Radioimmunodetection of Cancer with Radiolabeled Antibodies to a-Fetoprotein

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Abstract

Sixteen patients with histologically proven malignant neoplasia were investigated by radioimmunodetection, using goat anti-a-fetoprotein (AFP) antibody radiolabeled with 131I. Images of the chest and abdomen were made with a scintillation camera, usually at 24 and 48 hr following injection of 1 to 2.5 mCi of radioiodinated antibody. Computer-assisted processing for the subtraction of 99mTc background radioactivity was used to enhance the detection and localization of tumors visualized by immune scintigraphy. All 12 sites involved by five AFP-containing and putatively AFP-deficient tumors, respectively. The image contrast was significantly greater for the AFP-containing tumors, and the subtraction technique enhanced the image contrast more than 2-fold. Based upon these initial results, the sensitivity of the method (true-positive rate) was 100%, its specificity (true-negative rate) was 80%, and the accuracy of the technique was 85%. This study thus indicates that radioimmunodetection of cancer with radioactive AFP antibodies can be useful in the evaluation of patients with AFP-containing neoplasms.

Introduction

Following the encouraging results of the external detection of CEA-containing tumors by radioimmunodetection, using anti-CEA antibodies labeled with 131I (2–4, 8), we proceeded to evaluate the use of 131I-labeled anti-AFP antibodies to detect and localize AFP-containing tumors (5, 6), particularly since AFP is another widely studied tumor marker (3, 10, 12, 13). Elevated AFP levels in the serum have been found in patients with not only hepatocellular carcinoma and germ cell tumors of the testis and ovary, but also cancers of diverse histopathology, especially those derived from the embryonic foregut, such as pancreatic and gastric carcinomas (3, 10, 12, 13). This paper reports our clinical radioimmunodetection results with 131I-labeled goat anti-AFP antibody administered to 16 patients with histologically confirmed malignant tumors of various types. Our earlier studies have appeared elsewhere (5, 6).

Materials and Methods

Patient Selection and Preparation. Sixteen patients with histories of histologically confirmed malignant tumors of various types were entered into the study after appropriate informed consent was obtained. In the majority of the patients, the primary tumors had been surgically resected, and the metastatic lesions were documented by diverse objective clinical measures or by surgery. The patients were tested for anaphylactic hypersensitivity to goat IgG and were given 5 drops of Lugol’s solution twice daily for 7 days to reduce thyroid uptake of free 131I activity, as described elsewhere (4).

Radioantibody Preparation. Hyperimmune goat antiserum was prepared against human AFP purified from hepatocellular carcinoma tissue by the method of Nishi (11). The IgG fraction of the antiserum was purified chromatographically with DEAE-cellulose, followed by concentration over an Amicon PM-30 membrane. The specificity of the antiserum was confirmed by double-gel diffusion, immunoelctrophoresis, and radioimmunoassay. At a concentration of 4.2 mg/ml, the goat anti-AFP IgG had a radioimmunoassay titer of 3 × 10^5, using 50% binding of a 2-ng labeled antigen as the end point; the antibody IgG did not decrease in immunoreactivity after radiolabeling with 131I (Amersham/Searle, Arlington Heights, Ill.) by the modified chloramine-T method (9), resulting in a specific activity of 5 to 10 Ci/g of IgG protein. The radiiodinated IgG preparations were tested by an independent laboratory (Scientific Associates, Inc., St. Louis, Mo.) and were found to be sterile, pyrogen free, and nontoxic in acute tests. The normal goat IgG used in one patient was prepared in a fashion similar to that for the anti-AFP IgG, except that an additional affinity purification step for adsorbing goat IgG to a donkey anti-goat IgG solid-phase adsorbent column was performed. The anti-AFP IgG prepared was not an affinity-purified antibody.

Photoscanning Techniques and Analyses. All 16 patients received approximately 1 to 2.5 mCi of 131I-labeled anti-AFP IgG (130 to 350 µg protein) i.v. in 20 ml sterile 0.9% NaCl solution over 10 min. One patient received 2.2 mCi of normal goat IgG after the AFP antibody scan was completed. In order to subtract unnecessary blood pool and free radiolabeled activities in the stomach and urinary bladder, about 0.5 mCi each of 99mTc-sodium pertechnetate and 99mTc human serum albumin...
were injected i.v. at 30 and 5 min, respectively, prior to imaging. Images of the anterior, posterior, and lateral chest and abdomen were obtained with a scintillation camera (LFOV, Searle Radiographics) at various time intervals following administration of the radioactive IgG preparation, usually at 24 and 48 hr. After the data were stored in a digital computer, the background counts per unit area of the $^{131}$I and $^{99m}$Tc were equalized, the technetium image was subtracted from the iodine image pixel by pixel, and the difference image was displayed in 8 color levels, from blue to black (lowest radioactivity) to red to white (highest radioactivity). To evaluate the specificity of radiocalization with the $^{131}$I-labeled anti-AFP antibody, the tumor to non-tumor count density ratios were derived by outlining regions of equal area at positive and negative locations. The image contrast, defined as the difference between the ground counts per unit area of the $^{131}$I and $^{99m}$Tc, was equalized, the technetium image was subtracted from the iodine image pixel by pixel, and the difference image was displayed in 8 color levels, from blue to black (lowest radioactivity) to red to white (highest radioactivity). To evaluate the specificity of radiocalization with the $^{131}$I-labeled anti-AFP antibody, the tumor to non-tumor count density ratios were derived by outlining regions of equal area at positive and negative locations. The image contrast, defined as the difference between the ground counts per unit area of the $^{131}$I and $^{99m}$Tc, was calculated on both the $^{131}$I image and the subtraction image ($^{131}$I minus $^{99m}$Tc) as a means of assessing the value of the subtraction method in detecting tumors. The mean values for AFP-containing and putatively AFP-deficient tumors were statistically compared by Student's t test.

Results

Essential data pertaining to the photoscanning results and clinical findings of the patients studied are summarized in Table 1. In 10 of the 16 patients, tumors were localized by the anti-AFP antibody labeled with $^{131}$I. As was expected, all 12 sites involved by the AFP-producing tumors (2 hepatocellular carcinomas, 2 testicular embryonal carcinomas, and one ovarian endodermal sinus tumor) could be demonstrated by the AFP immune scintiscans, and these localizations were corroborated by other objective diagnostic measures. In patients with testicular and ovarian tumors with germ cell elements, serum AFP titers were markedly elevated, whereas the serum AFP levels were within the normal range (<40 ng/ml) in the patients with hepatocellular carcinoma. All 5 sites of lung metastasis detectable by chest X-ray in Patient 145 with embryonal carcinoma of the testes were successfully visualized with the radiolabeled anti-AFP antibody, but normal goat IgG labeled similarly failed to show comparable localization. Five patients (Patients 144, 248, 173, 174, and 178), in whom tumors were not expected to produce AFP, also demonstrated localization with the anti-AFP scans in 6 of 16 sites, which agreed with other diagnostic findings. These tumors lesions were for most part quite massive, and the radioactivity accreted in the tumor sites was less than that detected in the AFP-producing tumors. The average tumor/non-tumor count density ratio was 3.20 and 1.96 for the AFP-containing and the putatively AFP-deficient tumors, respectively (Table 2). The image contrast was significantly

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Serum AFP (ng/ml)</th>
<th>Primary site</th>
<th>Secondary sites</th>
<th>Clinical findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>161</td>
<td>Hepatocellular carcinoma, liver</td>
<td>2.5</td>
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<td>+, chest; +, L/S scan; +, CT (abdomen)</td>
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<td>29.1</td>
<td>+</td>
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<td>E</td>
<td>+, lung (2); +, PE; +, bone scan</td>
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<tr>
<td>145</td>
<td>Embryonal carcinoma, right testis</td>
<td>800.0</td>
<td>E</td>
<td>+, abdomen; +, US; +, Ga-scan</td>
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</tr>
<tr>
<td>158</td>
<td>Seminoma, left testis</td>
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<td>+, chest; +, CXR; +, Ga-scan; +, post-operative reaction?</td>
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<tr>
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<td>E</td>
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<td>173</td>
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<td>+ (lung)</td>
<td>+, abdomen; +, CXR; +, II</td>
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<td>E</td>
<td>+, abdomen; +, CXR; +, CXR</td>
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<td>174</td>
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<tr>
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<td>+, abdomen; +, CXR; +, Ga-scan (1); +, Ga-scan (1); +, Ga-scan (1)</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of lesion sites positive.
greater for the AFP-containing tumors, and the subtraction technique was found to enhance the image contrast by more than 2-fold (Table 3). Patient 158 showed focal radioactivity in the region having surgery 4 days prior to the photoscan study, which we believe accounted for the false-positive result. In 18 sites devoid of tumor localization by radioimmunodetection, tumors could not be demonstrated by other diagnostic means and were thus presumed to be true-negative sites.

The following 2 cases illustrate our radioimmunodetection results with radioactive AFP antibody.

**Case 1.** An 83-year-old female (Patient 183) was referred for treatment of a biopsy-proven well-differentiated hepatocellular carcinoma. She had been in relatively good health until about 3 months prior to the referral, when she developed a dull pain in the right upper quadrant of the abdomen that radiated to her right scapula. She had experienced some weight loss, but there was no history of alcohol abuse, toxic exposure, or jaundice. Her diagnostic evaluation included negative hepatitis titer by radioimmunoassay was 29.1 ng/ml. The immune scintigram performed after injection of 1.3 mCi of 131I-labeled anti-AFP IgG revealed a focal abnormal concentration of radioactivity in the right lobe of the liver (Fig. 1), consistent with the liver-spleen scan results.

**Case 2.** A 25-year-old male (Patient 143) had undergone an orchiectomy for teratocarcinoma of the left testis about 1.5 years prior to our radioimmunodetection study. About 1 month previously, he was found to have a superior vena caval syndrome due to tumor mass in the right main bronchus that was immediately irradiated. He developed left-sided weakness as well as loss of bladder control. On physical examination, he had a decreased sensation from L3 down; deep tendon reflexes were lost., as well as rectal tone were also decreased. It was felt that he probably had cauda equina compression from the metastatic tumor. A brain scan showed metastatic lesions in the right hemisphere. His serum AFP titer was 15,000 ng/ml, as determined by radioimmunoassay. Radioimmune scans made after injection of 1.8 mCi of 131I-labeled AFP antibody showed a large area of abnormal radioactivity in the right upper lung and smaller lesions in the left lung that corresponded with the metastatic nodules seen on the chest radiograph (Fig. 2). There was another area of abnormal concentration of AFP antibody radioactivity in the sacral region of the pelvis. A bone scan revealed a metastatic lesion involving the right sacrum, thus corroborating the radioimmunodetection findings.

**Discussion**

Previous studies of radioimmunodetection have been restricted to the use of tumor cell surface markers, such as CEA, as targets for radiolabeled antitumor antibodies (3, 4, 7, 8). Although AFP is a cytoplasmic tumor marker, we proceeded to evaluate the ability of radiolabeled anti-AFP antibodies to localize in AFP-containing tumors, either because of intracellular uptake or because the tumors had an extracellular milieu of AFP which would provide a gradient between tumor and nontumor, adjacent tissues. Our results in this and in former studies (5, 6) support the view that AFP and other intracellular tumor markers can serve as antibody targets for tumor detection and localization. All 12 sites involved by AFP-producing tumors were successfully localized with radioiodinated anti-AFP antibody. Moreover, extremely elevated serum AFP levels, such as 18,000 ng/ml in a patient with ovarian endodermal sinus tumor, did not prevent successful tumor radiolocalization, a finding which supports our experiments in CEA tumor radioimmunodetection (2, 4, 8). Conversely, hepatocellular carcinomas could be imaged in 2 patients without elevated serum AFP titers; it is known that about 28% of patients with this tumor type do not have increased circulating AFP levels (13).

The question of AFP tumor specificity of this technique was addressed indirectly. First, in one patient in whom all 5 metastatic sites in the lung localized after injection of the specific antibody, administration of radioactive normal goat IgG did not give similar radioimmunodetection results, thus supporting the view that the specific antibody was required for visualizing the antigen-containing tumors. Secondly, higher average tumor/ non-tumor count density ratios on nonsubtraction images in AFP-producing, as compared to putatively non-AFP-producing
tumors, further suggest an antigen-antibody reaction. The tumor localization seen in 5 patients with apparently AFP-deficient tumors was perhaps due to nonspecific uptake of the AFP antibody which was not an affinity-purified preparation similar to that used in our CEA radioimmunodetection studies (4). Indeed, these tumors demonstrated image contrast results which were significantly less than those of the AFP-producing tumors. Moreover, it is interesting to note that this apparent false-positive finding occurred in large tumors, which are known to absorb macromolecules such as immunoglobulins (3). We must also bear in mind, however, that these tumors may possibly contain sufficient AFP for radiolocalization without having elevated serum AFP levels. Indeed, it has been our experience in CEA radioimmunodetection that there is not a good correlation between blood CEA levels and immune scintigraphy results (4, 8). Finally, consideration of the true-positive and true-negative results indicates a high degree of immunological specificity despite the fact that the antibody used was not affinity purified. All 12 of the AFP-containing tumor sites could be demonstrated by AFP radioimmunodetection, indicating a sensitivity of 100%. Computing all of the putatively AFP tumor-negative sites found to be negative by radioimmunodetection results in a specificity of 80%. The accuracy of the method in this initial series, which is determined by dividing the number of true-positives and true-negatives by the total number of sites, was 85%. Thus, it appears that this stage of our development of AFP tumor radioimmunodetection provides a method of tumor detection and localization that compares well with other cancer detection methods currently available. Although AFP is not a tumor-specific marker, its quantitative increase in a number of neoplastic types does appear to offer a degree of tumor specificity in radioimmunodetection not achieved by other methods of external radionuclide imaging. Therefore, we are hopeful that the future use of affinity-purified AFP antibodies, or of hybridoma-derived monoclonal AFP antibodies, together with improved imaging facilities, such as emission tomography, will provide important advances for improved accuracy and resolution in cancer radioimmunodetection.

Acknowledgments

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References

Fig. 1. Anterior (ANT) view of liver on subtraction image of AFP immune scintiscan (A) shows focal abnormal activity (arrows) in the upper portion of the right lobe, corresponding to the focal defect (arrows) seen on liver scan with $^{99m}$Tc sulfur colloid (B) in patient with hepatocellular carcinoma (Patient 183). L, liver; H, heart.

Fig. 2. Anterior (ANT) view of chest on subtraction image of AFP radioscan (A) shows large focal abnormal activity (open arrow) in the right upper lung and 2 small areas of abnormal activity in the left lung (closed arrows), corresponding to lesions seen on chest radiograph (B), in Patient 143 with embryonal carcinoma metastatic to the lungs. H, heart; CXR, chest X-ray.
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