Cell-mediated Antitumor Immunity in Breast Cancer Patients Evaluated by Antigen-induced Leukocyte Adherence Inhibition in Test Tubes

N. Grosser and D. M. P. Thomson

The Montreal General Hospital Research Institute, Division of Clinical Immunology; Department of Medicine, McGill University, Montreal, Quebec, Canada

SUMMARY

Tumor antigen-induced inhibition of leukocyte adherence was adapted and modified for use in glass test tubes for the study of cell-mediated antitumor immunity to human adenocarcinoma of the breast. Peripheral blood leukocytes from 40 of 47 patients with proven breast cancer responded to an antigenic extract of breast cancer with significant leukocyte adherence inhibition, whereas only 2 of 32 controls showed a response. Further, 7 patients with histologically proven benign breast disease did not react. Moreover, in contrast to the results obtained by Halliday et al., they were unable to demonstrate immunological “blocking” with serum.

INTRODUCTION

Virtually all of the established methods for demonstrating cell-mediated antitumor immunity are tedious and suffer from complexity and lack of reproducibility. Recently, Halliday and Miller (10) developed a rapid assay of cell-mediated antitumor immunity called LAI (leukocyte adherence inhibition). This assay is based on the findings that nonsensitized leukocytes from both cancer patients and control subjects adhere to glass whereas leukocytes from cancer patients but not from control subjects, when mixed in vitro with antigenic extracts of tumors of the same histological type, undergo a diminution in their normal adherence to glass surfaces (9). The authors suggested that the LAI assay may have an immunological basis similar to the macrophage migration test (3) and lymphocytotoxicity assays (12).

Holan et al. (13) have described a modified version of the LAI assay in rats. In their system, the number of nonadherent cells in the presence of the tumor preparation is counted. Moreover, in contrast to the results obtained by Halliday et al., they were unable to demonstrate immunological “blocking” with serum.

In the studies described in this paper, we have adapted and modified the LAI procedure of Holan et al. (13) for the study of cell-mediated antitumor immunity to human adenocarcinoma of the breast. The present investigation was designed to determine whether this rapid and simple procedure was reliable for detecting the presence of cell-mediated antitumor immunity in a large number of breast cancer patients when compared to control subjects. Furthermore, the effect that tumor burden, surgery, and irradiation had on the phenomenon under consideration was examined.

MATERIALS AND METHODS

Subjects. Heparinized blood samples were obtained from 47 patients with adenocarcinoma of the breast. The group of 32 control subjects was composed of 7 patients with benign breast disease and 25 other patients suffering from a variety of nonmalignant diseases or cancers other than those of the breast. The diagnosis of all apparently benign breast lesions was also confirmed by histological examination of the surgical specimen.

Antigen-induced LAI in Test Tubes. The LAI assay of Holan et al. (13), originally described for rat cells, was modified for use in humans. A 20-ml sample of heparinized venous blood was placed in a glass universal bottle that was then incubated vertically at 37° for 1 hr. The resulting leukocyte-rich plasma fraction was aspirated and centrifuged at 200 × g for 5 min. The cell-free plasma was then removed and discarded. The cell button was suspended...
The resulting material was homogenized for 10 to 15 min in 5 volumes of PBS at 40,000 rpm in a VirTis 45 homogenizer. The homogenate was centrifuged at 20,000 \( \times g \) for 30 min and the supernatants were stored at \(-40^\circ\) in 2-mI aliquots. The protein concentrations of the stock extracts of breast cancer, malignant melanoma, and normal breast tissue were 7.3, 8.7, and 6.4 mg/ml, respectively. All tumor extracts were of similar protein concentrations.

Extracts of normal breast tissue, malignant melanomas, and all other tumors were prepared identically. For use in the assay, the concentrated stock extracts were thawed in a water bath at room temperature and an aliquot was diluted 1:4 with Medium 199. A 0.1-mI aliquot of the diluted stock extract was added to certain designated tubes containing the standard quantity of \(10^7\) PBL in 0.1 mI of Medium 199. The optimum tumor antigen concentration was established for the breast tumor extract and control extracts by determining the concentration that produced the most specific and least nonspecific inhibition of leukocyte adherence (Table 3). In this study, the breast cancer and control extracts were used at a 1:4 dilution.

Blocking of the LAI Phenomenon. Blood was taken from both patients with breast cancer and control subjects and was immediately stored at \(4^\circ\). After overnight retraction of the clot, the serum was separated and stored at \(-40^\circ\). Before use the serum was diluted 1:1 with Medium 199. In the LAI assay, equal volumes of 0.1 mI of the antigen preparation, leukocyte suspensions, and serum were mixed. Medium 199 was added to bring the final volume to 0.5 ml. After mixing and incubation, the number of nonadherent cells was counted, and the NAI was calculated for both the breast cancer patients and the control subjects.

LAI Assay of Breast Tumor Extract Chromatographed on Sepharose 4B. The breast tumor extract was concentrated by Diaflo ultrafiltration through Amicon PM 10 membranes (Amicon Corp., Hartwell, Mass.). Protein determinations were performed by the method of Lowry et al. (15).

An aliquot of tumor extract was dialyzed against the column buffer for 72 hr, and there was no loss of activity when the extract was retested in the LAI assay. A total of 500 mg of tumor extract in 15 ml was applied to a Sepharose 4B column (5 \( \times \) 90 cm) and was eluted by upward flow at 40 ml/hr with 0.05 m NaH\(_2\)PO\(_4\)-0.15 m NaCl, pH 5.0, and collected in 20-ml fractions. The collected fractions were then pooled selectively (see Chart 5), dialyzed against PBS, pH 7.3, and concentrated with Aquacide 11 (Calbiochem, La Jolla, Calif.) and ultrafiltration to 10 ml.

The 4 pools, and an aliquot of the original material applied to the column, were then tested for activity by the LAI test. In addition, the whole breast tumor extract was treated with 1.0 m perchloric acid. The supernatant and precipitate were extensively dialyzed against cold water and then PBS, pH 7.3. The perchloric acid extract of breast tumor was then tested by LAI.

RESULTS

Clinical Features of LAI Assay in Test Tubes in Breast Cancer. When PBL from breast cancer patients or control subjects were incubated in glass test tubes without antigen for 2 hr, about 10% of the cells were nonadherent and this percentage did not change with prolongation of the incubation period. The addition of either tumor extract or normal breast tissue extract to nonsensitized PBL inhibited adherence of 15 to 40% of the leukocytes. Incubation with breast tumor extract of PBL from patients with breast cancer caused a 40 to 65% LAI, whereas incubation of the same...
cells with normal breast tissue, melanoma extract, or other unrelated tumor extracts produced a 15 to 40% LAI. Table 1 illustrates typical data obtained when the PBL of 4 breast cancer patients and 4 control subjects were assayed.

LAI was similar whether the PBL of patients with breast cancer were exposed to different breast tumor extracts or to their own breast tumor extract (Table 2). It is possible, however, to demonstrate only a common reaction pattern due to the fact that each tumor extract has a variable amount of cancerous tissue and the patients could be tested only with an extract of their own tumor after surgery. The presence of tumor-specific transplantation antigens in individual tumor extracts cannot be excluded.

To achieve the optimum assay conditions, that is, the maximum specific inhibition of leukocyte adherence with the least nonspecific inhibition, the antigen extracts were tested at different concentrations with leukocytes from patients with malignant melanoma, patients with breast cancer, and control subjects (Table 3). As shown, the NAI falls with increasing dilutions. In this instance, however, a dilution of 1:4 of the breast cancer extract and melanoma extract gave a high NAI value for the patient with breast cancer and a low NAI to breast cancer for both the control subject and the patient with malignant melanoma. Hence, all subsequent assays were performed with all extracts diluted 1:4.

Results in 47 patients with adenocarcinoma of the breast and 32 control donors are shown in Charts 1 and 2. Significant LAI occurred with the breast cancer extracts with PBL from 40 of the 47 breast cancer patients. Twenty-three breast cancer patients showed a mean NAI of 58 to the breast cancer extract when the nonspecific control antigen was an extract of malignant melanoma (Chart 1). Another group of 24 breast cancer patients had a mean NAI of 189 to the breast cancer extract when the nonspecific antigen was an extract of normal breast tissue (Chart 2). The calculated NAI's for the breast cancer patients with melanoma as the nonspecific antigen, as opposed to those

### Table 1

| Patient | Diagnosis   | Breast tumor antigen | Melanoma antigen | No antigen | NAI
|---------|-------------|----------------------|------------------|------------|-----
| M. M.   | Breast cancer | 48 ± 3.5             | 26 ± 5.0         | 8 ± 2.0     | 84  |
| B. T.   | Breast cancer | 50 ± 3.0             | 31 ± 3.0         | 11 ± 1.5    | 61  |
| C. D.   | Breast cancer | 40 ± 2.8             | 22 ± 5.2         | 3 ± 1.0     | 82  |
| E. S.   | Breast cancer | 56 ± 4.5             | 36 ± 4.3         | 16 ± 2.5    | 56  |
| V. T.   | Fibroadenoma of breast | 26 ± 3.5             | 28 ± 3.5         | 10 ± 2.5    | 96  |
| R. M.   | Fibrocystic breast disease | 40 ± 3.5             | 35 ± 5.0         | 5 ± 2.1     | 13  |
| S. S.   | Melanoma     | 35 ± 6.0             | 70 ± 4.5         | 10 ± 2.8    | 50  |
| H. A.   | Melanoma     | 34 ± 4.0             | 63 ± 4.5         | 8 ± 1.8     | 44  |

* NAI using breast cancer antigen as specific antigen and melanoma as nonspecific antigen.

\[ \text{NAI} = \frac{\% \text{ nonadherent cells in presence of breast cancer} - \% \text{ nonadherent cells in presence of unrelated antigen}}{\% \text{ nonadherent cells in presence of unrelated antigen}} \times 100 \]

### Table 2

| Diagnosis of breast cancer patients | NAI to breast tumor extract
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A. S.</td>
<td>300 143</td>
</tr>
<tr>
<td>S. T.</td>
<td>50 48</td>
</tr>
<tr>
<td>C. B.</td>
<td>81 42</td>
</tr>
<tr>
<td>R. G.</td>
<td>65 52</td>
</tr>
<tr>
<td>N. T.</td>
<td>83 69</td>
</tr>
<tr>
<td>M. G.</td>
<td>73 78</td>
</tr>
<tr>
<td>C. T.</td>
<td>60 54 73</td>
</tr>
</tbody>
</table>

* Patients were tested against the various breast cancer extracts on separate days.

* NAI calculated using breast cancer antigen as specific antigen and melanoma as unrelated antigen.

### Table 3

| Diagnosis of leukocyte donor | Dilution of antigens | NAI to breast cancer
|-----------------------------|----------------------|---------------------
| Cancer of breast            | 1:4                  | 300                 |
| Melanoma                    | 1:4                  | -35                 |
| Cholelithiasis              | 1:4                  | -30                 |

* NAI using breast cancer antigen as specific antigen and melanoma as nonspecific antigen.
for normal breast tissue, showed significantly lower values (Charts 1 and 2). This occurred due to the greater inhibition of glass adherence produced by the melanoma extract on the leukocytes from breast cancer patients. Other tumor extracts have been used as the nonspecific antigens. The results have been similar to those obtained with the melanoma extract as the nonspecific control. Moreover, no evidence of cross-reaction has been observed between breast cancer antigen and tumor extracts of bowel, ovary, bladder, and epidermoid lung cancer (Table 4).

As shown in Chart 3 there was a striking difference in the NAI between breast cancer patients who had clinically localized disease to either the breast alone (Stage I) or the breast and lymph nodes (Stage II) and individuals with spread to multiple organs (Stage IV). The mean NAI of Stage I and Stage II breast cancer patients was 156 and 134, respectively, and this was significantly different from the mean NAI of 53 for the group of patients with disseminated breast cancer ($p < 0.01$).

A NAI value of greater than 18 was considered significant when the control extract was malignant melanoma on the basis that this value appeared best to separate the breast cancer population from the control subjects. Similarly, a NAI of greater than 60 was considered significant when normal breast tissue was used as the control extract. Six breast cancer patients showed a NAI below the cut-off level of 18 when the melanoma extract was used as a control (Chart 1). Three of the 6 patients had disseminated disease. One patient had axillary node involvement but no clinical evidence of metastasis and the other 2 patients had small breast lumps which, histologically, showed invasive adenocarcinoma. When normal breast tissue was used as the nonspecific control antigen, only 1 breast cancer patient had a NAI below the level of 60 (Chart 2). This patient had disseminated cancer. Thus 4 of the 7 patients with a NAI below the selected cut-off levels had disseminated breast cancer.

The group of 32 controls had no evidence of breast cancer although 7 subjects had benign breast disease. The controls
Detection of Breast Cancer by LAI in Test Tubes

Within the subsequent 8 weeks, both the surgery and irradiated patients have, in general, shown a return of their LAI responsiveness (Table 5).

Effect of Adding Serum. A series of experiments were performed in order to determine the effect that serum from the cancer patient had on the LAI assay. A representative experiment is shown in Table 6. It was found that serum alone in the absence of antigen caused a moderate degree of nonspecific inhibition of leukocyte adherence, and this was related to the serum concentration. As shown in Table 6, no specific abolition of LAI by the serum of breast cancer patients was found. In fact, no “blocking” of LAI was observed in 6 separate experiments with 6 different cancer patients and their respective sera.

failed to demonstrate breast tumor antigen-induced inhibition of leukocyte glass adherence. When the nonspecific control antigen was an extract of malignant melanoma, the controls had a mean NAI of -18 to the breast cancer extract. The controls had a mean NAI of 30 to the breast cancer extract when the nonspecific antigen was normal breast tissue. The mean NAI of the breast cancer patients was significantly different from the corresponding control group (p < 0.001). As shown in Chart 2, 2 of the 32 controls had a NAI above 60. These patients were not retested because they were studied early in the investigation and calculation of the NAI was not done until the end of the study. However, they had no evidence of breast cancer.

Within the control group were 7 patients with histologically proven benign breast disease. These patients showed a mean NAI similar to the other controls, and no individual patient demonstrated a high NAI to the breast cancer antigen (Charts 1 and 2).

PBL from 8 patients with breast cancer were studied in the LAI assay before and after surgery and/or irradiation. All patients showed a significant NAI to breast cancer antigen before any therapy was given. Four patients with a primary tumor had a mastectomy, and 7 to 14 days after surgery their leukocyte response was reexamined in the LAI assay. A marked depression in leukocyte responsiveness was observed (Chart 4). Similarly, 4 patients presenting for the first time with recurrent breast cancer had local irradiation therapy to the area of recurrence. The patients were retested 14 days after the commencement of irradiation therapy. As shown in Chart 4, irradiation therapy resulted in a dramatic decrease in their leukocyte responsiveness in the LAI assay.

Table 5
NAI of leukocytes of breast cancer patients within 8 weeks of therapy

<table>
<thead>
<tr>
<th>Breast cancer patients</th>
<th>Pre-surgery</th>
<th>After surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-2 wk</td>
<td>4-8 wk</td>
</tr>
<tr>
<td>S. C.</td>
<td>211</td>
<td>24</td>
</tr>
<tr>
<td>R. T.</td>
<td>186</td>
<td>13</td>
</tr>
<tr>
<td>G. K.</td>
<td>60</td>
<td>-5</td>
</tr>
<tr>
<td>Post irradiation, 7 days</td>
<td>Post irradiation, 14 days</td>
<td>Postadrenal-ectomy</td>
</tr>
<tr>
<td>A. S.</td>
<td>300</td>
<td>-13</td>
</tr>
</tbody>
</table>
Mediation of Antigen-induced LAI in Test Tubes. In the LAI assay in test tubes, $10^6$ cells were plated and only $10^6$ of the cells were nonadherent in medium alone. In the presence of a nonspecific antigen approximately $2.5 \times 10^6$ leukocytes from breast cancer patients were nonadherent. By contrast, the presence of breast cancer antigen resulted in 4 to $6.5 \times 10^6$ leukocytes from the sensitized breast cancer patient becoming nonadherent. The difference between nonspecific and specific nonadherent cells indicates that $1.5$ to $3.5 \times 10^6$ leukocytes of every $10^6$ cells of the breast cancer patient respond specifically to the breast cancer antigen. This represents a considerable number of responding or sensitized cells and for this reason experiments were carried out to determine whether mediators played a role in the observed response.

In the 1st experiment, leukocytes from patients with breast cancer or from normal subjects were incubated with breast tumor extract for 1 hr, and the resultant supernatants were collected and incubated with leukocytes from normal control subjects. Table 7 shows that the supernatant did not affect the adherence of PBL to glass.

In the 2nd experiment, LAI was tested where different proportions of leukocytes from the breast cancer patient were replaced by leukocytes from a normal control subject. As shown in Table 8, replacement of cancer patient's leukocytes by normal leukocytes produced a fall in the NAI that was roughly proportional to the percentage of the cancer patient's cells replaced.

The effect of late addition of antigen to preincubated leukocytes was examined in a final series of experiments. When antigen was added to leukocytes that had been preincubated in the glass tubes for 1 hr, it was found that the resultant percentage of nonadherent cells was low. Moreover, preincubated sensitized leukocytes showed no significant difference in nonadherence with the late addition of specific or control antigens.

Nature of the Antigen from Breast Tumor Extract Reactive in the LAI Assay. A PBS extract of breast tumor was
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Chromatographed on Sepharose 4B. The chromatographic fractions with antigenic activity were determined by LAI. The chromatographic profile obtained and the fractions that were pooled are shown in Chart 5. Pool 1, a fraction in the void volume of the Sepharose 4B column, contained all the antigenic activity (Table 9).

Solubility of the antigenic component in 1.0 M perchloric acid was examined. It was found that after treatment of the breast tumor extract with perchloric acid no antigenic activity remained as measured by LAI.

DISCUSSION

The results of the present study indicate that the LAI assay, modified for use in glass test tubes, is a simple and quantitative method for measuring cell-mediated immunity to tumor antigens in patients. The LAI assay for breast cancer appears to be immunologically specific and reproducible. PBL of 40 of 47 patients with proven breast cancer responded to an antigenic extract of breast cancer with significant LAI, whereas only 2 of 32 controls showed a response. Cell-mediated immunity to the breast tumor antigen as measured by LAI was shown to be depressed by a large tumor load, surgery, and irradiation therapy. A series of experiments suggests that the responding cells interact directly with the antigen and that as a result subsequent adherence to glass is inhibited. No evidence was obtained to indicate that the leukocyte nonadherence was produced secondarily by lymphokines.

The clear difference between patients with breast cancer and control donors with other cancers in the response of LAI to breast cancer antigen strongly suggests that the LAI assay detects a tumor antigen of breast cancer. Conversely, the patients with breast cancer showed no response to unrelated tumor extracts, including those of malignant melanoma and bowel, lung, ovarian, and bladder cancers. Moreover, the demonstration that breast cancer patients react to both allogeneic and autologous breast cancer extracts indicates that adenocarcinomas of the breast share a common tumor antigen. Furthermore, the lack of response to the normal breast tissue extract tends to rule out the possibility that a cell-mediated response to a normal organ-specific antigen was observed. Further, the 7 patients with histologically proven benign breast disease did not react to the breast adenocarcinoma extract, indicating that only breast cancer patients have leukocytes sensitized to the breast cancer antigen(s). These results agree with earlier reports of tumor-associated antigens on human breast carcinomas by migration inhibition assays (2, 6, 17, 19), microcytotoxicity assays with short-term cultured breast carcinoma cells (8, 12), and breast carcinoma patient skin tests (1, 14, 20). Whereas most studies have reported a common tumor-associated antigen, Segall et al. (19) found no cross-reactions among extracts of different breast carcinomas.

Chromatographic fractionation on Sepharose 4B of the PBS breast tumor extract showed that the antigenic component was greater than 10^6 daltons.

The cell-mediated antitumor immune response of the breast cancer patient was observed to be dependent upon the stage of the cancer. Patients with cancer confined to the breast, with or without the draining local nodes alone, demonstrated strong reactions. In contrast, patients with widely disseminated breast cancer, as a group, showed decreased responsiveness in the LAI assay. In addition, 4 of the 7 breast cancer patients who had no response in the LAI assay had disseminated breast cancer.

The depression of cell-mediated antitumor immunity with a large tumor burden has been observed in experimental animal models and in human tumors. Earlier, Cochrane et al. (5) observed less frequent migration inhibition reactions with leukocytes from patients who had visceral metastasis in breast cancer. In contrast, the papers of Halliday et al. (9) and Malvish and Halliday (16) have not reported any depression of LAI in the cancer patients studied. Our LAI assay appears to measure 1 aspect of the effector arm of the immune response to tumor. Hence, a decrease in this reaction in patients with a large tumor burden may be viewed as an indication of an impaired antitumor immune response.
response. One explanation for the observed depression of host-tumor immunity with increasing tumor burden is an abrogation of the effector arm of the tumor immune response by the release of excess soluble tumor antigen systemically (21–23).

In studies of the breast cancer patients, the LAI reactivity was observed to be greatly depressed within 14 days after surgery and/or irradiation. Most patients demonstrated an impaired LAI response from 7 to 14 days after surgery and after the 1st week of irradiation therapy. The mechanism of the LAI depression is not known. It is possible that the observed depression in cell-mediated tumor immunity results from a decrease in the percentage of circulating cells reactive to the breast cancer antigen or in their functional capability. With radiotherapy, a marked lymphopenia is produced, but even the cells present in the circulation do not respond actively in the LAI assay. Depression of tumor-specific and general cell-mediated immunity in breast cancer patients after surgery and/or irradiation, respectively, has been described (6, 7). It appears that these forms of therapy may be relevant to the survival of the last few breast cancer cells and/or their dissemination after operation or irradiation therapy.

O'Toole et al. (18) and Unsgaard and O'Toole (24) studied cell-mediated immune response to carcinoma of the urinary bladder and skin melanoma in man, respectively. They found that therapy influenced the microcytotoxicity assay for cell-mediated immunity by causing alterations in the amount of tumor material in the body. Hence, they observed that removal of tumors by surgery resulted in a loss of detectable cell-mediated immunity while recurrence of tumor after surgery resulted in the reappearance of cytotoxicity. It has not yet been determined whether tumor-free breast cancer patients show a progressive fall in their LAI reactivity in the year after surgery or if recurrence of tumor enhances LAI reactivity. If the LAI in test tube assay demonstrated significant reactivity only in the presence of recurrent tumor, it might be informative as to the presence or absence of tumor.

"Blocking" factors in the serum of tumor-bearers have been demonstrated and extensively studied in the lymphocyte cytotoxicity assay of Hellström et al. (12). Moreover, Halliday et al. (9) have demonstrated, in the serum of tumor-bearers, tumor-specific "blocking" factors by LAI. In our modified version of LAI, "blocking" was not demonstrable. Similarly, Holan et al. (13) have previously reported that the "blocking" effect was not obtained with a similar assay system in rats. The explanation for this difference is not clear but may be technical.

In this assay system, only 10% of PBL did not adhere to glass in a protein-free medium. The addition of unrelated tumor extract or serum of both control subjects and patients non-specifically inhibited the adherence of approximately 25% of PBL. The non-specific inhibition of adherence appears to be the result of protein coating of the glass surface which interferes with attachment by some of the leukocytes. However, approximately $10^4$ to $3.5 \times 10^4$ of the $10^8$ PBL of a breast cancer patient plated respond specifically to the breast tumor antigen by nonadherence.

Experiments were undertaken to characterize partially the mechanism by which LAI is mediated. The results suggest that the tumor antigen, upon interacting with sensitized peripheral blood cells, apparently produces cell surface alterations that inhibit attachment of the cells to glass. Once the leukocytes have adhered to glass, addition of specific antigen does not sufficiently alter their cellular membrane to cause detachment. Furthermore, no evidence was obtained to suggest that a factor analogous to the migration inhibition factor or to other lymphokines was released during the interaction of sensitized PBL with the antigen to cause inhibition of leukocyte adherence.

The LAI assay described in this paper is a comparatively simple and sensitive technique for demonstrating cell-mediated antitumor immunity. The ease and rapidity of performing the assay should make it useful in routinely monitoring the antitumor cellular immune response. Since LAI measures the strength of one aspect of the effector arm of the tumor-immune response of the host, it needs to be determined whether the intensity of the reaction reflects on the eventual outcome of the patient’s clinical course. Similarly, the significance of a therapeutically induced decrease in reactivity must be clarified. Two patients with small, locally invasive adenocarcinomas of the breast showed no response in the LAI assay. It appears, then, that a certain mass of tumor is required before systemic sensitization is detectable which is similar to the findings in experimental animal tumor models (21). Studies are now in progress to determine at what stage in tumor evolution systemic sensitization occurs. If sensitization occurs early, the assay could be an additional aid for screening high-risk individuals. If this is not the case, then the assay may be of assistance to the clinician in differentiating benign from malignant palpable breast lumps.

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