ABSTRACT

Monoclonal antibodies which bind to breast cancer have been used to evaluate the detection of metastatic disease in axillary lymph nodes. Three monoclonal antibodies (H59, H71, and H72) were reacted with tissue sections of primary tumors and axillary nodes from 24 mastectomy specimens and four specimens from glandular mastectomies for benign disease. All three antibodies had been shown to react with subsets of normal and malignant breast tissue; did not bind erythroid, myeloid, or lymphoid tissue; and recognized antigens in paraffin-embedded tissue. The antibodies recognized cell surface antigens, and H59 and H72 bound to glycoproteins which are either sloughed or secreted. Primary tumors and tumors in lymph nodes from the same specimen were always bound by the same antibodies. Antibodies detected unrecognized microscopic tumor in nodes from one previously node-negative specimen and two specimens with positive nodes. This suggests that monoclonal antibodies may be useful for detecting metastatic breast cancer in nodes which by microscopy are negative. Moderate binding of H59 and H72 antibodies to sinus histiocytes and perivascular cells was observed in all uninvolved nodes with sinus hyperplasia obtained from benign and malignant specimens. Thus, breast antigens can be identified in hyperplastic nodes in patients with no evidence of breast cancer. The antigens are detected predominately in the lymphoid sinuses and are bound to nonneoplastic cells. Therefore, breast antigens are regularly being processed and presented by normal lymphoid cells within the sinus. The binding of these monoclonal antibodies to axillary lymph nodes does not necessarily indicate the presence of metastatic disease. Dense binding to paraortical single cells was observed in tumor-containing lymph nodes and in uninvolved nodes obtained from mastectomy specimens with breast cancer. These cells are infrequent, and their number in an uninvolved node correlates with the pathological stage. They represent either binding to isolated lymphoid cells or metastatic tumor. Studies are under way to determine the origin of these cells.

INTRODUCTION

The most significant prognostic factor in breast cancer at the time of diagnosis is the extent of disease. In those patients with regional involvement, the number of nodes and the amount of metastatic disease in the node (microscopic versus macroscopic) correlate with the development of metastatic disease and survival (1–5). Patients without evidence of regional or distant metastasis have an excellent prognosis. However, 25% of patients without regional disease will eventually develop disseminated disease and die of breast cancer (2, 6, 7). To predict which patients with apparent local disease are at high risk for developing metastatic disease, many prognostic factors, including histological grade (5, 8, 9), intralymphatic extension (10–12), vascular invasion (5, 12), patient age and menopausal status (2, 13, 14), and estrogen and progesterone receptor levels (7, 15–18), have been evaluated. Other approaches have been to examine the regional lymph nodes in more detail by either reviewing more sections from each node (19, 20) or by using special techniques to find more nodes (21). These have been successful in detecting additional disease but rarely effect the pathological stage of the breast cancer. These results have led to an examination of the role of monoclonal antibodies in evaluating lymph nodes apparently free of tumor by microscopic studies.

The data reported herein are the results of binding 3 monoclonal antibodies (H59, H71, and H72) to fixed breast tissue and axillary lymph nodes obtained from mastectomy specimens. These antibodies bind to subpopulations of both benign and malignant breast tissue (22–24). At least one of these 3 antibodies will bind 85% of breast cancers and almost 100% of benign disease. The antigens recognized by the antibodies are localized to the cell surface; H59 and H72 antigens are either sloughed or secreted, and H71 is not secreted. The antibodies bind tissue sections which have been cryopreserved or paraffin embedded. The antibodies readily detect breast antigens in lymph nodes in the presence or absence of histologically identifiable tumor. The amount and location of the antigen appear to be associated with other prognostic factors. Therefore, monoclonal antibodies binding to negative axillary nodes may be a useful means of predicting which patients are at risk to develop recurrent disease.

MATERIALS AND METHODS

Monoclonal Antibodies. H59, H71, and H72 were selected from a series of murine monoclonal antibodies which bind breast cancer (22–24). The antibodies were produced by classical hybridoma techniques using the human hormone-dependent breast cancer cell line, ZR-75-1, as the source of antigen (22, 23). The antibodies bound subsets of breast cancer from 39 to 65% of all breast cancers studied (24). The 3 antibodies and their antigens have been partially characterized morphologically and biochemically. All 3 breast antibodies were IgM. The breast antibodies have no cross-reactivity, but all recognized differentiation antigens which were present on normal and malignant duct cells. The antigens bound to cell surface glycoproteins. H59 and H72 antigens have been detected on the apical surface of duct cells, the medium of cultured cells, breast cyst fluid, breast milk, and human sera (22–26). The antigens are either sloughed or secreted, whereas H71 antigen was diffusely located on the cell membrane and was not secreted. The antibodies reacted equally well with either cryopreserved or paraffin-embedded tissue. MTS, an IgM antibody secreted by a spontaneous mouse B-cell leukemia, which has no specific affinity for breast cancer cells, was used as an irrelevant antibody control for nonspecific binding (24).

Tissue Assay. Cryosections obtained from mastectomies performed at Baylor University Medical Center and Parkland Memorial Hospital were...
BREAST CANCER ANTIGENS IN LYMPH NODES

Patients 1 to 24 had modified radical mastectomies for breast cancer. The patients' prognostic factors evaluated were age and menopausal status (pre- and perimenopausal (pre), postmenopausal (post), and perimenopausal for 1 month of biopsy (P + H)). The specimen characteristics evaluated included tumor size (cm), number of axillary nodes detected at surgery (total nodes), number of nodes containing tumor (nodes positive), estrogen and progesterone receptor in fmol/mg of cytosol protein; the presence of lymphatic invasion in the primary tumor, histological grade, the presence of sinus histiocytosis, and the binding of monoclonal antibodies, H59, H71, and H72.

<table>
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<tr>
<th>Patient</th>
<th>Tumor size</th>
<th>Nodes positive</th>
<th>Total nodes</th>
<th>Age</th>
<th>Menopause</th>
<th>ER*</th>
<th>PR</th>
<th>Intralymphatic extension</th>
<th>Histological grade</th>
<th>Sinus histiocytosis</th>
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<td>11.1</td>
<td>+</td>
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</table>

*ER, estrogen receptor; PR, progesterone receptor; ND, not done.

RESULTS

Three monoclonal antibodies (H59, H71, and H72) which had been shown to bind to normal breast tissue and breast cancer and an irrelevant control antibody, MTS, were reacted with cryosections from breast biopsy specimens. Twenty-four cancer specimens were from 52 consecutive specimens (46%) from which cryosections bound at least 2 of the 3 breast antibodies. These 24 specimens and 4 mastectomy specimens with axillary nodal tissue available from patients who did not have cancer were selected for additional studies. The distribution of antibody binding with respect to nodal status, tumor size, histological grade, intralymphatic extension (none of the primary tumors studied had evidence of vascular invasion), sinus histiocytosis, patient age, and steroid hormone receptor is shown in Table 1. H59 bound 21, H71 bound 15, and H72 bound 21 of 24 primary breast tumor-containing specimens. All specimens were bound by at least 2 antibodies; 10 were bound by all 3 antibodies.

Tissue from the primary tumor and representative axillary nodes which had been formalin fixed and embedded in paraffin were reacted with the 3 breast antibodies and the irrelevant antibody. The results of a representative study of binding to a specimen which bound all 3 breast antibodies are shown in Figs. 1 to 4. The photomicrographs of the emulsion autoradiography demonstrated silver grains specifically overlaying the tumor tissue in the breast mass and an involved lymph node in sections reacted with all 3 breast antibodies; the control antibody demonstrated no specific binding (Figs. 1 and 2). Binding of breast antibodies was demonstrated in uninvolved nodes as well. Intense binding to rare single cells located in the paracortical region of lymph nodes was seen with the 3 breast antibodies (Fig. 3).

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fixed in 50% acetone-PBS (25 mM KCl-15 mM KH₂PO₄-80 mM Na₂HPO₄-137 mM NaCl) and reacted with the 3 breast antibodies and the irrelevant control antibody using a radioimmunoassay as previously described (22-24). Tumor specimens that were bound by at least 2 antibodies and from which axillary nodes were available were studied. In addition, breast tissue specimens with nodal tissue were obtained from 4 patients who had undergone total glandular mastectomies for each a high risk of developing breast cancer or severe fibrocystic disease. Paraffin-embedded tissue sections (6 to 8 µm) from the primary breast tumor, nodes containing tumor, and adjacent nodes which had no histological evidence of tumor were reacted with the 4 antibodies. The tissue obtained from areas of benign proliferation within the total glandular mastectomy specimen was reacted with the antibodies. At least 4 lymph nodes were studied from each mastectomy specimen. Tissue sections were obtained from only the lowest level of axillary nodes in specimens which had no evidence of axillary node tumor. When sinus histiocytosis was identified by the pathologist, sections were obtained from these nodes as well.

Tissues from the mastectomy specimens were preserved with 10% buffered formalin and embedded in paraffin. The paraffin was removed from the sections with xylene; the sections were subsequently incubated at 24°C for 5 min each in alcohol, methanol, 50% methanol-PBS, PBS, 50% acetone-PBS, and 4 washes of PBS. Tissues were air dried and either assayed directly or stored at -70°C. The sections were reacted with radiolabeled monoclonal antibodies (200,000 trichloroacetic acid-precipitable 125I cpm in 100 µl of 50% calf serum-PBS) and washed as previously described (21-23). Sections were dipped in photographic emulsion (NTB-2; Kodak), exposed for 96 h prior to development, and stained with hematoxylin-eosin (23, 26, 27). Sections were reviewed by 3 observers independently to identify the presence of tumor and the cells bound by the antibody and to quantitate the amount of antibody bound. Two sections from tissue block containing axillary nodes were reacted with each antibody.

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The abbreviation used is: PBS, phosphate-buffered saline.
Less intense binding of H59 and H72 antibodies was detected in areas of sinus hyperplasia (sinus histiocytosis) in nodes which did not contain tumor (Fig. 4), whereas H71 binding in these regions was not significantly greater than that observed with the irrelevant antibody. The binding of the antibodies in the sinuses was to mononuclear cells, which morphologically appear to be sinus histiocytes, and to free antigens in perivascular spaces (Fig. 4).

The distribution of antibody binding to the 24 breast cancer specimens and axillary nodes studied is shown in Table 2. For all specimens, the antibodies which bound the primary tumor bound the regional metastatic disease. In addition, micrometastatic tumor was detected with antibodies in one axillary node from a specimen which had been judged to be histologically free of disease. In 2 specimens with more than 3 nodes positive, an additional positive node was detected. These 3 nodes had tumor detected by antibody in all sections reacted with antibodies that bound to the primary tumor. Nine of 13 specimens which had no evidence of tumor metastatic to lymph nodes had binding to single cells located in the paracortical region of lymph nodes (Chart 1). In the node-negative patients where single cell binding could be detected (9 of 13), an average number of 1.9 ± 2.8

Table 2

<table>
<thead>
<tr>
<th>Axillary nodes positive</th>
<th>Specimens</th>
<th>Tumor</th>
<th>Sinus histiocytes</th>
<th>Perivascular</th>
<th>Single cells</th>
</tr>
</thead>
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<tr>
<td>0</td>
<td>13</td>
<td>-</td>
<td>6</td>
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<td>+</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
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</table>

Chart 1. The relationship of single cell binding to tumor size. Tissue sections containing uninvolved lymph nodes were reacted with the 4 monoclonal antibodies and processed according to "Materials and Methods." The number of intensely bound single cells was counted. The number of intensely binding single binding in uninvolved lymph nodes detected with antibodies which bound the primary tumor was screened and divided by the number of sections reviewed and the number of uninvolved lymph nodes observed. The average number of single cells bound per node per tissue section is plotted with respect to the size of the primary tumor.

Table 3

<table>
<thead>
<tr>
<th>No. of positive axillary nodes</th>
<th>Specimens with single cell binding</th>
<th>Single cells bound/uninvolved node</th>
<th>P value</th>
</tr>
</thead>
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<td>0</td>
<td></td>
<td>1.9 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>1-3</td>
<td></td>
<td>2.0 ± 1.5</td>
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</tr>
<tr>
<td>&gt;3</td>
<td></td>
<td>16.3 ± 2.49</td>
<td></td>
</tr>
</tbody>
</table>

Binding to single cells

Monoclonal antibodies were reacted with tissue sections of axillary lymph nodes and processed according to "Materials and Methods." Lymph nodes which did not contain tumor from specimens which did and did not have evidence of involved axillary nodes were evaluated for binding to individual paracortical cells (single cell binding as shown in Fig. 3). The data were segregated on the basis of: nodal involvement; primary tumor size; and pathological stage. The single cells bound were counted for all antibodies which reacted with the primary tumor and are normalized on the basis of lymph nodes evaluated and the number of tissue sections studied. Only those node-negative tumors (9 of 13) with evidence of single cells which bound the antibody are included in the calculations of cells per uninvolved lymph node. Similarly, 4 tumors were excluded from the calculations when specimens are segregated according to tumor size or stage. The P value was determined for the number of single cells bound by analysis of variance (29), and the brackets indicate which results were statistically compared.

(SE) cells per node was identified (Table 3). The single cells were detected only by those antibodies which bound to the primary tumor, and the numbers of single cells identified by antibody binding usually were not identical, since most sections were not serial but did not vary significantly when the different antibodies were compared statistically (29). Hence, the numbers of single cells detected were averaged per node for each tissue section reacted with the antibodies that bound the primary tumor. In all nodes which contained tumor where the nodal architecture was not completely effaced by tumor binding, intense binding to many individual paracortical cells was observed. Since the portion of the node which did not contain tumor in the involved nodes varied, it was impossible to quantify the number of single cells bound within involved lymph nodes. Single cell binding was observed in negative lymph nodes from specimens with histological evidence of nodal tumor; 2.0 ± 1.5 cells were bound per negative node studied from specimens with one to 3 axillary nodes containing breast cancer, and 16.3 ± 2.49 cells were bound in negative nodes from specimens with greater than 3 nodes containing tumors. Only those negative nodes observed in specimens which contained more than 3 positive nodes were statistically different by analysis of variance (P < 0.001) when the single cell binding was related to the tumor size (Chart 2; Table 3). There was no statistical difference between the small tumors and larger tumors studied (≤2 versus >2 cm). However, when the specimens are segregated according to pathological stage, the number of single cells bound per node to Stage I was 0.7 versus 5.1 per node for Stage II (P < 0.001).

Binding to sinus histiocytes and in a perivascular pattern was
observed in 6 of 13 specimens with no axillary node tumor and in 6 of 11 with axillary node involvement (Table 3). All specimens with sinus histiocytosis and binding to sinus histiocytes had perivascular binding as well. H59 and H72 bound to the sinus histiocytes, perivascular lymphoid cells, and antigens within ves-
sels, whereas H71 did not bind significantly above background (Fig. 4).

Antibody binding was studied in 4 total glandular mastectomy specimens in which axillary nodes were located (Table 4). None of these 4 breast specimens had evidence of cancer. The 3 antibodies bound fibrocystic disease and normal ducts in the breast tissue to varying degrees. Axillary nodes from 3 of 4 specimens had evidence of sinus histiocytosis. Binding of H59 and H72 antibodies to sinus histiocytes and perivascular regions was present to the same degree as in corresponding regions in

nodes obtained from 24 mastectomy specimens. These antibo-
dies were selected for study because they recognized both cell
surface (H59, H71, and H72) and secreted antigens (H59 and
H72) (23, 24). All 3 antibodies are IgM, and were selected
because of their ability to detect antigens in subsets of breast
cancer in cryopreserved and paraffin-embedded tissue (23, 24).

The specimens included in this study were selected from those
breast cancer specimens which had reacted with at least 2 of
the 3 monoclonal antibodies so that binding data could be
corroborated. Because the antibodies recognize differentia-
tion antigens and steroid-hormone receptor-positive tumors (23, 24),
and because of the requirement that at least 2 of the 3 antibodies
had to bind a tumor specimen, a selection bias has been intro-
duced toward better-differentiated tumors which contained es-
trogen and progesterone receptor. None of the primary tumors
had evidence of vascular invasion; few tumors had evidence of
intramythaphnic invasion, and none of the tumors was Stage III or
IV. The glandular mastectomy specimens from patients with
benign breast disease were selected on the basis of at least 3
axillary nodes having been identified by the pathologist. This
may have biased selection of these specimens in favor of those
with reactive lymph nodes.

The binding of antibody to an invasive primary breast tumor
was associated with binding to the metastatic tumor in the
regional lymph node (11 of 11 specimens). In one of 14 speci-
mens which were originally determined to have negative axillary
lymph nodes by the pathologist, micrometastatic involvement
of lymph nodes was detected by the antibodies. In 2 specimens
with histological evidence of nodal tumor, additional micrometa-
static nodal involvement was detected with the antibodies. Thus,
the antibodies detect micrometastatic tumor. These results are
consistent with that observed by Wells ef al. (30) using a milk fat
globule antigen to detect axillary node metastasis. Since increas-
ing the number of histological sections increases the likelihood
of discovering metastatic disease (17, 20), the detection of small
foci of tumor within nodes may in part have been associated
with increased sampling as at least 8 tissue sections were
required for each tissue block studied. However, the same
increased sampling failed to detect evidence of additional tumor
metastasis to lymph nodes in the 21 other specimens.

In addition to tumor in lymph nodes, binding of antibodies to
sinus histiocytes, to perivascular cells within the sinuses, and to
individual cells in the paracortical region of the lymph node was
observed. The binding to sinus histiocytes and perivascular cells
was seen only with H59 and H72, the 2 antibodies which bind
secreted antigens. The binding was always observed in hyper-
plastic lymph node sinuses. The degree of lymph node sinus
hyperplasia varied; only 7 of the 24 axillary node dissections
from the mastectomies for cancer had been reported initially as
sinus histiocytosis by the surgical pathologist at the time of
diagnosis. On review by our pathologists, 12 mastectomy spec-
imens had binding to sinus histiocytes in areas of sinus histio-
cytosis. In the 12 specimens which had significant binding to
sinus histiocytes, all had binding of H59 and/or H72 to antigen
localized to the region of the blood vessels within the sinuses.
Although it is possible that these nodes contained viable tumor
cells, these were undetected by either light microscopy or anti-
body binding in the 8 sections studied. Similar binding was
observed in the hyperplastic nodes from 3 of 4 total glandular
mastectomy specimens. The fourth glandular mastectomy spec-

DISCUSSION

Three monoclonal antibodies (H59, H71, and H72) which had
been shown to bind to both benign and malignant breast cells
have been reacted with primary breast masses and axillary lymph

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BREAST CANCER ANTIGENS IN LYMPH NODES

imen had no evidence of sinus hyperplasia and no binding. These 4 specimens were obtained from patients who underwent mastectomy for either a high risk of developing breast cancer or hyperplastic fibrocystic changes (31); they had no histological evidence of breast cancer in the mastectomy specimen. Since we have previously shown that the 3 breast antibodies do not bind normal or reactive lymphoid cells (22-24), these data demonstrate that breast antigens, particularly those which are secreted, frequently reach the axillary lymph nodes. The antigens were bound and presumably presented by lymphoid mononuclear cells. The amount of antibody bound indicates that the antigen present on the mononuclear cells is considerable (½ to ½ of the antibody bound by tumor). These antigens were only detected in areas of sinus hyperplasia and are presumably bound to sinus histiocytes. The presence of antigens bound to mononuclear cells indicates that these cells are reactive consistent with Hirschel et al. (32) and not banal histiocytes as suggested by Fisher et al. (33). The binding of antibodies to sinus histiocytes supports the thesis that sinus histiocytosis in axillary nodes of patients with breast diseases is at least in part due to the presence of breast antigens in the lymph node. Although it is controversial as to whether sinus histiocytosis is associated with a better prognosis in breast cancer (34, 35), the data presented suggest that the presence of breast antigens in nodes has stimulated the immune system, and subsequently, this may affect the development of distant metastasis. The binding of antibodies to the efferent vessels suggests that all the antigen in the lymph node is not bound and that some antigens are about to egress from the lymph node into the peripheral circulation. Since the binding to sinus histiocytes is detected in lymph nodes obtained from mastectomy specimens which did or did not contain primary breast cancer, these studies cannot distinguish as to whether the binding to sinus histiocytes reflects antigens being secreted by benign or malignant tissue. These events could only be discriminated by antigens which were both specific for breast cancer and secreted by the tumor.

These data suggest that breast monoclonal antibodies may be useful in delineating the relationship of the immune response to the prevention or the development of metastatic breast cancer. In addition, the presence of breast antigens in hyperplastic lymph nodes establishes 2 criteria which may be necessary for monoclonal antibodies to be used for immunoscintigraphy to detect cancer in axillary nodes: (a) the antibodies selected for these purposes should recognize breast antigens which are not secreted; and (b) the antigens should be found only on malignant breast cells. If antibodies not meeting these criteria were used and sinus hyperplasia were present, significant false-positive binding would be detected, depending on the amount of antigen bound to the sinus histiocytes, and, as a result, studies might be uninterpretable. Monoclonal antibodies not meeting these criteria would still be useful in detecting metastatic breast cancer, especially in patients who had had their primary tumor regional lymph nodes removed.

Single cells that are located among the small lymphocytes in the paraaortal region were bound by all 3 antibodies. These cells bound antibody so intensely that we have not been able to identify them morphologically. In subsequent studies utilizing less iodinated antibody and thinner sections, the degree of antibody binding to single cells decreased concordantly with the amount of antibody reacted, but the decreased binding of the antibody prevented the single cells from being identified morphologically. Similar studies using immunoperoxidase have also not been successful. It is our speculation that these individual cells may represent malignant breast cancer cells. These cells are not found in the nodes from patients who have had glandular mastectomy for benign disease. In 4 of 13 specimens without nodal disease, no intensely binding cells were detected in the paraaortal region. At least one of the uninvolved lymph nodes from all specimens with axillary node containing tumor had single cell binding. The number of cells per node which are bound by the antibodies correlated with the pathological stage of the tumor (P < 0.001).

An alternative explanation is that these cells which bind antibody intensely are of lymphoid lineage and are located in the paraaortal region of the lymph node. Given the large amount of antigen detected on their surface, this seems unlikely. The amount of antigen bound would require the cell to be one capable of antigen concentration. Studies which simultaneously use antibodies to lymphoid, macrophage, and breast tissue are necessary to identify the origin of these cells.

Recently, Redding et al. (36) have demonstrated the presence of isolated tumor cells that bind breast antigens within the bone marrow of patients with and without regional node involvement, and this binding appeared to correlate with tumor burden. These studies used a monoclonal antibody to milk fat globule. Considering the observations in the present study of large numbers of mononuclear cells within the lymph node sinus which were detected in both benign and malignant disease, the results of Redding et al. are subject to an alternative interpretation, i.e., some of the cell detected by the milk fat globule antibody may be lymph node-derived mononuclear cells with breast antigens bound to their surface which have migrated to the bone marrow, or even resident marrow mononuclear cells which have bound circulating breast antigens. Studies using lymphoid antigens and/or more specific breast cancer antigens might discriminate whether these circulating cells with breast antigens are tumor cells.

The data presented indicate that monoclonal antibodies can detect micrometastatic breast cancer. The antibodies are capable of detecting breast cancer antigens in lymph nodes which do not contain histological evidence of tumor. These studies suggest that the presence of single cells which bind antibody is associated with the pathological stage of the tumor, an important prognostic factor reflecting the tumor burden. Thus, the antibodies may be useful in detecting metastatic disease or predicting those patients who are more likely to develop metastatic disease in the future. They may also be useful in studying antigen presentation and the development of the immune response. However, since these antibodies are IgM, it is unlikely that they will be useful for immunolymphangiography and scintigraphy. Other antibodies directed against antigens which are not secreted will probably be more useful for imaging axillary lymph nodes.

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Fig. 1. Binding of monoclonal antibodies to primary breast cancer. Radiolabeled monoclonal antibodies which bind breast tissue (H59, H71, H72) and a control antibody (MTS) were reacted with a primary tumor from a representative mastectomy specimen according to “Materials and Methods.” The tissue had been formalin preserved and paraffin embedded. The photomicrographs are of the emulsion autoradiographs, and the silver grains represent the reaction with the following antibodies: H59 (a); H71 (b); H72 (c); MTS (d). × 630.
Fig. 2. Binding of monoclonal antibodies to nodal metastases of breast cancer. Four monoclonal antibodies were reacted with a lymph node which contained metastatic breast cancer from the same representative mastectomy specimen described in Figs. 1, 3, and 4. The photomicrographs demonstrate the reactivity with the following antibodies: H-50 (a, b); H-71 (c); H-72 (d); MTS-1 (e) x 630.
Fig. 3. Binding of monoclonal antibodies to individual cells in the paracortical region of an uninvolved lymph node. Four monoclonal antibodies were reacted with an auxiliary lymph node which had been determined to be free of tumor cells by histologic means. The lymph node was reacted as described in Fig. 2. The photomicrographs show the reactivity of the following antibodies to cells within the paracortical region of the lymph nodes: 165 (a), 167 (b), 167 (c); 167 (d), 167 (e), 167 (f). MTB 60 x, 630.
Fig. 4. Binding of monoclonal antibodies to skin biopsies from the uninvolved lymph node. The photographs were taken of the lymph node sections from the same lymph node shown in Fig. 4. a—normal peripheral blood lymphocytes; b, c, and d—liver, spleen, and lymph node, respectively.
Presence of Breast Cancer Antigens in Uninvolved Axillary Lymph Nodes

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