ABSTRACT

Lymphocyte subset phenotypes in peripheral blood and axillary lymph node cell isolates from 28 patients undergoing surgery for breast cancer were determined by two-color immunofluorescence with monoclonal antibodies and flow cytometric analysis. Lymphocyte subpopulation proportions were determined with combinations of monoclonal antibodies directed against the Leu 2, Leu 3, Leu 7, Leu 8, Leu 11, Leu 12, Leu 15, Leu M3, and HLA-DR surface markers. Patients were staged according to the postsurgical-pathological modification of the Tumor-Node-Metastases staging system, for analysis of tissue source (lymph node versus peripheral blood) and stage of disease as factors influencing lymphocyte subset size. Activated Leu 2+DR+ and Leu 3+DR+ T-cells were elevated in Stage 2 carcinoma compared to Stage 1. Elevation of Leu 2+8+ circulating T-cells and a reciprocal depression of Leu 2+8- T-cells were also seen in Stage 2 patients when compared to Stage 1. Total T-cells, B-cells, Leu 2+, and Leu 3+ T-cell subsets and natural killer phenotypes defined by Leu 7 and Leu 11 were unchanged in the peripheral blood of Stages 1 and 2 breast cancer. Regional lymph nodes from Stage 1 were found to contain a high frequency of Leu 3+ cells which dropped significantly in Stage 2 patients; this was found to be numerically due to a sharp decrease in the Leu 3+8- subpopulation in Stage 2 patients. Elevated B-cells (Leu 12+), activated T-cells (Leu 2+DR+ and Leu 3+DR+), total Leu 2+ cells, and Leu 7–11+ natural killer cells were demonstrated in Stage 2 lymph nodes when compared to Stage 1. Generally, no differences in subpopulations were seen when level 1 (low axillary) lymph node cells were compared to level 3 (high axillary) lymph node cells at each stage of the disease. These findings demonstrate substantial differences in the profile of lymphocyte phenotypes between Stage 1 and Stage 2 breast carcinoma, especially in the ipsilateral regional nodes. The findings presented in this study suggest that changes in local-regional immunocompetent cell subsets may be related to metastasis of tumor to the regional nodes and progression of disease without being fully reflected in the systemic circulation.

INTRODUCTION

Investigations of cell-mediated immunity in the peripheral blood of breast cancer patients have generally shown little evidence of immune impairment, except in advanced metastatic disease (1–6). In contrast, differences in the reactivity in vitro to soluble tumor extracts by various leukocyte subsets in the early stages of breast cancer have been reported (7, 8). Regional lymph node cells have been reported to demonstrate impaired in vitro immune responses when isolated from patients with Stage 2 breast cancer (9, 10). Correlation of changes in lymph node architecture and broad subset analysis with the presence of nodal metastases, however, has been variable (11, 12).

Recently, monoclonal antibodies defining functionally relevant lymphocyte subsets have become available. These surface markers include Leu 7 (13, 14), Leu 8 (1, 15), Leu 11 (13), and Leu 15 (16). These antibodies are directed against determinants which span multiple major cell types and have proven useful when paired with antibodies which define different but overlapping distributions of surface markers, in the resolution of subsets which contain specific functional capabilities. The present study was designed to evaluate the hypothesis that extent of disease (stage of breast cancer), tissue source of monoclonal cell isolates (lymph node versus blood), and proximity to the primary tumor (low versus high axillary lymph node) are significant factors in the frequencies of phenotypically defined subsets of immunocompetent cells detectable by immunofluorescence and flow cytometry in patients with operable breast carcinoma.

MATERIALS AND METHODS

Patients. Twenty-eight patients with invasive breast carcinoma undergoing mastectomy with axillary dissection or excision with axillary dissection entered this study with informed voluntary consent. Heparinized venous blood samples were obtained prior to surgery in all 28 subjects, and lymphocytes were isolated from one level 1 and one level 3 (17) lymph nodes in the surgical specimens from 18 patients. Breast carcinoma was staged in each patient according to the postsurgical-pathological modification of the Tumor-Node-Metastases staging system (18). Briefly, pathological Stage 1 breast cancer (axillary nodes not containing macrometastases) and pathological Stage 2 (nodal metastases present) breast cancer are operable early breast cancers, while Stage 3 features extensive local disease (irrespective of nodal status), and Stage 4 indicates distant metastases.

Peripheral Blood Lymphocytes. Fifteen ml of heparinized blood from each patient were layered onto Ficoll-Hypaque (Pharmacia, Piscataway, NJ) and then centrifuged at 400 × g for 30 min. The lymphocyte layer was harvested, washed three times with HBSS(4) (Gibco Laboratories, Chargin Falls, OH), and resuspended in HBSS with 20% minimal essential medium (Grand Island Biological Company, Grand Island, NY) to a concentration of 5.0 × 10⁶ cells/ml. Cell viability by the trypan blue exclusion test was greater than 97%.

Lymph Node Cells. Lymphocytes were harvested from axillary lymph nodes following removal of a thin slice of one level 1 lymph node and one apical lymph node from the surgical specimen which were not grossly involved with tumor. The lymph node slices were each minced finely with a surgical blade and then passed through a fine filter to obtain a single cell suspension. The lymphocytes were isolated by Ficoll-Hypaque flotation and washed and resuspended as above. In all cell suspensions viability as determined by trypan blue exclusion exceeded 90%. Typical cell yields from peripheral blood samples were 1–1.5 × 10⁶ cells/ml of blood and 1.5 × 10⁶ cells from each lymph node slice sampled.

Monoclonal Antibody Staining. All monoclonal antibodies with the exception of Leu 7 were conjugated directly with either FITC or PE. Antibodies used in this study included Leu 2a, Leu 3a, Leu 8, Leu 11a, and Leu 12 FITC conjugates, Leu 2a, Leu 3a, Leu 15, and Leu M3 PE conjugates, and a Leu 7a biotin conjugate developed with avidin-PE (Becton Dickinson Monoclonal Antibody Center, Mountain View, CA). The full panel of combinations of monoclonal antibodies depicted in

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2 Surgical Research Fellow. The Jane Forbes Clark Surgical Research Laboratories, St. Luke's-Roosevelt Hospital Center, New York, NY.
3 To whom requests for reprints should be addressed, at the Surgical Service, St. Luke’s-Roosevelt Hospital Center, Amsterdam Ave. at 114th St., New York, NY 10025.
4 The abbreviations used are: HBSS, Hank’s balanced salt solution; DR+, cells bearing the surface antigens detected by monoclonal anti-HLA-DR antibody; FITC, fluorescein conjugated antibody; PE, phycoerythrin conjugated antibody; NK, natural killer.

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Table 1 was used with each cell isolate (lymph node and peripheral blood) in this study. For direct immunofluorescence 5 x 10^5 cells were incubated with 5 μl of the appropriate antibody (see Table 1) and incubated at 4°C for 15 min. The second antibody, always the PE conjugated antibody, was added in the same concentration to the cell suspension, and incubation was carried further for 15 minutes at 4°C. The cells were then washed with HBSS and resuspended in 0.4 ml of HBSS. Two-color immunofluorescent staining was determined by flow cytometry.

Flow Cytometric Analysis. Fluorescent antibody staining was detected on a FACS IV four parameter flow cytometer/cell sorter (Becton Dickinson, Sunnyvale, CA), using dual fluorescence log amplifiers, two-color compensation network, and excitation by the 488-nm line of a 5-W argon laser at 200 mW. Fluorescence detectors were situated behind a 560-nm long pass dichroic beam splitter, with green (FITC) signals detected through a 520-nm short pass dichroic filter, and the orange-red (PE) signals were detected through a 580-nm bandpass dichroic filter. Signal acquisition was triggered on forward light scatter with simultaneous acquisition of forward and wide angle light scatter and two color log-fluorescence signals. Data were acquired in list mode (serial recording of the four signals for each cell counted) by the ACQ4 module of the LACEL flow cytometry program (Consort 40 analysis and data acquisition software, Becton-Dickinson) and stored on 8-inch floppy disks by a Consort 40A microcomputer (Becton-Dickinson). For each experiment a minimum of 10,000 cells was counted.

Data Reduction. List mode files were analyzed by the QUAD4 program developed in our laboratory for simultaneous determination of two-color fluorescence data on samples gated on forward and 90° scatter. Briefly, the QUAD4 program processes recorded, digitized list mode data by gating through a window established for lymphocytes on forward and 90° scatter characteristics and calculates the percentages of cells in the four possible combinations of two-color positive and negative classifications. This method is analogous to quantitating the cells on a bivariate (scatterplot) histogram of red versus green fluorescence intensity, segmenting the histogram into four contiguous areas (quadrants). The QUAD4 program yields results which agree precisely with those obtained with the DISP2D module of the Consort 40 analysis software (Becton-Dickinson) but is better suited to rapid processing of multiple samples, as in the present study. Statistical analysis was performed on an IBM PC computer utilizing the Systat Version II Multivariate Statistical package (Systat, Inc., Evanston, IL). Because of unequal sample sizes, posthoc testing was performed using the method of Scheffé, which provides rigorous protection against Type I errors (19, 20).

RESULTS

Pathology. Nine patients were found to have Stage 1 breast cancer; 11 had Stage 2; 6 had Stage 3; and 2 had Stage 4. Two ipsilateral axillary lymph nodes were harvested from surgical specimens of each of 8 patients with Stage 1, 8 with Stage 2, one with Stage 3, and one with Stage 4 disease. The Stage 3 and 4 patients were included in calculations of the frequencies of cell surface markers and major subpopulation types for purposes of illustration (below) but were then excluded from subsequent analyses.

Surface Markers. Frequencies of cells expressing the surface antigens defined by monoclonal antibodies in the regional lymph nodes and peripheral blood of patients in this study are shown in Table 2. Overall, there were significantly fewer cells expressing Leu 2 (P = 0.001), Leu 8 (P = 0.000), and Leu 15 (P = 0.002) in the lymph nodes than in the peripheral blood samples. Expression of Leu 3 (P = 0.000), Leu 12 (P = 0.000), and HLA-DR (P = 0.000) was more frequent, however, in lymph node cells than in peripheral blood in the patients studied. Cells bearing Leu 7, Leu 11, and Leu M3 were not significantly different in frequency in regional lymph nodes than in peripheral blood. Two-way analysis of variance with tissue (lymph node versus peripheral blood) and stage of disease confirmed the significance of the tissue origin of cells as a factor for frequency of expression of Leu 3 (P = 0.002), Leu 8 (P = 0.000), Leu 12 (P = 0.000), and HLA-DR (P = 0.003). Stage of disease was a significant factor for Leu 12 (P = 0.005) and

Table 1: Monoclonal antibody combinations used in this study and major cell phenotypes defined

For references, see “Discussion.” Monoclonal antibodies were purchased from Becton-Dickinson, Mountain View, CA. Mouse IgG1 — FITC specific for nonhuman determinants and HLE-1 — FITC (pan leukocyte) monoclonal antibodies were used as negative and positive controls respectively for each cell sample.

Table 2: Mononuclear cell surface markers in regional lymph nodes and peripheral blood from breast cancer patients

![Table 2: Mononuclear cell surface markers in regional lymph nodes and peripheral blood from breast cancer patients](attachment:image.png)
HLA-DR ($P = 0.0028$) as well. The interaction of tissue origin of sample and stage was significant for Leu 2 ($P = 0.028$) and for Leu 15 ($P < 0.001$).

Multiple comparisons by the method of Scheffé showed that Leu 2+, Leu 8+, and Leu 15+ cells were less frequent in Stage 1 nodal cells than in peripheral blood ($P < 0.05$), whereas Leu 12+ cells were significantly more frequent in nodal samples than in the peripheral blood ($P < 0.001$) of Stage 2 patients. There were more HLA-DR+ cells in the lymph nodes than the blood of Stage 2 patients ($P < 0.10$). Leu 3+ cells were significantly higher in Stage 1 than in Stage 2 lymph nodes ($P < 0.05$). There were fewer Leu 12+ cells in Stage 1 lymph nodes than in Stage 2 nodes ($P < 0.01$). Significant differences by stage of disease were not seen in either tissue source for Leu 2, Leu 8, Leu 15, and HLA-DR.

Lymphocyte Subpopulations. T-cells, determined by the sumation of Leu 2+ and Leu 3+ cells for each sample, constituted 65 ± 18% (SD) of lymph node cells and 69 ± 16% of cells in these Ficoll-Hypaque density separated specimens. Non-T-cells expressing HLA-DR, determined by the difference between the frequencies of Leu 3-DR+ and Leu 2+DR+ cells for each sample, were the next largest group in both tissues (Table 3). Small monocytes (Leu M3+) and NK cells (Leu 11+) were present in low and similar frequencies in lymph node and peripheral blood isolates. B-cells (Leu 12+) were higher in lymph nodes than in peripheral blood, especially in samples from Stage 2 patients ($P < 0.001$).

Expression of HLA-DR on T-Cells. Leu 3+ cells coexpressing HLA-DR were detected in both lymph nodes and blood (Table 4) but with a higher frequency in Stage 2 than Stage 1 ($P < 0.01$). Leu 3+DR+ cells constituted a subset of Leu 3+ cells of similar size in lymph nodes and blood of Stage 1 and Stage 2 patients.

| Table 3 Subpopulations of mononuclear cells in regional lymph nodes and peripheral blood from breast cancer patients
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>% of lymphocyte region cells</td>
<td>Stage 1</td>
<td>Stage 2</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Regional lymph nodes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-cells: [Leu 2+] + [Leu 3+]</td>
<td>n = 15</td>
<td>n = 16</td>
</tr>
<tr>
<td>B-cells: Leu 12+</td>
<td>70 ± 10</td>
<td>60 ± 24</td>
</tr>
<tr>
<td>Monocytes: Leu M3+</td>
<td>6 ± 6</td>
<td>9 ± 8</td>
</tr>
<tr>
<td>NK cells: Leu 11+</td>
<td>5 ± 2</td>
<td>10 ± 10</td>
</tr>
<tr>
<td>Non-T-cells, HLA-DR+: [Leu 2-3-DR+]</td>
<td>25 ± 11</td>
<td>25 ± 8</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-cells: [Leu 2+] + [Leu 3+]</td>
<td>n = 9</td>
<td>n = 11</td>
</tr>
<tr>
<td>B-cells: Leu 12+</td>
<td>70 ± 9</td>
<td>69 ± 20</td>
</tr>
<tr>
<td>Monocytes: Leu M3+</td>
<td>4 ± 2</td>
<td>6 ± 5</td>
</tr>
<tr>
<td>NK cells: Leu 11+</td>
<td>9 ± 5</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Non-T-cells, HLA-DR+: [Leu 2-3-DR+]</td>
<td>13 ± 5</td>
<td>16 ± 14</td>
</tr>
</tbody>
</table>

* Sum of Leu 2+ and Leu 3+ cells.
* Mean ± SD.
* $P < 0.001$, stage 1 versus stage 2 lymph nodes.
* $P < 0.001$, stage 2 lymph node cells versus stage 2 peripheral blood.
* Difference between frequencies of Leu 2−DR− cells and Leu 2+DR+ cells.

| Table 4 HLA-DR expression on regional lymph node and peripheral blood T-cells in breast cancer
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>% of Leu 3+ in Leu 3+DR+ subset of helper/inducer T-cells</td>
<td>% of Leu 2+ in Leu 2+DR+ subset of suppressor/cytotoxic T-cells</td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Level 1 nodes</td>
<td>Level 3 nodes</td>
<td>Pooled nodes</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Stage 1 15 ± 12</td>
<td>13 ± 6</td>
<td>14 ± 9</td>
</tr>
<tr>
<td>Stage 2 41 ± 20</td>
<td>30 ± 25</td>
<td>36 ± 23</td>
</tr>
<tr>
<td>$P &lt; 0.01$</td>
<td>$&lt;0.01$</td>
<td>$&lt;0.01$</td>
</tr>
</tbody>
</table>

* Mean ± SD.
* NS, not significant.
* Peripheral blood versus each lymph node column, respectively.

Total Leu 3+ cells were less frequent in lymph nodes of Stage 2 than in lymph nodes of Stage 1 patients ($P < 0.05$). This decrease was noted to be a reflection of a significantly smaller Leu 3+DR− subset in Stage 2 lymph nodes ($P < 0.001$). There was no corresponding decrease in the Leu 3+DR− subset of peripheral blood T-cells in Stage 2, however, and total Leu 3+ cell levels were the same in Stages 1 and 2. There were significantly more Leu 3+DR− cells in lymph nodes than in blood, expressed either as percentage of total lymphocytes or as a percentage of total Leu 3+ cells.

Expression of Leu 8 on Leu 3+ Cells. Leu 3+ T-cells were divided into subsets based on coexpression of the Leu 8 marker. Similar levels of Leu 3+8− cells, expressed as percentages of total lymphocytes in the gated region of analysis, were found in lymph nodes and peripheral blood of both Stage 1 and Stage 2 breast cancer (Table 5). Expressed as a percentage of all Leu 3+ cells, however, there were significantly fewer Leu 3+8+ cells in the lymph nodes than the peripheral blood of Stage 1 ($P < 0.001$) and Stage 2 ($P < 0.001$) patients. There were no significant differences between Stage 1 and Stage 2 lymph node Leu 3+8+ cells (level 1, level 3, or pooled) expressed either as a percentage of total lymphocytes or as a percentage of total Leu 3+ cells.

Expression of Leu 8 on Leu 2+ Cells. Expression of Leu 8 on Leu 2+ cells, as a percentage of total lymphocytes, was not significantly different in lymph nodes or peripheral blood, in either Stage 1 or Stage 2 disease (Table 6). Similarly, the size of the Leu 2+ subset was not significantly different in lymph nodes or in the blood of patients with either stage of disease. The Leu 2+8− subset did constitute a significantly larger fraction of the Leu 2+DR− population in blood than in pooled lymph nodes ($P < 0.05$) and was a slightly larger component of peripheral blood Leu 2+ cells in Stage 2 than in Stage 1 disease ($P < 0.10$).

Cells of the Leu 2+8− phenotype were significantly fewer as a percentage of total lymphocytes in lymph nodes than in peripheral blood ($P < 0.01$) of patients with Stage 1 disease, but they constituted a virtually identical fraction of the Leu 2+ subset in both nodal and peripheral blood cells. In Stage 2 breast carcinoma, the Leu 2+8− subset was slightly smaller as a fraction of Leu 2+ cells in peripheral blood than in Stage 1 ($P < 0.10$) and constituted a smaller fraction of the Leu 2+ cells in peripheral blood than in lymph nodes ($P < 0.05$).
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Table 5 Expression of Leu 8 antigen on Leu 3+ regional lymph node and peripheral blood T-cells in breast cancer

<table>
<thead>
<tr>
<th></th>
<th>Level 1 nodes</th>
<th>Level 3 nodes</th>
<th>Pooled nodes</th>
<th>Peripheral blood</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Leu 3+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>11 ± 9b</td>
<td>8 ± 3</td>
<td>9 ± 7</td>
<td>25 ± 6</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>19 ± 14</td>
<td>17 ± 8</td>
<td>18 ± 12</td>
<td>22 ± 13</td>
<td></td>
</tr>
<tr>
<td>Leu 2+8+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>4 ± 5</td>
<td>3 ± 2</td>
<td>4 ± 4</td>
<td>9 ± 6</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>11 ± 10</td>
<td>7 ± 7</td>
<td>9 ± 8</td>
<td>12 ± 8</td>
<td></td>
</tr>
<tr>
<td>Leu 2+8—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>6 ± 4</td>
<td>5 ± 2</td>
<td>5 ± 3</td>
<td>17 ± 6          &lt;0.01b</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>9 ± 5</td>
<td>10 ± 5</td>
<td>9 ± 5</td>
<td>10 ± 5</td>
<td></td>
</tr>
</tbody>
</table>

Table 6 Expression of Leu 8 antigen on Leu 2+ regional lymph node and peripheral blood T-cells in breast cancer

<table>
<thead>
<tr>
<th></th>
<th>Level 1 nodes</th>
<th>Level 3 nodes</th>
<th>Pooled nodes</th>
<th>Peripheral blood</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Leu 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>36 ± 12</td>
<td>32 ± 11</td>
<td>34 ± 11</td>
<td>34 ± 19</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>50 ± 14</td>
<td>41 ± 16</td>
<td>46 ± 15</td>
<td>52 ± 13          NS, NS, &lt;0.05b</td>
<td>&lt;0.10b</td>
</tr>
<tr>
<td>Leu 2+8+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>64 ± 12</td>
<td>68 ± 11</td>
<td>66 ± 11</td>
<td>66 ± 19          NS, NS, &lt;0.05b</td>
<td>&lt;0.10b</td>
</tr>
<tr>
<td>Stage 2</td>
<td>50 ± 14</td>
<td>59 ± 16</td>
<td>54 ± 15</td>
<td>48 ± 13          NS, NS, &lt;0.05b</td>
<td>&lt;0.10b</td>
</tr>
</tbody>
</table>

Expression of Leu 15 on Leu 2+ Cells. In this experiment, the Leu 2+ subpopulations were quite similar in size to those preceding, as would be expected (Table 7). The larger number of lymphocytes that expressed Leu 2 in the peripheral blood than in lymph nodes was significant (P < 0.05) in Stage 1 disease. Also, the percentage of Leu 2+ cells in Stage 2 lymph nodes was higher than in Stage 1. Leu 15 expression identified a small subset of Leu 2+ cells which constituted approximately 20% of Stage 1 lymph node Leu 2+ cells, 36% of Stage 2 lymph nodes Leu 2+ cells, and 50% of peripheral Leu 2+ blood cells. Leu 2+15- cells constituted about 80% of Stage 1 and 64% of Stage 2 nodal Leu 2+ cells.

Expression of Leu 7 and Leu 11. Coexpression of Leu 7 and Leu 11 surface markers was examined in these Ficoll-Hypaque density flotation lymphocyte preparations. These samples were not enriched for the larger granular lymphocyte subsets. By simultaneous two-color fluorescence, the Leu 7 and Leu 11 phenotypes were enumerated (Table 8). There were no significant differences in the expression of Leu 7 by tissue or stage, or of Leu 11 by tissue or by stage when analyzed as total Leu 7+ and total Leu 11+ cells. Analysis of the three phenotypic subsets (Leu 7+11-, Leu 7+11+, and Leu 7-11+) showed that the largest subset was the Leu 7+11- subset, in both lymph nodes and peripheral blood of both Stages 1 and 2 disease. The Leu 7+11+ subset was small and not significantly affected by tissue source or stage of disease. The Leu 7-11+ subset of lymph node cells was larger in Stage 2 than Stage 1 disease (P < 0.05).

DISCUSSION

Immunological phenotypes for lymphocytes from the peripheral blood and regional lymph nodes of breast cancer patients...
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were determined utilizing two-color flow cytometric analysis of monoclonal antibody defined cell surface markers. Sufficient numbers of patients with Stage 1 and 2 disease have been entered into the present study to permit statistical analysis of the effects of the tissue source of the lymphoid cell isolates (lymph node versus blood) and the presence or absence of axillary nodal metastases. In general, the presence of Stage 2 disease was accompanied by changes in a number of subpopulations in lymph node cells but only a few changes in peripheral blood cells. Stage 2 breast cancer was associated with increases in circulating activated DR+ (14, 21) T-cells in both the helper/inducer (Leu 3+) and suppressor/cytotoxic (Leu 2+) subpopulations. Increased circulating Leu 2+8+ and reciprocally decreased Leu 2+8- subsets were also seen in Stage 2 breast cancer. There were no significant differences between Stages 1 and 2 in peripheral blood total T-cells, B-cells, Leu 3+, or Leu 2+ T-cell subsets, in agreement with the results of others (2-6, 12). Detection of activated peripheral blood T-cells, Leu 2+DR+ and Leu 3+DR+ (22), in Stage 1 patients, and to a greater extent in Stage 2 patients, is consistent with previously reported findings of T-cell reactivity to soluble extracts of breast adenocarcinoma in the peripheral blood samples from patients with the early stages of breast cancer (7, 8). The design of the study presented here did not permit further phenotypic characterization of these activated Leu 2+ and Leu 3+ cells.

Regional ipsilateral lymph node cells from breast cancer patients also coexpressed HLA-DR with Leu 2+ and Leu 3+ in proportions comparable to those in peripheral blood in patients without axillary nodal metastases (Stage 1). Increased percentages of Leu 2+ cells and Leu 2+DR+ cells were seen in Stage 2 breast cancer lymph nodes, compared to Stage 1. Proportions of Leu 2+ cells coexpressing Leu 8 were not significantly different in Stage 1 or 2 lymph nodes. Both the Leu 2+8+ and Leu 2+8- subsets are required in combination to significantly suppress Leu 3-induced immunoglobulin synthesis (15, 23), and both Leu 2+8+ and Leu 2+8- subsets have been shown to contain precursor and effector T-killer cells (23). Coexpression of Leu 15 with Leu 2 was not significantly different in the lymph nodes (or peripheral blood) of patients with either stage of breast cancer. Leu 15 has been shown to functionally subset Leu 2+ cells (16); the Leu 2+15+ subset contains suppressor cells for T-cell proliferative responses.

Leu 8 surface antigen status has been shown to represent a functional characterization of Leu 3+ cells (1, 15) as well as Leu 2+ T-cells and B-cells (1). In the present study, significantly higher frequencies of Leu 3+ cells were found in lymph nodes from Stage 1 breast cancer than from Stage 2 lymph nodes. The decrease in Leu 3+ cells in Stage 2 lymph node cells was shown to be numerically due to a significant drop in the Leu 3+8- subset; the Leu 3+8+ subset remained essentially unchanged. The Leu 3+8- subset has been shown to contain nearly all of the helper function for immunoglobulin synthesis in response to pokeweed mitogen stimulation and to autologous mixed leukocyte reaction (1, 15). The decrease in Leu 3+8- Stage 2 lymph node cells was accompanied by a significant increase in Stage 2 lymph node B-cells, as determined by Leu 12 (1).

Natural killer cell phenotypes were determined for peripheral blood and lymph node cell isolates with Leu 7 (13, 14) and Leu 11 (13). While the major NK subpopulation in both tissue sites and both stages of disease carried the Leu 7+11- phenotype, this subset has been shown to have only weak NK activity (13). There was a significant increase in the level of Leu 7-11+ Stage 2 lymph node cells, compared to Stage 1 lymph node cells. This subset has been shown to have a high level of NK activity (13).

Changes in the microarchitecture of axillary lymph nodes draining breast cancer have been reported; however, evidence of a correlation of phenotype patterns with clinical/pathological categorization (i.e., nodal metastases) was not demonstrated (11). The findings of significant differences in the surface marker-defined subpopulations in the present study are more consistent with many prior reports correlating functional changes in lymph node cells from patients with Stage 2 disease (2, 10).

Caution must be exercised in interpretation of the findings of differences in lymphocyte subpopulation frequency in the axillary lymph nodes of breast cancer patients in the present study. Due to the design of this study, only axillary lymph nodes draining the breast in which the cancer was found were studied. While the findings discussed above can be clearly related to the presence or absence of axillary nodal metastases (Stage 1 versus Stage 2), for example, the study design does not address the question of whether these subset frequency differences are unique to the ipsilateral regional lymph nodes. It may be that similar changes would be found in lymph nodes distant to the site of the primary cancer, if such lymphoid tissue from other body sites in these patients was available for study. The observation that level 1 lymph node cells from Stage 2 breast cancer patients of the Leu 2+DR+ and Leu 3+DR+ phenotypes were significantly more frequent than in the peripheral blood, whereas the level 3 subsets expressing these phenotypes were not is suggestive of the possibility that at least some of the lymph node subset frequency differences may be related to proximity of the nodes to the tumor. Although the mean frequency of Stage 2 level 1 lymph node Leu 2+DR+ and Leu 3+DR+ cells was higher than the mean frequencies of these subsets in Stage 2 level 2 nodes, comparison of the means was not statistically significant, due in part to sample size.

Secondly, it was not possible in this study to compare subset frequencies in lymph nodes of breast cancer patients to those of lymph nodes from normal controls. The design of this study did not permit determination of how many of the observed differences in frequencies between phenotypically defined subsets of lymph node and peripheral blood cells are attributable to normal tissue distribution of lymphocyte subsets. Nor did the experimental design allow determination of changes in lymphocyte subsets which could be related to the presence of breast cancer and which would not be found in normal individuals or women with benign breast disease. Analysis of the data presented here, however, does show that the frequency in Stage 1 and Stage 2 breast cancer of lymph node cells expressing Leu 3+, Leu 3+DR+, Leu 3+8-, Leu 2+, Leu 2+DR+, Leu 7-11+, and Leu 12+ are unlikely to represent samples of underlying common distributions.

The findings of this study show that substantial differences in the profile of lymphocyte phenotypes, as determined by monoclonal antibodies directed against cell surface determinants, may be demonstrated in the early stages of breast cancer. Significant differences between the lymphocyte subsets in the peripheral blood of patients with only local or limited regional nodal breast cancer were in general restricted to Leu 2+ T-cells bearing the Leu 8 surface marker. More extensive differences in the lymphocyte subsets defined by pairs of monoclonal antibodies in dual color flow cytochemistry were seen in cells isolated from regional axillary lymph nodes draining the tumors. The effects of proximity to the tumor primary on nodal lymphocyte marker expression was limited to T-cells bearing the DR marker. A significantly larger subset of T-cells which...
has been shown to contain helper activity for B-cell differentiation
was seen in lymph nodes from Stage 1 than Stage 2 breast
cancer, while a higher frequency of B-cells, as defined by the
Leu 12 marker, was seen in nodes from patients with Stage 2
disease. No significant differences in T-cell subsets known to
cancer, while a higher frequency of B-cells, as defined by the
activation markers but not showing significant changes in the
petent cell subsets may be related to metastasis of breast cancer
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