Peripheral Leukocyte Migration Inhibition Reactivity to Breast Cancer Antigens in Patients with Breast Cancer and in Normal Controls

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SUMMARY

The peripheral leukocyte migration inhibition test has been used to assess cellular immunity to soluble antigen extracts of breast cancer in patients and normal controls. In sequential tests over several weeks, 23 of 23 patients with breast cancer in remission reacted intermittently, with 67 of 139 tests (48%) being positive (>20% migration inhibition). Similarly, 6 of 10 patients in relapse reacted intermittently showing 16 of 61 positive tests (26%) and 126 of 129 normal females reacted intermittently showing 135 of 512 positive tests (26%). The mean percentage of migration inhibition for all tests in patients in remission was 16.4 ± 1.2% and that for normal controls was 7.2 ± 0.7%; this difference was highly significant (p < 0.001). The value for all tests in patients in relapse was 11.8 ± 1.4%; this was statistically lower than that for patients in remission (p < 0.05) but statistically higher than that for normal controls (p < 0.05). A few normal women, some with high risk factors such as a strong family history and/or fibrocystic and proliferative disease, had a mean percentage of migration inhibition value in the range of that for patients with breast cancer. Mean values of sequential tests may be a more meaningful index of cellular immunity against breast cancer antigen in all groups.

INTRODUCTION

The PLMI assay has been used by many investigators as an in vitro test of cellular immunity to a variety of soluble antigens (12,13) as well as to extracts of tumors such as melanoma (5), nasopharyngeal carcinoma (3), and breast cancer (2,4,6). We have recently reported a critical evaluation of the PLMI test as an index of delayed cutaneous hypersensitivity using PPD as a purified antigen (10). Variation of PLMI reactivity occurred in all subjects irrespective of skin test status. However, Mantoux-positive subjects, both as a group and in most cases individually, demonstrated greater reactivity than Mantoux-negative subjects. The conclusions reached from this study suggested that PLMI does in fact detect cellular immunity in a well-defined system, but that the variation in reactivity that occurred from week to week meant that sequential testing rather than a single determination was essential if the test was to provide a meaningful index of cellular immunity. In this report, which is an extension of the work done using PPD, sequential testing of leukocytes of breast cancer patients and normal female controls was carried out against normal and malignant breast tissue antigen extracts. The results of these tests indicate that: (a) single tests are not as reliable as sequential tests in detecting cellular immunity to breast cancer; (b) variation in reactivity occurs both in patients with breast cancer in remission or relapse and in normal subjects; (c) the mean percentage of migration inhibition observed in sequential tests in breast cancer patients as a group is statistically higher than that seen in a group of normal females; and (d) some normal women have a mean migration inhibition score on sequential tests in the range of that observed in breast cancer patients.

MATERIALS AND METHODS

Twenty-three patients with breast cancer in apparent remission from 6 months to 10 years after surgery, 10 patients in relapse with widespread disease, and 129 normal female controls were studied. The patients were volunteer members of a mastectomy group and/or patients treated through the facilities of the Manitoba Cancer Treatment and Research Foundation. Female control subjects were nurses, technicians, and support staff of the Foundation as well as some outside volunteers.

Methodology concerning antigen preparation and the PLMI assay have been reported previously (4). Patients and normal controls were tested at weekly intervals against 0.7% NaCl extracts of breast cancer antigens. A few subjects were also tested at more frequent intervals, sometimes both morning and afternoon 3 to 5 days a week against similar antigens. A total of 16 antigens were obtained from tumor specimens that were all histologically diagnosed as infiltrating duct adenocarcinoma; they have been designated BCA-3 through BCA-18. Protein concentration of the tumor extracts was determined by the method of Lowry et al. (8), and dose-response curves for each extract were obtained on patients and normals to determine antigen concentrations required to effect statistically significant migration inhibition. Parallel experiments determined that...
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centers of each antigen effect the same degree of inhibition in several subjects. For example, 100 μg of BCA-11 antigen per ml were determined to be equivalent to 250 μg of BCA-14 antigen per ml in its ability to inhibit migration. Control antigens were prepared in the same manner from specimens of fibroadenoma, benign cystosarcoma phylloides, normal breast tissue obtained from a reduction mammoplasty (NBA-1), and macroscopically normal tissue (NBA-2) from an area adjacent to the breast cancer that was used to prepare the BCA-5 antigen. Dose-response studies using concentrations of 50 to 360 μg/ml of these benign antigens failed to demonstrate significant inhibition of leucocyte migration of either the patients or normal controls. For sequential testing, the protein concentration of the benign breast tissue antigen was always adjusted to the same level used with breast cancer antigen.

The percentage of migration inhibition of replicate tests was calculated by the formula:

\[
100 - \left( \frac{\text{area of migration in presence of antigen}}{\text{area of migration in absence of antigen}} \right) \times 100
\]

While preliminary tests were done in duplicate, many of them in a nonblind fashion, subsequent tests were done in quadruplicate in a blind fashion. The overall results of mean migration inhibition were tabulated by averaging the migration inhibition observed with the sequential tests and were analyzed using Student’s 2-tailed t test.

RESULTS

If migration inhibition equal to or greater than 20% was used as a positive result, then 23 of 23 breast cancer patients in apparent complete remission were intermittently reactive, with 67 of 139 tests (48%) being positive (Table 1). Using a similar criterion, 6 of 10 breast cancer patients in relapse were intermittently reactive, with a total of 16 of 61 tests (26%) being positive, and in sequential tests of 129 normal females, 135 out of 512 tests (26%) were positive; only 3 controls were consistently unreactive by this criterion.

The use of statistical analysis of results (p < 0.05 in Student’s 2-tailed t test) rather than an arbitrary value of ≥20% migration inhibition as a criterion of positivity, greatly increased the percentage of positive tests in all groups. A comparison of these 2 criteria of positivity in 131 quadruplicate tests in 20 normal controls shows that 30 of 131 tests (23%) were positive using the arbitrary value, while 74 of 131 tests (57%) were positive using statistical analysis. Similarly, in 40 quadruplicate tests in 5 patients with breast cancer in remission, 19 (48%) were positive using the arbitrary value, while 32 (80%) were positive using statistical analysis; in 29 quadruplicate tests in 7 patients in relapse, 6 (21%) were positive using the arbitrary value, while 17 (59%) were positive using statistical analysis.

The migration inhibition (mean ± S.E.) in all tests in the 29 patients in apparent remission was 16.4 ± 1.2% (Table 1) and that for normal controls was 7.2 ± 0.7% and this difference was highly significant (p < 0.001). The mean migration inhibition in all tests for the 10 patients in relapse was 11.8 ± 1.4%, and this value was statistically different from both the patients with breast cancer in remission (p < 0.05) and from the normal controls (p < 0.05).

In contrast to the reactivity seen against breast cancer antigens, significant reactivity was not observed against any of the 4 benign breast tissue antigens in either normals or patients. In 16 patients in remission, the mean migration inhibition in 85 sequential tests against cystosarcoma phylloides antigen was 2.3 ± 6.0% and in 98 concurrent tests against BCA-8 it was 18.1 ± 1.5%; this difference was highly significant (p < 0.001). Similarly, significant reactivity against any of the benign antigens was not observed in normal controls tested concurrently to breast cancer antigen; the mean migration inhibition in 40 tests against fibroadenoma was 0.8 ± 1.7%, in 52 tests against NBA-1 it

Table 1

<table>
<thead>
<tr>
<th>Disease status</th>
<th>No. of subjects</th>
<th>No. of tests</th>
<th>No. of positive tests*</th>
<th>Migration inhibition (%) for all tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer (remission)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>9</td>
<td>57</td>
<td>29</td>
<td>14.3 ± 2.0*</td>
</tr>
<tr>
<td>Stage II</td>
<td>14</td>
<td>82</td>
<td>38</td>
<td>17.5 ± 1.3*</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>139</td>
<td>67 (48)*</td>
<td>16.4 ± 1.2*</td>
</tr>
<tr>
<td>Breast cancer (relapse)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>61</td>
<td>16 (26)</td>
<td>11.8 ± 1.4*</td>
</tr>
<tr>
<td>Normal female controls</td>
<td>129</td>
<td>512</td>
<td>135 (26)</td>
<td>7.2 ± 0.7*</td>
</tr>
</tbody>
</table>

* Positive leukocyte migration inhibition is defined as inhibition of migration ≥20% of that observed in control chambers not containing antigen.

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was 2.1 ± 1.4%, and in 85 tests against NBA-2 it was −6.8 ± 2.0%.

To elucidate the variation in reactivity, 7 individuals were studied over a 2- to 4-week period during which time blood was taken on at least 3 occasions per week, and often morning and afternoon blood samples were obtained on the same day. The subjects included 5 patients in remission with breast cancer and 2 normal female subjects who had previously shown occasional reactivity to breast cancer antigen on the weekly sequential study. Variation in reactivity was again observed in all those tested. A total of 23 morning and afternoon samples was obtained on the 7 subjects. Statistical analysis revealed that 16 of the 23 morning and afternoon samples were not significantly different from each other while 7 were significantly different (p < 0.05). Overall paired t test analysis of the 23 morning versus afternoon tests showed no significant difference.

Following tabulation of the data, the charts of the 23 patients in remission were reviewed and evaluated with respect to pathological staging and to overall disease status. Thirteen of the remission patients were found to have had pathological Stage II (node-positive) disease at the time of surgery. All received postoperative radiotherapy. One of these subjects subsequently underwent a 2nd mastectomy for a primary tumor in the opposite breast (Stage I pathologically) and has since been tumor-free clinically. An additional remission patient was found to have extensive bilateral multifocal lobular carcinoma; she did not receive postoperative radiotherapy.

The other 9 patients in remission had pathological Stage I (node-negative) disease at the time of surgery. Five of these patients received postoperative radiotherapy. The mean migration inhibition for all sequential tests in the 14 patients with Stage II disease was 17.5 ± 1.3% and that for the 9 patients with Stage I disease was 14.3 ± 2.0% and this difference was not significant (Table 1). Previous irradiation did not appear to affect reactivity.

The 10 patients in relapse who had widespread disease at the time they were tested all had Stage II disease initially. Six had been treated with postoperative radiotherapy. One of these patients was in apparent complete remission for 11 weeks of testing and, on the 12th week, suddenly developed chest wall recurrence. She was tested for a further 7 weeks. Mean migration inhibition to BCA prior to developing obvious metastases was 15.1 ± 2.5% and over 7 weeks following relapse it was 13.9 ± 6%; the difference between these 2 values was not significant.

Chart 1 shows the percentage of distribution of mean reactivity for 6 successive weekly tests in 21 breast cancer patients in remission and in 46 normal female controls.

Chart 2 shows the percentage of distribution of mean reactivity for 4 successive weekly tests in 23 patients with breast cancer in remission and in 58 normal female controls.

Seven normal subjects with a high mean inhibition score (>13.0%) were examined by xeromammography. Three of these subjects, all over age 50, had radiological evidence of cystic and proliferative disease but no evidence of cancer. The other 4 subjects, all under age 35, had normal mammograms. Four of the 7 also had a family history of breast cancer.

One woman previously included in the normal control group has since developed breast cancer and, pathologically, there was both multifocal infiltrating and in situ lobular carcinoma involving 1 breast, with mammographic changes of severe cystic and proliferative disease in the opposite breast. Her mean score prior to diagnosis of cancer was 7.0 ± 7.2% and was therefore within the range for the normal group of women.

**DISCUSSION**

The results suggest that, when tested sequentially at weekly intervals, all patients in remission, the majority of
those in relapse and most normal subjects may react positively, although not consistently to breast cancer antigens but not to normal breast tissue or benign breast tumor antigens. The observation that variation occurs in sequential tests is consistent with the single test reports of other investigators with regard to both cancer patients and normal subjects. For example, Cochran et al. (4) using PLMI reported that 74 of 138 patients (54%) and 7 of 26 normal controls (27%) reacted against breast cancer antigens when a criteria of greater than 20% inhibition of migration was considered a positive result. Other investigators, using the same criteria, have reported similar degrees of reactivity in the 2 groups of women tested (1, 9). Jones and Turnbull (7) have reported that in sequential tests of patients with breast cancer studied 4 times in the year following their mastectomy, variability in the degree of reactivity in all patients has been seen and in some cases tests have varied from positive to negative. Rieche et al. (11) using >30% inhibition as a positive result claimed that PLMI reactivity to BCA disappeared at about 40 days postmastectomy and reappeared when the disease relapsed. The results reported here suggest that, on the contrary, a high level of mean reactivity persists in remission patients; the average disease-free interval was in excess of 4 years in the 23 patients studied. Moreover, reactivity persists in most relapse patients; the overall mean reactivity is significantly lower than for patients in remission but significantly higher than for normal controls.

The results suggest that instead of approximately 50% of breast cancer patients and 20% of controls reacting as reported in the various studies, all cancer patients tested sequentially react about 50% of the time and 98% of the normal female controls react about 25% of the time if >20% inhibition of migration is used as a criterion of positivity. This increases substantially in all groups if statistical analysis is used to determine a positive test result. The reason for the variability in test results observed against well-defined antigens such as PPD (10) or crude tumor extracts such as breast cancer antigen is not readily apparent. While technical artifact might account for the differences noted, the finding of positive reactivity against malignant but not normal breast antigens suggests that variability is not simply due to technical factors.

This study is in agreement with previous reports that greater reactivity is found against breast cancer antigen in patients with breast cancer compared with normal women, but as with PPD, variation of reactivity limits the value of a single test; the mean value of sequential tests may be a more meaningful index of cellular immunity than a single determination. When sequential tests are performed, it can be seen that reactivity occurs in all groups, but the mean percentage of migration inhibition for all tests is significantly lower in normals than in patients with breast cancer either in relapse or remission. Although the mean percentage of migration inhibition for patients in relapse is significantly lower than that for patients in remission, PLMI tests would seem to be of little value as an in vitro correlate of disease course as shown by the observation that 7 of 10 patients in relapse had a mean level of reactivity greater than 10%.

Those normal females with a mean PLMI score of >10.0% may simply represent an overlap in the frequency distribution curves between normals and patients with breast cancer. However, the finding of increased mean reactivity in certain normal individuals, some of whom apparently have risk factors such as a family history of breast cancer and/or underlying fibrocystic disease, raises the question as to whether sequential testing might be predictive of undergoing breast pathology.

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