Lymphoscintigraphy with Radionuclide-labeled Antibodies to Carcinoembryonic Antigen

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Abstract

Upper- and lower-extremity lymphoscintigraphy was performed on 50 patients with proven carcinoma of the breast, gastrointestinal system, genitourinary tract, lung, and vulva; 42 patients had 131I-antibodies to carcinoembryonic antigen (CEA) and 8 patients had 131I-normal goat immunoglobulin G. All metastases in the axillary or inguinal lymph nodes were detected with the CEA antibodies. In nine patients without metastases, radioactivity was observed in inguinal or axillary nodes. It is postulated that the nodal sequestration of antibody in the absence of metastatic carcinoma is due to captured CEA in lymph nodes draining the tumor site. This study has demonstrated the high sensitivity for detecting nodal metastases by labeled antibodies to CEA draining from primary or recurrent tumors.

Introduction

The s.c. application of radionuclide-labeled colloids for the visualization of lymph nodes (lymphoscintigraphy) has been used successfully to visualize the internal mammary lymph nodes (3), inguinal nodes (20, 21), and pelvic nodes. Although lymphoscintigraphy is now used as a routine procedure for the visualization of lymph nodes in many nuclear medicine departments, early investigations were directed towards therapy. Several researchers evaluated colloid 198Au for the radiotherapeutic treatment of malignant neoplasms in nodes (9, 23). Since the sequestration of the radioactive colloid was the most efficient in normal lymph nodes, the desired therapeutic effect was not achieved.

It was soon apparent that external lymphoscintigraphy was possible with colloidal 198Au. Because of the high-radiation dose from the β-radiation of colloidal 198Au, however, the quantity that could be administered was limited; thus, the insufficient data density (photons) resulted in images of poor quality. To achieve in vivo images of better diagnostic quality, colloids were labeled with radionuclides that emitted a more suitable photon, i.e., lesser radiation to the patient and increased compatibility with current imaging instrumentation. Sulfur colloid labeled with 99mTc was shown to provide satisfactory definition of lymph nodes following s.c. administration (3). Unfortunately, the 6-hr physical half-life of 99mTc limits its usefulness if images are required beyond 1 day. To overcome the time limitation of 99mTc, Goodwin et al. (6, 7) proposed

111In-labeled colloid since the 2.8-day half-life of this radionuclide will provide an adequate residual photon density for imaging beyond 24 hr. For improved lymphatic channel transport to the nodes, Garzon (4) developed the smaller aniony sulfur colloid labeled with 99mTc. For certain diagnostic problems, such as demonstration of the internal mammary lymph nodes, this colloid labeled with 99mTc has proved to be very useful (3).

Radio colloids are sequestered in lymph nodes by means of phagocytosis and provide evidence similar to that obtained with contrast lymphography. Pearlman (17) studied the pelvic and abdominal lymph nodes in 100 patients with proven lymphoma by radiocolloid and contrast lymphangiography. Although he found a good correlation between the 2 methods, radiocolloid lymphoscintigraphy was considered to be a supplement to the radiological method. He did observe, however, that in certain cases unsuspected disease was detected in the abdominal nodes. Abnormal nodes are manifest by absence or decreased concentration of radioactivity or loss of expected configuration. Occasionally, lymph nodes without tumor may fail to sequester the radiocolloids. In a study of the regional lymph nodes from radical mastectomies for carcinoma of the breast, Seaman and Powers (22) found that normal lymph nodes may fail to sequester the colloid and, also, that lymph nodes with metastatic carcinoma may sequester the colloid appreciably. These findings illustrate the nonspecificity of lymphoscintigraphy with radiolabeled colloids.

A more tumor-oriented radiotracer is 67Ga citrate. Although it is not a “tumor-specific” agent, it does frequently concentrate in several types of tumors (e.g., lymphoma, bronchogenic carcinoma, and hepatoma), including metastatic sites in lymph nodes (11). The mechanism by which 67Ga concentrates in tumor tissue has been investigated extensively, but an understanding still remains elusive (13). Other agents with a variable degree of tumor orientation have been evaluated. Day et al. (1) demonstrated that a radiolabeled antibody to fibrin would localize in tumors; however, the concentration of the antibody depended on a tumor-associated inflammatory process, not a tumor-associated antigen. Herrera et al. (12) observed that L-[75Se]selenomethionine would concentrate in lymph nodes infiltrated by lymphoma. Radiolabeled bleomycin has been used also to detect tumors with only partial success (19).

The development of antibodies to tumor-associated antigens with sufficient purity to ensure adequate target-to-nontarget radioactivity for external scintillation imaging (5) has provided the basis for lymphoscintigraphy of malignant lymph nodes. Although not tumor-specific in the strictest sense, antibodies to tumor-associated antigens are an important advancement in the pursuit of a tumor-specific agent.

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2 To whom requests for reprints should be addressed, at the University of Kentucky Medical Center, Room N-7, Lexington, Ky. 40536.
3 W. Kaplan, personal communication.

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Materials and Methods

Purified antibodies to CEA were labeled with $^{131}$I (5). Patients were given s.c. injections, in the web of the fingers or foot (or both), of approximately 250 µCi of labeled antibody (25 to 50 µg of IgG protein). To facilitate absorption of the antibody, the sites of injection were massaged for 2 to 3 min. Images were obtained with a γ-camera usually at 6, 24, and 48 hr after administration of the labeled antibody. The data were stored in a laboratory computer. If sufficient radioactivity had been absorbed systemically at 24 and 48 hr to produce an interfering background of activity, $^{99m}$Tc-labeled human serum albumin and sodium $^{99m}$Tc-pertechnetate were given i.v. to minimize nontarget $^{131}$I (2) by computer processing.

Fifty patients were entered into the study who had documented carcinoma, and included carcinoma of the breast (12 cases), gastrointestinal carcinoma (14 cases), genitourinary carcinoma (14 cases), pulmonary carcinoma (5 cases), and squamous cell carcinoma of the vulva (3 cases). Forty-two subjects received $^{131}$I-labeled antibodies to CEA and 8 received $^{131}$I-labeled normal IgG (goat). Patients with carcinoma of the breast or vulva were selected since the nodal groups in the axilla or inguinal area are in the usual drainage pattern for metastases from these cancers. Those with carcinoma of the gastrointestinal, genitourinary, or pulmonary systems were chosen because the inguinal and axillary lymph nodes do not lie within the usual lymphatic drainage pathway of these organs.

Results

The results from lymphoscintigraphy with $^{131}$I-labeled antibody to CEA are summarized in Table 1. Of 9 patients with carcinoma of the breast, 8 demonstrated concentration of radioactivity in the axillary nodes. The one patient with no evidence of antibody sequestration in the axillary area was proved subsequently to have mammary duct ectasia with peri-ductal mastitis. In 8 patients, metastatic carcinoma to the ipsilateral axillary nodes was confirmed by surgery (4 cases) or clinically by hard enlarged masses that were unquestionably tumor (4 cases). In 3 patients, concentration of radioactivity was observed in the contralateral axilla. Extensive systemic metastases were present in 2 of these latter cases, and a massive, fixed, inoperable breast carcinoma was found in the third. Fig. 1 illustrates the typical appearance of radioactivity concentrated in the axillary nodes.

Four of 11 patients who had had resections for carcinoma of the rectum or sigmoid colon had visible inguinal lymph nodes by scintigraphy. In none were the nodes palpable. In 5 other patients with cancer of the gastrointestinal tract (colon, 2 cases; pancreas, 1 case; biliary tree, 1 case; and esophagus, 1 case), radioactivity in the axillary nodes was demonstrated in one patient with carcinoma of the left colon (left axilla) and in the patient with carcinoma of the esophagus (both axillae).

In 2 of 9 patients with genitourinary tract cancer (ovary, 4 cases; urinary bladder, 2 cases; prostate, 2 cases; and endometrium, 1 case), the radiolabeled antibody was sequestered by inguinal lymph nodes. Pelvic metastases were present in both of these patients, one with urinary bladder carcinoma and the other with prostatic carcinoma.

<table>
<thead>
<tr>
<th>Primary site of carcinoma</th>
<th>No. of cases</th>
<th>Inguinal lymph nodes</th>
<th>Axillary lymph nodes</th>
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<tbody>
<tr>
<td>Breast</td>
<td>9</td>
<td>+</td>
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</tr>
<tr>
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</tr>
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No concentration of radioactivity was observed in the axillary or inguinal regions of 5 patients with carcinoma of the lung. In 3 patients with squamous cell carcinoma of the right vulva, all showed bilateral radioactivity in the inguinal regions. In one, bilateral inguinal node metastases were found; in one, metastases to the right nodes only (Fig. 2) were found; and in one, no metastases were found. The lymph nodes were dissected from the 3 bilateral inguinal node resections, and portions of each node were measured for radioactivity. All lymph nodes demonstrated the presence of $^{131}$I. In the specimen with ipsilateral metastases only, the distribution and radioactivity of each node was plotted. On the ipsilateral side of the carcinoma, a narrow channel of nodes leading from the malignant vulvar lesion to the femoral nodes showed high levels of radioactivity. The remaining lymph nodes on the ipsilateral side and those on the contralateral side had variable levels of radioactivity but less than that in the described channel.

In 3 patients with breast carcinoma, $^{131}$I-labeled normal IgG (goat) was used (Table 2). In 2 cases, concentration of the radioactivity was observed in the ipsilateral axilla. Both of these patients had massive axillary metastases. In 5 patients with genitourinary carcinoma (ovary, 2 cases; oviduct, 1 case; cervix, 1 case; and testis, 1 case), only the patient with a testicular carcinoma demonstrated inguinal radioactivity on the ipsilateral side. The lymphoscintigraphy was performed about 2 weeks following an orchietomy and inguinal node dissection.

Discussion

Order et al. (16) reported one of the first investigations of lymphoscintigraphy by means of a radionuclide-labeled antibody to an antigen. They used $^{131}$I-labeled antiferritin immunoglobulin to demonstrate sequestration of the antibody in lymph nodes of patients with breast carcinoma and lymphomas. In this study, the sensitivity of labeled antibodies to CEA for detecting CEA-producing tumor metastases to lymph nodes was 100%. The specificity, however, was variable. Three patients with breast carcinoma showed concentration of radioactivity in the contralateral axilla without palpable lymph nodes.

Table 1

Lymphoscintigraphy with $^{131}$I-labeled antibody to CEA

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Table 2

Lymphoscintigraphy with $^{131}$I-labeled normal IgG (goat)

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The abbreviation used is: CEA, carcinoembryonic antigen.
Six of 16 patients with gastrointestinal cancer, 2 of 9 with genitourinary cancer, and 3 with vulvar cancer had either axillary or inguinal sequestration of the labeled antibody. In most of these patients, no lymph nodes were palpable. Since there were 116 axillary and inguinal sites that did contain within the lymph nodes but also in cancer-free germinal centers genitourinary cancer, and 3 with vulvar cancer had either CEA was observed not only in the metastatic carcinoma cells and lymph nodes out of the drainage pathway of the tumors. It appears quite probable that the concentration of antigen-reactive lymphocytes with natural antibodies. Potomski et al. (18) studied the location of CEA in tumor, adjacent normal tissue, and lymph nodes in the drainage region of gastrointestinal cancers by immunofluorescent techniques. As expected, CEA was demonstrated in the centroglandular regions of gastric and colonic adenocarcinoma, in the cytoplasm of the more malignant carcinomata, and in the mucosa surrounding the tumors but not in adult lung, liver, spleen, kidney, thymus, and lymph nodes out of the drainage pathway of the tumors. CEA was observed not only in the metastatic carcinoma cells within the lymph nodes but also in cancer-free germinal centers of nodes with metastases and prominently in nonmetastatic nodes that lie in drainage pathways from the primary tumors. From these findings, it is highly probable that CEA is released from the tumor, transported through the regional lymphatic channels, and then sequestered by the germinal centers of lymph nodes for a transient period before the CEA gains access to the blood. In one of our patients who had had a carcinoma of the rectum resected 15 months earlier, the only positive diagnostic findings were a plasma CEA of 500 ng/ml and a concentration of 131I-labeled antibody to CEA in a nonpalpable left inguinal node. This lymph node was excised 2 months later (elsewhere), and no tumor was found, only hyperplasia of the germinal centers. Two months after this biopsy, the abdomen was explored and recurrent tumor in the pelvis and metastasis to the liver were found. The deposition of labeled antibody by inguinal lymph nodes in patients with pelvic metastases (prostate or urinary bladder) and recurrent tumor in the pelvis (rectum and sigmoid colon) is comprehensible in view of the directional changes in lymphatic drainage by the tumors and by surgery. Since Potomski et al. (18) demonstrated CEA in lymph nodes draining sites of carcinoma and never in lymph nodes out of the drainage area, the lymph node deposition of 131I-CEA-antibody in the absence of metastatic tumor definitely suggests that a tumor site is proximal to the nodes. Although our data are still incomplete, they reinforce the findings of Potomski et al.

The concentration of radioactivity in the contralateral axilla in 3 of our patients with carcinoma of the breast is not as easily explained. The observations by Haagensen (8) and Handley and Thackray (10) that metastases to the contralateral axilla do occur in the absence of regional skin recurrence or disease in the opposite breast provide substantiating evidence for our findings. Of considerable interest to this problem are the investigations of Vendrell-Torné et al. (24). By injecting 198Au colloid in the 4 quadrants of the breast, they found that the colloid migrated to the internal mammary nodes in 62% of the patients treated in the upper inner quadrant, 86% of patients treated in the lower inner quadrant, 36% of patients treated in the upper outer quadrant, and 64% of patients treated in the lower outer quadrant. These percentages are much higher than the published comparable figures for metastases to the internal mammary nodes from breast carcinoma (10). Presumably, then, antigen from a breast carcinoma can migrate medially in a relatively high percentage of patients. Since Haagensen (8) reports that about 5% of his patients with breast carcinoma had contralateral axillary metastases at autopsy, it is quite possible that a higher percentage of patients may have sequestered antigen in the contralateral axilla.

This investigation has demonstrated the feasibility of visualizing metastatic carcinoma in lymph nodes by lymphoscintigraphy with radiolabeled antibodies to tumor-associated antigens. It has also suggested the possibility of detecting sequestered antigen in lymph nodes from a regional tumor. These findings should contribute to the diagnosis of cancer and to patient evaluation with respect to therapy. Although many questions remain, this new approach, lymphoscintigraphy by means of radiolabeled antibodies to tumor-associated antigens, should provide an important diagnostic tool in oncology.

References

Fig. 1. Adenocarcinoma of left breast. Posterior view of γ camera image shows concentration of 131I-antibody (arrow, CEA in left axilla). Fourteen of 24 lymph nodes contained metastatic carcinoma.

Fig. 2. Squamous cell carcinoma of right vulva. Anterior view shows concentration of 131I-antibody (CEA) in 2 nodes of right inguinal region and one node of left inguinal region. Metastases found only in right inguinal nodes.
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