Human chorionic gonadotropin radioantibodies in the radioimmunodetection of cancer and for disclosure of occult metastases

(tumor markers/radioscanning/immunoscinography/tumor localization/testicular cancer)

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ABSTRACT Radioimmunodetection (RaID) of tumors containing human chorionic gonadotropin (hCG; choriogonadotropin) was evaluated in 25 patients by injecting 125I-labeled goat antibody IgG against hCG and performing total-body photoscans with a γ scintillation camera 24 and 48 hr later. All 10 testicular cancer patients with proven tumor sites had positive RaID results, whereas three cases without known tumor were negative. Four patients with hydatidiform mole and one with degenerative products of conception showed positive RaID results consistent with elevated serum hCG titers. Two putatively false-positive results were obtained in patients with lung or ovarian cancer, whereas a false-negative metastasis to the liver of a patient with lung cancer and an elevated serum hCG titer was observed. Of 14 tumor sites found by RaID in 10 testicular cancer patients, 4 were revealed by RaID prior to any other detection method used and provided a lead time to definitive diagnosis by other measures of a few days to > 1 yr. Although a number of patients had high serum hCG levels, even exceeding 3 μg/ml, the xenogeneic antibody was capable of localizing in tumor. No adverse effects were noted in any of the patients studied. Thus, hCG RaID appears to be a safe and effective method of detecting and locating hCG-producing tumors and has been found to disclose occult testicular cancers.

Radioimmunodetection (RaID) is the method by which antibodies are used to transport diagnostic amounts of radioactivity to specific sites of the body for the external scintigraphic demonstration of abnormal sequestration of radioactivity in pathological tissues. In cancer, the use of antibodies directed against qualitatively distinct or quantitatively increased substances (tumor "markers") contained in neoplasms permits the visualization of tumor sites by this technology (1, 2) and has been applied clinically to detect tumors bearing carcinoembryonic antigen (CEA), α-fetoprotein, and human chorionic gonadotropin (hCG) (3–14).

The application of RaID appears to be particularly indicated in patients in whom a tumor marker is shown to be increased in the blood, and where the sites of tumor or of marker production are suspected or unknown. We now communicate our experience with RaID using hCG antibodies, emphasizing the use of this approach to disclose occult metastases of testicular cancer.

MATERIALS AND METHODS

Subjects. RaID was applied to 25 consecutive patients with putative hCG-producing lesions. Thirteen had a history of histologically confirmed testicular cancer and were evaluated for the presence of primary, recurrent, or metastatic disease. In addition, five patients with hCG-secreting hydatidiform moles or uterine choriocarcinoma, patients with diverse ovarian and lung tumors, and a case with degenerative products of conception were included. Tumor sites were confirmed by surgery, biopsy, ultrasonography, radiological studies including computed tomography (CT), arteriography, venacavography, intravenous pyelography, and by other firm clinical evidence. Some subjects were preoperative, while others had a history of treatment and suspected recurrence because of a rising serum hCG titer. Serum hCG levels were determined in all patients both before and during the RaID studies by a double-antibody radioimmunoassay (RIA) with an antibody against the β-subunit of hCG. The RIA had a sensitivity of less than 1 ng/ml and was considered to be normal above a titer of 1 ng/ml (15). Once informed consent was obtained, the patients were tested for anaphylactic hypersensitivity to goat IgG. To prevent or reduce accretion of 131I in the thyroid, Iodine Solution Strong (Lugol's solution; Purepack Pharmaceutical, Elizabeth, NJ) was given by mouth, 5 drops daily for 7 days, beginning 1 day before injection of the radiolabeled antibody.

Anti-hCG Antibody IgG Preparation. Hyperimmune goat antiserum was prepared with purified urinary hCG. The antigen to hCG was adsorbed with human urinary protein by using an automated chromatography system with a solid-phase (Sephrose 4B, Pharmacia) immunosorbent column (16). After heat inactivation of complement, the antiserum was absorbed with erythrocytes of blood groups A and B. The IgG fraction of the antiserum was isolated by DEAE-cellulose chromatography. The immunoreactivity of the antiserum against hCG was confirmed by gel immunodiffusion, immunoelectrophoresis, and RIA. The purified goat IgG antibodies were labeled with 125I by the chloramine T method (17), yielding a specific activity of 5–10 Ci/g (1 Ci = 3.7 × 1010 becquerels) of IgG protein at the onset of our study and between 10 and 20 Ci/g of IgG protein in the later phase. Sephadex G-200 (Pharmacia) column chromatography indicated that 90–95% of the radiolabeled antibody was a homogenous peak at the molecular size of IgG. The IgG was diluted in 1% human serum albumin (Hyland, Costa Mesa, CA) in 0.9% NaCl and sterile-filtered with a 0.22-μm filter. The hCG antibody had an RIA titer of 0.5 × 106 after radioiodination. Each lot of labeled antibody was tested for sterility, pyrogenicity, and acute toxicity by an independent laboratory and found to be suitable for clinical use.

Photoscanning Technology. The radioiodinated anti-hCG

Abbreviations: RaID, radioimmunodetection; hCG, human chorionic gonadotropin (choriogonadotropin); CEA, carcinoembryonic antigen; RIA, radioimmunoassay.

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IgG was administered intravenously at a dose of 2–3 μg/kg of body weight (usually between 1 and 2.5 mCi adult radiation dose) in 20 ml of sterile 0.9% NaCl over a period of 10–15 min. To subtract blood-pool activity and free iodine activity in the stomach and urinary bladder, 0.5 mCi of 99mTcO4⁻ (Squibb) and 0.5 mCi of 99mTc-labeled human serum albumin (Union Carbide, Tuxedo, NY) were injected intravenously 30 and 5 min, respectively, before each imaging study. Anterior, posterior, and lateral views of the chest and abdomen were made with a gamma scintillation camera (LFOV, Searle, Chicago, IL), usually at 24 and 48 hr after administration of the radioactive antibody.

The scans were stored in a minicomputer (PDP 11/40, Digital Corp., Maynard, MA), which generated digital images of the radioiodinated antibody alone (at 364 keV), of the Tc components (at 140 keV), and of the 123I radioactivity minus the 99mTc activity as described (3). We have further refined the subtraction method for certain organs, such as liver, by use of an organ-specific radionuclide (e.g., 99mTc-S-Sodium colloid), whereby the organ’s image is selectively extracted from the region of interest, and then the 99mTc radioactivity is subtracted, pixel by pixel, from that of 123I. The organ-specific radionuclide is given after the routine examination with 99mTc-labeled human serum albumin and 99mTc-pertechnetate as nontarget subtraction agents. In other instances, such as for the lungs, selective organ extraction was accomplished by outlining the organ of concern electronically and then processing according to the standard subtraction method. By this means, greater confidence in identifying tumor-related radioactivity (as compared to nontarget background) was achieved.

RESULTS

Our RaID findings in the 25 patients evaluated are presented in Table 1. True-positive results were determined on the basis of whether the serum hCG titer was elevated and a tumor lesion was known or subsequently confirmed. True-negative findings were based on a negative RaID result in a patient without an elevated serum hCG titer, even if a tumor site was disclosed by another detection measure. Conversely, false-positivity and false-negativity were defined as positive or negative RaID results, respectively, in patients in whom either no tumor sites were known or found later or who did not have elevated serum hCG titers. It is known from other studies with CEA RaID, however, that CEA-positive tumors can be localized by RaID even if the plasma CEA titer is not elevated (3–5, 7), so that the category of false-positivity may need to be modified once tissue levels of the hCG target molecule are determined. Nevertheless, 10 out of 10 testicular cancer patients with proven tumor sites had positive RaID results, whereas three cases without known tumor were negative, thus yielding a sensitivity (true-positive rate) of 100% and a specificity (true-negative rate) of 100%. Four patients with hydatidiform mole and one with degenerative products of conception also showed positive RaID consistent with elevated hCG titers. When the RaID study was repeated after surgical removal of the hCG-producing tissues, the scans did not show any areas of abnormal radioactivity, thus confirming that the localization was specific for the hCG-bearing tumors or tissues. Two putatively false-positive results were obtained in a patient with an ovarian adenocarcinoma and in one with a lung carcinoma because neither showed an elevated serum hCG titer, whereas two other lung cancer patients had abnormal serum hCG levels and positive RaID results. However, a liver metastasis was missed by RaID in one of these lung cancer patients. Thus, of the total of 25 patients, 7 had normal hCG titers. All 18 with elevated hCG titers had evidence of tumor revealed by RaID, whereas two out of seven cases were putatively false-positive and five out of seven were true-negative on the basis of circulating hCG titers (71% specificity). Only one false-negative tumor site was present. On a patient-based, however, an overall sensitivity of 100% was achieved.

Table 2 indicates the radioscan results and correlations with other detection methods for 13 testicular cancer patients. Analysis of this table affords an opportunity to evaluate the results on a tumor-site basis, in contrast to the results reported above on a patient basis. All 14 tumor sites in patients with elevated hCG titers were disclosed by RaID. Although patient no. 200/165/145 (three successive studies) had a previous positive a-fetoprotein radioscan, the hCG RaID study was unrevealing, consistent with the patient’s normal serum hCG level. Likewise, normal goat IgG given to this patient did not reveal the lung metastases seen in the chest roentgenogram (9). In terms of the method’s specificity in this series, 21 sites known to be free of tumor (as determined by other detection methods) were all negative by hCG RaID. Of the 14 sites of tumor found by RaID, 4 sites in four patients were revealed by RaID before other methods used at the time of the RaID study were positive (Tables 2 and 3), and these tumor sites were ultimately confirmed by surgery, chest roentgenogram, or computed tomography scans between a few days and >1 yr later. The case in which a lead time of more than 1 yr between the RaID finding and the subsequent confirmation of metastasis is worthy of further description.

In December 1978, at age 25, patient no. 252 was admitted for evaluation because of an enlarging right testicle noticed about 4–5 mo earlier. A right radical orchiectomy was performed and the diagnosis of pure seminoma, stage I, was made. His preoperative hCG level was elevated at 6 ng/ml and decreased to 4 ng/ml after surgery. The patient received 2500 rad radiation to the right inguinal area and para-aortic nodes. In August, 1979, he was referred for evaluation of a gynecomastia. The gynecomastia was evident on the right side, but no lymphadenopathy, hepatosplenomegaly, or abdominal masses were noted. The remaining testicle was normal in size and consistence. At this time his serum hCG was 1.8 ng/ml. Computed tomography scans of the abdomen and pelvis were normal, as was also a bipedal lumphangiogram for demonstration of pelvic and para-aortic lymph node involvement. By the end of August, the patient’s hCG level had risen to 45 ng/ml and also showed a 3-fold increase in his luteinizing hormone activity. Chest
The use of radioactive antibodies against certain tumor products for RaID has proved to be a new cancer detection modality (1–14). With affinity-purified antibodies to CEA, for example, RaID has been found to be a highly sensitive and specific method, even demonstrating tumor sites that were not revealed by more conventional techniques (3–5, 7). Although similarly highly purified antibodies to hCG were not used in these studies, the antibody preparation appears to be of sufficiently high titer and specificity required for selective and increased sequestration in hCG-containing tumor sites. This is consistent with earlier findings in an experimental tumor model (18).

Current results with hCG RaID appear to be quite similar in terms of safety and efficacy to our experience with CEA RaID. The patients tolerated the goat IgG well, with no adverse reactions noted. Although a number of patients had high serum levels, even exceeding 3 µg/ml, the xenogenic antibody was capable of localizing in tumor. Previous studies with CEA antibodies have indicated that the goat antibody can complex with

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**Table 2. Radioimmunodetection findings in 13 testicular cancer patients evaluated for disease progression**

<table>
<thead>
<tr>
<th>Study no.</th>
<th>Cell type</th>
<th>Serum hCG, ng/ml</th>
<th>Radioimmunodetection results*</th>
<th>Correlations†</th>
</tr>
</thead>
<tbody>
<tr>
<td>30/266/243</td>
<td>Embryonal</td>
<td>16.3</td>
<td>–, chest –, CXR; –, liver –, L/S; +, abdomen –, US; –, CT; –, –, –, –, CT</td>
<td>–, –, –, –, –, –, –, CT</td>
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<tr>
<td>317</td>
<td>Embryonal</td>
<td>5.0</td>
<td>+, chest +, CXR; +, abdomen +, CT</td>
<td>–, –, +, +, CT</td>
</tr>
<tr>
<td>200/165/145</td>
<td>Embryonal</td>
<td>0.5</td>
<td>–, chest +, CXR; –, liver –, L/S; –, abdomen –, US</td>
<td>–, –, –, –, –, CT</td>
</tr>
<tr>
<td>256†</td>
<td>Embryonal + teratoma</td>
<td>5.1</td>
<td>–, chest –, CXR; +, abdomen +, CT</td>
<td>–, –, +, +, CT</td>
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<td>–, –, –, –, –, CT; –, –, –, –, –, CT</td>
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<td>–, –, –, –, –, CT; –, –, –, –, –, CT</td>
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<tr>
<td>363</td>
<td>Embryonal + teratoma</td>
<td>330.0</td>
<td>+, chest +, CXR; +, abdomen +, CT</td>
<td>–, –, +, +, CT</td>
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<tr>
<td>252†</td>
<td>Seminoma</td>
<td>400.0</td>
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<tr>
<td>231†</td>
<td>Embryonal + choriocarcinoma</td>
<td>1721.0</td>
<td>+, lungs +, CXR; +, abdomen –, CT; –, –, –, CT; –, –, –, –, –, CT</td>
<td>–, –, –, –, –, CT; –, –, –, –, –, CT</td>
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<tr>
<td>221/223</td>
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<td>–, –, –, –, –, CT</td>
</tr>
<tr>
<td>230</td>
<td>Teratocarcinoma</td>
<td>117.0</td>
<td>+, pelvis +, US; –, +, Op</td>
<td>–, –, –, –, –, CT</td>
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<tr>
<td>235</td>
<td>Embryonal + choriocarcinoma</td>
<td>0.5</td>
<td>–, –, –, –, –, –, –, CT; –, –, –, –, –, CT; –, –, –, –, –, CT</td>
<td>–, –, –, –, –, CT</td>
</tr>
</tbody>
</table>

* +, Positive scan; –, negative scan.
† Radioimmunodetection findings of tumor preceded other clinical results.

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The patient was treated with Delatestryl and the β-hCG dropped to 17.4 ng/ml and his gynecomastia diminished. He was referred to us for a hCG RaID study in October 1979 (10 mo after surgery) and received 300 µg of antibody IgG (1.98 mCi of 131I). The scans revealed a focal area of abnormal radioactivity in the midline aspect of the right lung, in the lower posterior portion (Fig. 1). Chest roentgenograms performed a week earlier and 2 mo later were still negative (Fig. 2). Finally, in November 1980, >1 yr after the RaID study, a chest roentgenogram disclosed a 6-cm mass in the mediastinum in proximity to the bronchus leading to the right lower lobe of the lung (Fig. 3). He was then placed on chemotherapy and had an excellent response, including a decrease in his β-hCG titer from 38.3 ng/ml in October 1980 to <1.0 ng/ml in January 1981. Thus, the RaID study was able to reveal occult metastasis to the lung of this patient more than 1 yr before it was finally confirmed by chest roentgenography. The metastasis was suspected because of a rising hCG titer, which then fell with successful tumor treatment.
circulating antigen but that this complexing did not prevent successful RaID (19, 20). These same relationships appear to be true also for hCG RaID, as has been suggested recently by others (13). Whether this is due to an excess of antibody or the localization of antigen-antibody complexes having a free antibody arm remains to be clarified.

We have reported that CEA RaID can disclose occult cancer (3–5, 7), and this also appears to be true for hCG RaID as indicated by this study. Of 14 tumor sites found by RaID in 10 patients with testicular cancer, 4 were revealed by RaID prior to any other detection method used and provided a lead time to definitive diagnosis by other measures of a few days to >1 yr. We believe that this lead time would have been even longer had the RaID results not been known to the referring clinicians, particularly in patients nos. 231 and 256, who underwent surgical exploration shortly after our RaID studies.

The series of patients reported here is still too small for assessing the sensitivity (true-positive rate) and specificity (true-negative rate) of this new cancer detection method, but it is nevertheless encouraging that, among the testicular cancer patients, a value of 100% was achieved for each. Two putative false-positive findings were obtained in patients with ovarian and lung cancers, based upon their having normal serum hCG titers. However, both these tumor types have been known to result in elevated blood hCG levels (21–23), suggesting that the tumors may indeed have hCG available for binding the radioactive antibody without increased amounts of hCG circulating in the blood. Indeed, this has occurred in patients with CEA tumors (3–5, 7).

hCG RaID is not cancer-specific but is only as cancer-re-
stricted as tissue hCG is. This is emphasized by the positive hCG RaID results found in patients with nonmalignant lesions, such as hydatidiform mole and, in one case, degenerative products of conception. Because both instances have elevated tissue hCG, the lesions could be disclosed by hCG RaID. From this viewpoint, hCG RaID may have applications in such other circumstances, where a complete elimination of pathological tissues containing hCG could be controlled after surgery by a method that complements the determination of serum hCG titer. Indeed, after complete excision of hydatidiform moles, it has been our experience that the hCG RaID results become negative (11).

Serum markers such as α-fetoprotein and hCG have come to play an important role in the detection of occult disease in patients with nonseminomatous testicular cancer (24, 25), even though more than one-third of such patients have normal titers of these markers (26). Also, patients with elevated marker titers that return to normal during therapy do not always show a rise in the titer upon tumor recurrence. Thus, these markers sometimes become positive indicators after there is overt clinical evidence of tumor recurrence, suggesting that in such instances the use of RaID would be indicated.

The excellent response of testicular cancers to treatment makes this tumor type a particularly good candidate for RaID studies, where disclosure of occult metastases could have an effect on both patient survival and the cure rate. This is unfortunately less true for the majority of metastatic tumors identifiable by CEA RaID. Another advantage of hCG RaID over CEA RaID is the high sensitivity of serum hCG, as compared to blood CEA, in reflecting tumor burden. Serum hCG levels can increase rapidly and considerably when tumor recurrence or spread begins, thus alerting the clinician to the need for instituting or changing therapy. The mode of therapy, however, is often dependent upon a careful evaluation of the extent of disease, whether the tumor is localized to a single area or to numerous secondary organs. As we have emphasized elsewhere (12), hCG RaID can contribute to a therapeutic decision. Another important application is for the interpretation of the functional and, in turn, histogenetic character of germ cell tumors. Although one of the patients included in this study had a testicular cancer diagnosed as a pure seminoma, positive hCG RaID indicated that there had to be syncytiotrophoblastic elements in the tumor and, thus, could not be a pure seminoma. From the clinical standpoint, the two entities are treated differently, with the hCG-producing mixed seminoma having a poorer response to standard seminoma therapy.

Although the results reported here appear to have clinical usefulness in the management of patients with hCG-containing neoplasms, we appreciate that RaID's limitation of only disclosing tumors larger than 1.5–2.0 cm in diameter (4, 5, 12, 14) will need to be improved before it can be used more routinely in the identification of occult tumor. The other cancer detection methods employed, however, such as ultrasound and computed tomography, have similar limitations in resolution and, in addition, do not differentiate cancer from other masses identified. Since hCG is present most frequently in malignant tumors, particularly of the genital system, RaID presents an adjunctive method for determining the character and functional properties of an abnormal mass. This is particularly important after a tumor has been treated and the question of whether a fibrous mass or a viable, marker-producing tumor is present cannot be resolved by computed tomography and other noninvasive tests.

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