Preliminary Studies of Monoclonal Antibody Lymphoscintigraphy in Malignant Melanoma


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Lymphoscintigraphy was performed at 3 and 20 hr following subcutaneous injection of $^{131}$I anti-melanoma antibody (Fab) in 11 patients who had surgical resection of lymph nodes (neck, axilla, groin) at 24 hr for suspected metastatic melanoma. Comparable amounts of $^{125}$I nonspecific control antibody (Fab) were co-administered. Six patients had nodal metastases and three showed positive images at both time periods. Five patients had no metastases though one was image positive. Four other nondiseased inguinal node groups were image negative. A total of 28 tumored nodes and 110 normal nodes were removed, counted and histologically examined. All metastatic tumors expressed antigen against which the specific Fab was directed. The concentrations of both specific and nonspecific Fab were similar in tumored nodes and both were significantly greater than in normal nodes. Dual isotope autoradiography with video densitometric analysis of tumored nodes showed essentially identical intranodal spatial distribution of the specific and control Fab in areas containing tumor. These preliminary results suggest the increased concentration of murine immunoglobulin (Fab) retained in diseased nodes was a nonspecific phenomenon.


Most malignant melanomas arise as pigmented skin tumors and are diagnosed by local excision. Subsequent surgical treatment commonly proceeds to a wide local excision (WLE) of the lesion site and excision of regional lymph nodes draining the area if they are palpably enlarged and the patient is otherwise free of disease (Class II designation). In certain patients WLE and lymph node resection of nonpalpable nodes may also be beneficial (Class I designation) when the melanoma is of intermediate depth (0.75–4.0 mm). In this setting it is presumed there is a high probability for nodal micrometastases and removal of nodes may significantly increase long-term survival (1).

When malignant melanoma occurs on an extremity, the regional nodes draining the area are in the ipsilateral groin or axilla. If the initial lesion is truncal near the midline or midway between axilla and groin or on the scalp, the sites of drainage may be unpredictable and multiple. In this setting we and others have performed lymphoscintigraphy with technetium-99m ($^{99m}$Tc) antimony sulfide colloid to determine which node groups are potentially involved and could be resected (2). Although very useful for indicating nodal areas for surgical resection, the labeling of nodes by colloid is nonspecific.

Several investigators have advanced the idea of using subcutaneously injected radiolabeled antitumor antibodies to delineate metastatic deposits in regional lymph nodes (3–5). If successful in melanoma, such target-specific lymphoscintigraphy would have a great advantage for determining pre-operatively if metastatic disease were present and, in the case of drainage to multiple sites, which node groups should be excised. The purpose of this study was to determine if subcutaneously administered antimelanoma monoclonal antibody (Fab) labeled with radioiodine could detect the presence of lymph node metastases as visualized by gamma camera imaging. We also compared the distribution and uptake of coadministered radiolabeled nonspecific (control) monoclonal antibody (Fab).
TABLE 1
Basic Data on Patients Studied

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Class</th>
<th>Lesion depth/mm</th>
<th>Site injected</th>
<th>Site resected</th>
<th>#Pos. nodes</th>
<th>Antigen\textsuperscript{1} content</th>
<th>#Neg. nodes</th>
<th>Nodal images</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>II</td>
<td>4.0</td>
<td>Thigh</td>
<td>Groin</td>
<td>9</td>
<td>2-3+</td>
<td>4</td>
<td>Positive</td>
<td>Gross metastases</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>II</td>
<td>0.8</td>
<td>Temple</td>
<td>Neck</td>
<td>6</td>
<td>2-4+</td>
<td>0</td>
<td>Positive</td>
<td>Gross nodal and parotid metastases</td>
</tr>
<tr>
<td>3</td>
<td>58</td>
<td>I</td>
<td>3.6</td>
<td>Calf</td>
<td>Groin</td>
<td>9</td>
<td>1-3+</td>
<td>7</td>
<td>Positive</td>
<td>Multiple microscopic metastases</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>I</td>
<td>4.7</td>
<td>Temple</td>
<td>Neck</td>
<td>2</td>
<td>1-4+</td>
<td>8</td>
<td>Negative</td>
<td>Gross metastases</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>I</td>
<td>3.0</td>
<td>Triceps</td>
<td>Axilla</td>
<td>1</td>
<td>2+</td>
<td>17</td>
<td>Negative</td>
<td>2 mm Metastasis leg injected, image neg.</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>I</td>
<td>3.0</td>
<td>Clavicular</td>
<td>Axilla</td>
<td>1</td>
<td>2+</td>
<td>14</td>
<td>Negative</td>
<td>One microscopic focal metastases</td>
</tr>
<tr>
<td>7\textsuperscript{2}</td>
<td>63</td>
<td>I</td>
<td>1.2</td>
<td>Sacral</td>
<td>Bilateral groins</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>38</td>
<td>I</td>
<td>1.0</td>
<td>Triceps</td>
<td>Axilla</td>
<td>0</td>
<td>0</td>
<td>19</td>
<td>Negative</td>
<td>Leg injected, image negative</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>I</td>
<td>1.0</td>
<td>Thigh</td>
<td>Groin</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>Negative</td>
<td>Uninvolved leg injected, image negative</td>
</tr>
<tr>
<td>10\textsuperscript{2}</td>
<td>27</td>
<td>I</td>
<td>1.0</td>
<td>Occiput</td>
<td>Neck</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>43</td>
<td>I</td>
<td>4.5</td>
<td>Occiput</td>
<td>Groin</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>Negative</td>
<td>Uninvolved leg injected, image negative</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Patient received Fab 96.5.

\textsuperscript{2} Based on visual inspection of immunoperoxidase stained sections. 0 = no antigen present, 4\textsuperscript{*} = intense staining.

MATERIALS AND METHODS

Patient Selection

Eleven patients (Table 1) were studied who had a diagnostic but limited excision of a cutaneous malignant melanoma, and who were selected to have a subsequent WLE of the cutaneous site and resection of regional lymph nodes. The original lesions ranged from 1 to 4.7 mm in depth. The sites of the original lesions and of node resections are indicated in Table 1. In two patients regional lymph nodes were palpable (Class II) and in nine patients, no regional nodes were palpable (Class I).

In Patient 7 whose primary lesion site was over the sacrum, \textsuperscript{99m}Tc microcolloid lymphoscintigraphy indicated drainage to both inguinal regions, and a bilateral inguinal node resection was done. In the three patients with scalp lesions, microcolloid lymphoscintigraphy showed drainage only to ipsilateral neck nodes.

In two other Class I patients with malignant melanoma, antibody lymphoscintigraphy was done but surgery was not performed (patients’ preference). In these individuals we obtained biodistribution data only which is included in Table 2. Gamma camera imaging was negative but the image results are not included in this data because surgical confirmation is lacking.

Monoclonal Antibodies and Radioiodination

Murine antibody 96.5 (IgG\textsubscript{2a}) directed against the p97 antigen of melanoma was used in two patients and in all other instances antibody 48.7 (IgG\textsubscript{1}) directed against a high molecular weight (HMW) antigen of melanoma was used. A nonspecific murine control antibody 1.4 (IgG\textsubscript{1}), specific for murine leukemia 9P70 but not reactive with human tissues or melanoma, was co-administered with the specific antibody.

All antibodies were administered as the Fab fragment. The purified antibodies were prepared as previously reported (6). For 11 administrations (including the two unoperated Class I patients), the specific Fab was labeled with iodine-131 (\textsuperscript{131}I) and the control with iodine-125 (\textsuperscript{125}I), using approximately equal activities of each radionuclide (0.5–1.0 mCi). These amounts of activity were chosen to facilitate dual isotope counting of excised nodes and especially to permit delayed \textsuperscript{125}I autoradiography. In two instances, \textsuperscript{131}I was labeled to the specific antibody and the amount of \textsuperscript{131}I on the control Fab was reduced tenfold to permit \textsuperscript{125}I imaging.

Radioiodination was done by the chloramine-T (CT) reaction starting with 5–10 mCi labeling grade radioiodine.\textsuperscript{7} Five milligrams of Fab was reacted with 125 \(\mu\)g of CT in phosphate buffer (pH 7.3) for 5 min. Purification of labeled product was

TABLE 2
Biodistribution Data Following Subcutaneous Injection of Fab—% Dose

<table>
<thead>
<tr>
<th></th>
<th>3 hr</th>
<th></th>
<th>20 hr</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Unine excretion</td>
<td>Specific Fab</td>
<td>7</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Control Fab</td>
<td>6</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Total blood content\textsuperscript{1}</td>
<td>Specific Fab</td>
<td>12</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Control Fab</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>% Dose remaining in injection site</td>
<td>18</td>
<td>71</td>
<td>23</td>
<td>15</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Calculated using estimated blood volume.
done using a Sephadex G-10 column and the final product typically showed >98% protein bound activity by cellulose acetate electrophoresis. Under these conditions the final product achieves a 0.1 I/Fab molar ratio which has been shown not to interfere with the immunonintegrity of the antibody (7). Sterility was achieved by micro filtration (0.22 µm) and apyrogenticity was established using the limulus amebolysate test. 

The immunoreactivity of the final product was confirmed by a cell binding assay using a suspension of formalin fixed melanoma tumor cells known to have high expression of both the HMW and P97 antigens (8).

Briefly, ~5 x 10⁶ fixed melanoma cells (M2669) from an explant of human melanoma are incubated with ~50 ng of antibody for 30 min. Under these conditions whole antibody 48.7 shows an average of 53% binding to cells and the Fab shows 20% binding. Whole antibody 96.5 shows an average of 80% cell binding and the Fab is 74%. Nonspecific Fab 1.4 cell binding averages 2%. We ascribe these binding differences to differences in avidity of the antibodies and their Fabs (8). For the studies reported herein the post-labeling cell binding was 80% or greater of the established normal binding value.

Our experience in vivo with both the 96.5 and 48.7 Fabs (labeled under the conditions described above) has been confirmatory that the antibodies maintain good immunoreactivity both in tumor containing nude mice (6) and in humans (9,10).

Patient Protocol

The studies were carried out in conjunction with IND-BB 1609 after approval by the University of Washington Human Subjects Committee and with the patient’s informed consent.

Prior to injection of antibody, each patient had a negative intradermal skin test for sensitivity to the specific and control Fab. Approximately 0.5 to 1 mg of the specific and control Fab was co-mixed in a single syringe in a volume of 1 ml. (volume adjusted with physiologic saline) and 0.1 ml was removed as a quantitative standard. All patients received Lugol’s solution prior to injection and daily for one week to block thyroid uptake of free iodine.

The Fab mixture was injected subcutaneously around the margin of the original lesion in 6-8 equal injections. Immediate gamma camera imaging of the specific Fab at the injection site was done at a distance of 10 cm and the observed count rate in the injection area was recorded and compared with the count rate at 10 cm from an 131I solution (11) in two instances where the specific Fab was so labeled. The 125I and 131I solutions served as comparator standards to account for decay and any changes in camera sensitivity at the subsequent counting times 3 and 20 hr later. Blood samples were drawn at 3 and 20 hr and urine was collected for the 0-3- and 3-20-hr intervals to determine their radioactive content using dual channel gamma ray spectrometry.

Gamma camera imaging of lymph node drainage sites was carried out at ~3 and 20 hr. In four instances additional subcutaneous injections of 131I specific antibody were made in a nontumored lower extremity not associated with the melanoma, and disappearance of activity from the injection site was recorded and imaging of normal nodes in the groin was performed.

In the Patients 4 and 9 (Table 1) where 1 mCi of 125I was used on the specific antibody and 100 µCi of 131I was labeled to the control antibody the following pertained. Gamma camera images of the injection site were of good quality and 80% of the counts in the 125I window were from 125I and 20% were from 131I down scatter. The 131I counts were subtracted for quantitation of the disappearance of specific antibody from the injection site. Imaging of the lymph nodes was negative in both patients. Since the nodes were very near the surface and little tissue attenuation was present and the imaging times were prolonged any focal accumulation of 125I in nodes would have been observed with this technique.

Analysis of Excised Lymph Nodes

In each instance the patients had surgery ~24 hr after the injection of antibody. At surgery the nodes were removed in a tissue block and the individual nodes were identified and their location mapped. Each node was dissected free of fat, weighed, and 131I and 125I activity was measured in a well counter using gamma spectrometry. Enlarged nodes were cut into smaller sections as needed for counting. Node weights ranged from 10 mg to 21 g.

Each node was inspected for gross tumor and one half of each node was processed, stained and examined microscopically at 40-µ intervals. The other half of each node was frozen and 20-µ sections were examined histologically for tumor which, when present, was stained for the presence of HMW and P97 antigen by immunoperoxidase staining techniques previously described (11). The amount of antigen present (based on visual inspection) is expressed semiquantitatively as 0-4+ where 4+ means intense antigen staining of the cells. In cases where gross tumor was apparent, 2 mm whole sections of the nodes were obtained for autoradiography. A total of 138 nodes were examined. Metastases were present in 6 patients who had 28 tumor nodes and 50 normal nodes. Five patients had no metastases in 60 normal nodes.

Autoradiography was performed on 21 nodes from three tumored patients. Whole node sections (2 mm thick) were placed on Cronex mammography x-ray film and exposed at 4°C for 1 wk. In the first week, the concentration of 131I was such that >90% of the film darkening was due to 131I specific antibody, and less than 10% was produced by 125I. After 40 days of decay (five 131I half-lives) the specimen were autoradiographed for an additional 40 days to detect the 125I labeled control antibody.

The dual autoradiographs were visually inspected and compared to adjoining histologic sections of the nodes. In addition, selected autoradiographs were analyzed for the spatial distribution of specific and control radioactivity using a digitized video densitometry system developed in our department (12). The paired digitized autoradiographs were compared by both digital profile analysis and by comparison of relative activity on a pixel by pixel basis. This is further explained in the legends accompanying Figures 4 and 5.

RESULTS

All patients tolerated the procedures easily and there were no reactions to injection of antibody. Table 2 shows biodistribution data on the patients. After 3 hr 70% of the injected radioactivity remained at the injection site, and at 20 hr 40% remained. Blood concentration of radioactivity rose to 10% of the dose in the estimated blood volume at 3 hr and was similar at 20
hr. Blood levels for specific and control Fab were similar. Urinary excretion of radioactivity was 5% at 3 hr and 20–30% at 20 hr. In two patients the mean fraction of plasma radioactivity of specific and control antibody bound to protein (TCA precipitation) was 60% at 3 and 20 hr while protein bound activity in the urine was 23% and 50% at 3 and 20 hr, respectively. No other precipitations were done in the course of this study.

The absorbed radiation dose at the subcutaneous injection site from the $^{131}$I antibody was estimated based on the work of Bergqvist and associates (13). Our data indicated the disappearance rate of activity was $\sim 0.046$ hr$^{-1}$ and that the dispersed volume of injectate as estimated from gamma camera images approximated 20 cm$^3$. Accordingly the dose to the subcutaneous tissues and skin would be in the range of 300 rad for a 750-$\mu$Ci injection of $^{131}$I antibody. This is slightly $<10\%$ of the skin tolerance dose (13). The estimated dose to the skin and subcutaneous tissues from 750 $\mu$Ci of $^{131}$I antibody would be $\sim 50$ rad.

Imaging of nodal activity is outlined in Table 1. Of the six patients with lymph node metastases, the two Class II Patients 1 and 2 and Class I Patient 3 had distinct nodal activity seen by gamma camera imaging at 3 and 20 hr. In the other three patients with positive nodes, the images were negative at both times. In Patients 5 and 6 the nodal metastases were present in only one of their axillary nodes and were microscopic (Table 1). Patients 7–11 had no metastatic disease, though one, Patient 7, following a mid-sacral injection showed distinct positive bilateral uptake of the specific antibody at 3 and 20 hr in groin nodes. The additional four subcutaneous injections of specific antibody made in an unaffected lower extremity all resulted in negative images of the groin.

Representative images from Patients 1 and 2 are shown in Figures 1 and 2, respectively. In Patient 1 antibody accumulated in palpable nodes in the groin and also in a deep nodal group in the left pelvis just superior and lateral to the bladder, an area not previously suspected of disease.

A total of 138 lymph nodes were surgically excised and examined (Table 1). The six patients with metastases had a total of 28 positive nodes (average 4.7 nodes per patient and range 1–9, Table 1). In all positive nodes immunoperoxidase staining showed that the tumor expressed the antigens (HMW and p97) against which the specific antibody was directed (Table 1). Fifty normal nodes were removed from these six patients. In the five patients (7–11) without metastatic tumor a total of 60 nodes were removed and examined. Particular attention was paid to Patient 7, the one "falsely" positive scan. In this case the nodes were not enlarged and neither tumor cells nor shed antigen were identified on histological examination.

A summary of the results of gamma counting of the nodes is shown in Table 3. The amount of radioactivity in nodes varied greatly from essentially zero to values $>2,300 \times 10^{-3}\%$ dose/g of node. This can be attributed to variable lymphatic delivery of antibody to individual nodes within a fairly large drainage area. The concentrations of antibody in nodes were not related to node size. Because of the wide variation in node activity, statistical analysis of the concentrations of specific and control antibody in the nodes was performed using the method of Wilcoxon for groups of non-normally distributed data points (14). This showed that in the 28 tumored nodes in Patients 1–6 there was no significant difference between the concentrations of specific and control antibody ($p > 0.05$), while the concentration of both specific and control antibody in the 28 tumored nodes was significantly higher than in the 50 normal

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**FIGURE 1**
Anterior groin image of Patient 1, 3 hr after subcutaneous injection of $^{131}$I specific Fab. The lower arrow represents palpable groin nodes containing gross metastases. The upper arrow indicates intrapelvic nodes adjacent to bladder, also grossly positive but nonpalpable. Both node groups were surgically removed.
nodes removed from this group of patients (p < 0.01). Also in the 50 normal nodes from Patients 1–6 the concentration of specific versus control antibody was not significantly different (p > 0.05). Likewise, in the five patients in which 60 normal nodes were harvested, the concentrations in the nodes of specific versus control Fab were not different (p > 0.05).

Dual autoradiography of both specific and control Fab was done on 21 tumored nodes from Patients 1, 2, and 3. By visual inspection radioactivity was associated with gross tumor deposits within the node and at the surface of the node in the marginal lymphatic sinuses in juxtaposition with tumor. In individual nodes this pattern was the same for both the specific and control Fab. A representative dual isotope autoradiograph and histological section of a tumored node is shown in Figure 3.

In addition, several dual autoradiographs were analyzed using digitized videodensitometry. These results confirmed the nearly identical spatial distribution of specific and control antibody activity within the nodes. Such analyses are shown in Figures 4 and 5 which display this identity in both a linear profile mode and on a pixel basis for the total node over a matrix of 1,000 pixels.

DISCUSSION

These studies show that after s.c. administration of Fab antibody (molecular weight 50k daltons) ~60% of the dose was removed from the injection site after 20 hr and material was transported through lymphatics to regional lymph nodes. In any given patient there was great variation in the amount of Fab in individual nodes indicating the variable lymphatic delivery to nodes within a wide spread nodal group.

Lymph nodes with metastatic tumor were visualized with specific antibody but our data show that this was a nonspecific phenomenon. Post-surgical examination of nodes showed that tumored nodes retained increased amounts of both specific and control Fab to the same degree (Table 3). As well, dual isotope autoradiography of whole node sections showed essentially identical spatial distribution of the specific and control Fab within tumored nodes. The reason for such nonspecific

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Concentration of Specific (S) and Control (C) Antibody in Lymph Nodes—% Dose/G (×10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Six patients with metastases</strong></td>
<td><strong>Concentration of specific Fab</strong></td>
</tr>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Positive nodes</td>
<td>28</td>
</tr>
<tr>
<td>Negative nodes</td>
<td>50</td>
</tr>
<tr>
<td>Ratio specific Fab in tumored nodes/specific Fab in normal nodes</td>
<td>3.1</td>
</tr>
<tr>
<td>Ratio nonspecific Fab in tumored nodes/nonspecific Fab in normal nodes</td>
<td>2.2</td>
</tr>
</tbody>
</table>

| **Five patients without metastases** | **Concentration of specific Fab** | **Concentration of control Fab** |
| | No. | Mean | Range | ±s.d. | Mean | Range | ±s.d. | Ratio |
| Negative nodes | 60 | 128 | 0–1452 | 288 | 69 | 0–1549 | 217 | 1.8 | NS |

1 Standard deviation.
2 Ratios are those for the mean concentrations of antibody in the groups as shown in columns 3 and 6.
FIGURE 3
A: Whole mount histologic section from a groin lymph node from Patient 1. Tumor is present throughout the node, especially in the subcapsular regions. The arrow indicates a nodular area near one end (B). Iodine-131 specific antibody autoradiograph of an adjacent section of the same lymph node showing major antibody deposition in the subcapsular sinuses and in the nodular tumor mass. Other areas of darkening correspond to intra-nodal tumor. C: I251 autoradiograph of control antibody in the same tissue section showing a pattern of deposition identical to that of the I311 specific antibody.

FIGURE 4
Digitized images of the lymph node autoradiographs in Figure 3, showing the I311 image (specific Fab) in the upper portion of the photograph and the I251 image (control Fab) in the lower portion of the photograph. The corresponding linear profiles of relative activity are shown to the right of each image. The linear profiles represent a 5-pixel-wide slice through the center of each image, marked by the white line. Note the identity of the images and the linear profiles of activity for specific and control Fab.
uptake is unclear but a possible explanation would be that tumor growth within a lymph node stimulates local factors which cause removal of murine gamma globulin or Fab per se from the lymphatic fluid.

On the other hand imaging of nondiseased nodal areas was negative in eight of nine instances (Table 1), consistent with the finding of lesser amounts of antibody in normal nodes. Patient 7, who had a positive image, had an advanced 4-mm-thick primary melanoma. His nodes were normal sized, had an average uptake of $1.5 \times 10^{-5}$ % dose/g of specific antibody (compared with an average of $1.64 \times 10^{-3}$ for the 28 tumor nodes) and no tumor cells or shed antigen were seen. Histologically the nodes showed variable degrees of proliferation of phagocytic histiocytes in the interstitial spaces, a phenomenon sometimes seen in lymph nodes without metastases that are situated in the drainage pathway of melanoma and other cutaneous malignancies (15). Again, one might infer a nonspecific removal of murine immunoglobulin.

Our results showing nonspecific uptake of antibody in tumor nodes are in contrast to data reported by Weinstein and associates where metastatic hepatocarcinoma in guinea pig lymph nodes showed convincing localization of specific anti-tumor whole antibody when compared with that of an irrelevant control antibody (5). Previous human studies have been reported to show positive imaging of presumed metastatic disease in nodes using anti-CEA antibody, though tissue confirmation was lacking and control antibody was not co-administered (4). Positive nodal imaging also has been shown to occur after subcutaneous injection of radiolabeled anti-T-cell antibody in patients with cutaneous T-cell lymphoma (16). Again the possibility of nonspecific localization of antibody was not tested. In this setting the antigen is expressed on normal as well as malignant T-cells in the nodes and it is of some interest that predominantly enlarged diseased nodes appear to have been visualized.

These observations are preliminary and it is possible the specific localization is achievable using alternate strategies. For example, the tumor retention of specific antibody may be sufficiently long that imaging beyond 24 hr would be more specific. Perhaps nonspecific binding sites can be saturated by injecting large amounts of nonspecific antibody prior to giving specific antibody. Would it be better to use a smaller amount of antibody? For example, if one were concerned about saturating all the antigen binding sites on tumor cells from too high a dose it might be logical that a smaller dose, i.e., a subsaturating dose, could be beneficial since a larger fraction of the dose would adhere to tumor. On the other hand, from examination of the histologic sections and autoradiographs it is obvious that penetration of the antibody from the lymphatic channels into the tumor areas is highly incomplete and there may be equal argument for increasing the amount of antibody. Such studies of alternate strategies are currently underway in our laboratory.

NOTES

* Du Pont Company, No. Billerica, MA.
* Associates of Cape Cod. Woods Hole, MA.

REFERENCES