon within 48 hr after scanning. The size and anatomical location of both the primary tumor and histologically confirmed sites of metastatic disease were recorded. Ovarian tumor CEA concentrations were determined by radioimmunoassay of water and perchloric acid extracts of the tissues. The immune scintiscan findings were compared to those of other diagnostic measures as well as to the surgical findings. Following surgery, the patients were evaluated in the Gynecologic Oncology Clinic at monthly intervals. Amounts of CEA in the circulating plasma were assayed before and at various intervals during the study. The Roche CEA radioimmunoassay was utilized throughout the study.

Our methods of antibody preparation, radiolabeling, patient preparation, and photoscanning have been described previously (2, 7, 8, 10). Briefly, antibodies to CEA were produced in a goat by hyperimmunization with CEA purified from a colon carcinoma metastasis to the liver. The final antibody titer was...
found by radioimmunoassay, at 50% binding of the labeled antigen as the end point, to be $2 \times 10^6$. After heattreatment of complement, the antiserum was absorbed with A- and B-group human erythrocytes and was then purified by affinity chromatography to remove CEA-cross-reactive components and to increase the affinity to CEA (7). Polymerized IgG was removed by column chromatography. The specificity of the antiserum was confirmed by gel immunodiffusion, immunoelectrophoresis, and radioimmunoassay. Radioidination with $^{131}$I performed by the chloramine-T procedure resulted in a specific activity of 5 to 10 Ci/g of IgG protein for each preparation. This was then diluted with human serum albumin in 0.9% NaCl solution and sterile-filtered before use. Sephadex G-200 chromatography indicated that 90 to 95% of the radioiodinated antibody chromatographed with IgG. The radioactive antibody to CEA was determined by affinity chromatography to be about 70% reactive with CEA. Before use, the radioantibodies were tested for pyrogenicity, sterility, and acute toxicity and were found to be appropriate for clinical application. Normal goat IgG utilized in the study was treated similarly, including being subjected to affinity chromatography with donkey antibody to goat IgG.

Prior to injection of the radioiodinated anti-CEA IgG, the patients were tested for anaphylactic hypersensitivity to goat IgG i.d. and then were given Lugol’s solution daily, 10 drops p.o., to block or reduce thyroid uptake of radioactive iodine. The radioactive antibody was administered i.v. at an average dose of 1.0 mCi (180 to 250 μg IgG protein).

Total-body photoscans were made with a LFOV gamma camera (Searle) at frequent intervals, particularly at 24 and 48 hr after injection of the radioantibody. In order to compensate for blood pool and nontarget background radioactivity, a computer-assisted subtraction method was developed, whereby $^{99m}$Tc-labeled human serum albumin or $^{99m}$Tc-pertechnetate was administered just before imaging. By means of computer processing, the $^{99m}$Tc radioactivity (140 keV) was subtracted from the $^{131}$I (364 keV) radioactivity to remove background, nontarget activity, and the data were stored in a minicomputer. Chest and abdominal images were made in 3 different views (anterior, posterior, and lateral) on at least 2 occasions, and these were evaluated by 3 different observers. Every effort was made to obtain scintigrams of computer-assisted subtraction of different subtraction levels, so that images of apparent ‘over’ and ‘under’ subtraction could also be evaluated among a series of pictures.

RESULTS

Our radioimmunodetection results in the 13 patients with ovarian cancer are summarized in Table 1. Eleven of the 13 patients had serous cystadenocarcinomas containing between 115 and 670 ng CEA per g tissue. The highest tumor CEA concentration (5314 ng/g) occurred in a single patient with a mucinous cystadenocarcinoma (Case 13). CEA plasma titers were elevated (>2.5 ng/ml) in only 3 of the 13 patients (Cases 1, 5, and 7). The immune scintigrams were successful in detecting and localizing all 15 tumors subsequently confirmed by surgery. The tumors localized by radioimmunodetection varied from 4 × 2 to 15 × 10 cm in dimension. Prior to scanning, all of these tumors were demonstrable on pelvic examination, ultrasonography, or i.v. pyelography. Plasma CEA values appeared to have no bearing on either size of the tumor or the radioimmunodetection findings.

Nine of these 13 patients had evidence of metastatic spread,

Table 1

<table>
<thead>
<tr>
<th>Case</th>
<th>Cell type</th>
<th>Plasma CEA (ng/ml)</th>
<th>Tumor CEA (ng/g)</th>
<th>Surgical findings (primary lesions)</th>
<th>CEA scan findings</th>
<th>Scan and</th>
<th>Surgical correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Serous</td>
<td>3.7</td>
<td>670</td>
<td>Ovarian tumor (10 × 10 cm)</td>
<td>Pelvic tumor</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Serous</td>
<td>1.1</td>
<td>ND</td>
<td>Ovarian tumor (15 × 10 cm)</td>
<td>Pelvic tumor</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Serous</td>
<td>0.8</td>
<td>585</td>
<td>Ovarian tumor (9 × 6 cm) (L)</td>
<td>Bilateral pelvic tumors</td>
<td>+</td>
<td></td>
</tr>
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<td></td>
<td>Ovarian tumor (6 × 3 cm) (R)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>4</td>
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<td>0.7</td>
<td>166</td>
<td>Ovarian tumor (9 × 7 cm) (L)</td>
<td>Bilateral pelvic tumors</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ovarian tumor (4 × 2 cm) (R)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Serous</td>
<td>8.4</td>
<td>ND</td>
<td>Ovarian tumor (4 × 5 cm)</td>
<td>Pelvic tumor</td>
<td>+</td>
<td></td>
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<tr>
<td>6</td>
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<td>1.9</td>
<td>ND</td>
<td>Ovarian tumor (5 × 8 cm)</td>
<td>Pelvic tumor</td>
<td>+</td>
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<td>7</td>
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<td>4.3</td>
<td>115</td>
<td>Ovarian tumor (20 × 5 cm)</td>
<td>Pelvic tumor</td>
<td>+</td>
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<td>8</td>
<td>Serous</td>
<td>0.9</td>
<td>320</td>
<td>Ovarian tumor (5 × 7 cm)</td>
<td>Pelvic tumor</td>
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<tr>
<td>9</td>
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<td>2.2</td>
<td>ND</td>
<td>Ovarian tumor (10 × 10 cm)</td>
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<td></td>
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<td>10</td>
<td>Serous</td>
<td>0.8</td>
<td>510</td>
<td>Ovarian tumor (6 × 5 cm)</td>
<td>Pelvic tumor</td>
<td>+</td>
<td></td>
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<tr>
<td>11</td>
<td>Serous</td>
<td>1.4</td>
<td>285</td>
<td>Ovarian tumors (15 × 10 cm)</td>
<td>Pelvic tumor</td>
<td>+</td>
<td></td>
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<tr>
<td>12</td>
<td>Sertoli-Leydig</td>
<td>1.2</td>
<td>170</td>
<td>Ovarian tumor (6 × 5 cm) (R)</td>
<td>Pelvic tumor</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Mucinous</td>
<td>0</td>
<td>5314</td>
<td>Ovarian tumor (4 × 4 cm)</td>
<td>Pelvic tumor</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

a ND, not done; L, left; R, right.
as determined at the time of surgery and confirmed histologically. The ability of the CEA radioimmunodetection method to detect these metastatic sites is demonstrated in Table 2. Photoscans identified metastatic disease in 6 of these 9 patients (67%). It appears that the efficacy of the radioantibody preparation to localize metastatic disease was directly related to the size of the tumor and was independent of the histological type or anatomical site of the metastasis. In this series of patients, it was found that the resolution of the scanning method was at 2 cm diameter for tumor detection, since no lesion below this size could be localized by photoscanning. It is important to note, however, that other noninvasive methods, including ultrasonography, computer-assisted tomography, and angiography, were unable to detect metastatic disease in more than 2 of these 9 patients (Table 3). Thus, the sensitivities of the radioimmunodetection method in this small series of patients with ovarian cancer were 100 and 67%, respectively, for primary and secondary tumors. Radioimmune scans were also performed on 5 patients with benign ovarian tumors. Although all of these tumors were 7 cm or greater in diameter, only one (Case 14) was localized successfully (Table 4). This was a cystic teratoma with a tumor CEA content of 400 ng/g. The image quality in radioimmune scintiscans of this patient is given in Fig. 1 and indicates the important contribution of computer-assisted subtraction for tumor localization. In this small series, only one patient had an elevated plasma CEA titer.

Normal goat IgG radiolabeled with 131I failed to show tumor localization in the 4 patients studied, all of whom had tumors of considerable size (Table 5). Five months after Case 13 was studied with normal goat IgG, a tumor recurrence was evaluated with anti-CEA radioantibody, and a carcinoma measuring 4 x 4 cm was localized (Table 1), thus supporting the specificity of the CEA radioantibody for a CEA-containing tumor. Interestingly, this patient’s CEA level in the plasma declined from 10 ng/ml in May 1978 to 0 in October 1978, at the time of the second surgery and the successful localization of tumor recurrence by radioimmunodetection. Thus, at the time of the initial evaluation, radiiodinated normal goat IgG failed to localize a primary ovarian carcinoma measuring 16 x 17 cm (Table 5), whereas this patient’s recurrence measuring 4 x 4 cm could be detected with the anti-CEA radioimmunodetection method.

**DISCUSSION**

The development of radioactive antibodies that localize in tumors and can be detected by external scintillation imaging has been the goal of many investigators for over 20 years (1, 12). One of the first successful reports involving tumor-localizing antibodies was with antibodies against human fibrinogen.
Localization was achieved in over 50% of more than 300 diagnostic studies in humans; however, these antibody preparations also localized in areas of thrombosis and secondary inflammation due to rheumatoid arthritis (14). More recently, the method of radioimmunodetection was proven to be of clinical value in the detection of localization of tumors containing CEA after 131I-labeled, highly specific antibodies to CEA were administered to various patients with primary, recurrent, and/or metastatic cancers of diverse histopathology (2, 6–8, 10, 11). In a small series of patients with colorectal cancer, a 93% accuracy rate for primary and secondary tumors could be achieved (11).

The current study was undertaken to assess the results of CEA immune scintigraphy in the detection and localization of primary and metastatic ovarian cancer. It was also of interest to determine the use of radioactive anti-CEA antibodies for the detection of benign ovarian tumors and also whether a similarly prepared normal goat IgG would localize in ovarian neoplasms. Thirteen patients with ovarian cancer showed anti-CEA radioantibody localization in all of their primary tumors and in two-thirds of the cases with metastases. Plasma CEA appeared to be a less reliable indicator of extent of disease than was CEA radioimmunodetection, since only 3 of 13 patients with malignant ovarian tumors had elevated plasma CEA titers. The CEA radioisotopes were able to detect sites of proven metastasis in 6 of 9 patients, whereas other noninvasive methods could detect metastases in only 2 of these patients. Thus, CEA radioimmunodetection in this series of patients appeared to be a more sensitive tumor detection method than ultrasonography, computer-assisted tomography, and angiography. The results obtained were found to be directly related to size of the tumor; lesions below 2 cm in diameter failed to localize by radioimmunodetection.

On the other hand, only 1 of 4 benign ovarian tumors could be localized with radioactive antibody to CEA, although all of these tumors were 7 cm or greater in diameter. The cystic teratoma which was localized successfully had a CEA content of 400 ng/g, and among this series of cases with benign and malignant ovarian tumors it appears that a tumor CEA content of 115 ng/g or higher was sufficient for localization of anti-CEA antibody. However, one must also take into consideration the total tumor volume containing CEA.

Radioiodinated normal goat IgG failed to show tumor localization when administered to 4 patients with malignant or benign ovarian tumors, although these neoplasms were of considerable size. This is the contrast to previous animal experiments (9) as well as some findings in clinical radioimmunodetection, where large tumors occasionally show accretion of radioactive normal immunoglobulin. In one of the patients included in this study (Case 13), both normal goat IgG and goat anti-CEA IgG were administered on different occasions. After failure of the normal goat IgG to localize radioactivity in the 16 × 17-cm mucinous cystadenocarcinoma, radioiodinated anti-CEA antibody did detect a tumor recurrence in this patient 5 months later. At this time, the tumor was 4 × 4 cm, and the patient had no detectable CEA in her plasma. The results in this patient thus support the view that tumor localization by radioimmunodetection of CEA in these ovarian neoplasms was due to an antigen-antibody reaction.

A major concern in this and in earlier studies was the possible inhibitory or complicating effect of elevated plasma titers of CEA which could complex the xenogeneic antibody. Neither here nor in previous studies (7, 8, 10, 11) did the level of CEA in the blood appear to hinder successful tumor localization. By analyzing the blood of patients receiving radiolabeled antibody, we have found evidence supporting the formation of immune complexes in these individuals (13), but these complexes do not seem to prevent tumor radiolocalization, even when mg quantities of CEA are present per liter of blood. Nevertheless, no untoward reactions have been observed in the more than 220 patients studied by radioimmunodetection to date.

Although CEA radioimmunodetection, in its current form, provides valuable information concerning the presence and location of ovarian cancer, particularly its metastases, the theoretical implications of these findings are even more important. CEA is not a specific marker substance for ovarian cancer but merely serves as a quantitatively increased target for 10-skeletonous antigen-antibody reaction.

Moreover, methods achieving an increased specific activity of the radionuclide to the IgG may enhance tumor resolution so that neoplasms smaller than 2 cm in diameter can be detected and localized. These and other approaches to improve cancer radioimmunodetection are now being investigated.

REFERENCES


5 D. M. Goldenberg, F. DeLand, and E. E. Kim, unpublished results.

Table 5

<table>
<thead>
<tr>
<th>Case</th>
<th>Cell type</th>
<th>Plasma CEA (ng/ml)</th>
<th>Tumor CEA (ng/g)</th>
<th>Surgical findings</th>
<th>IgG scan findings</th>
<th>Scan and surgery correlation</th>
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<tbody>
<tr>
<td>13a</td>
<td>Mucinous carci-noma</td>
<td>10</td>
<td>17,800</td>
<td>Ovarian tumor (16 × 17 cm)</td>
<td>No localization</td>
<td>–</td>
</tr>
<tr>
<td>19</td>
<td>Mucinous carci-noma</td>
<td>22</td>
<td>210</td>
<td>Ovarian tumor (20 × 10 cm)</td>
<td>No localization</td>
<td>–</td>
</tr>
<tr>
<td>20</td>
<td>Serous ade-noma</td>
<td>0</td>
<td>100</td>
<td>Ovarian tumor (7 × 10 cm)</td>
<td>No localization</td>
<td>–</td>
</tr>
<tr>
<td>21</td>
<td>Follicular cyst</td>
<td>0</td>
<td>160</td>
<td>Ovarian tumor (10 × 12 cm)</td>
<td>No localization</td>
<td>–</td>
</tr>
</tbody>
</table>

* Repeat of case which showed tumor localization with 131I-labeled anti-CEA IgG scan.
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