Heterogeneity in the Expression of HLA and Tumor-associated Antigens by Surgically Removed and Cultured Breast Carcinoma Cells

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ABSTRACT

Surgically removed normal and malignant mammary tissues and human breast carcinoma cell lines were tested in binding assays with monoclonal antibodies to HLA-A,B,C antigens, \( \beta_2 \)-microglobulin, HLA-DR antigens, and tumor-associated antigens; the latter included a \( M \), 280,000, a \( M \), 94,000, and a \( M \), 85,000 membrane-bound glycoprotein and a cytoplasmic antigen. HLA-A,B antigens, \( \beta_2 \)-microglobulin, HLA-DR antigens, and the cytoplasmic antigen are expressed by normal mammary cells. Their malignant transformation may be associated with quantitative changes in the expression of these antigens and with the appearance of \( M \), 94,000 and \( M \), 85,000 glycoproteins. The \( M \), 280,000 glycoprotein was detected on only one of the breast carcinoma cell lines tested. Analysis of primary tumors and autologous axillary lymph node metastasis from 13 patients has shown differences in the expression of all the antigens tested between primary and metastatic lesions.

INTRODUCTION

Malignant transformation of human cells may be associated with changes in the expression of histocompatibility antigens and with the appearance of antigenic structures undetectable in their normal counterparts. Until recently, the serological analysis of the antigenic profile of human tumor cells has relied on the use of conventional antisera; the heterogeneity of antibody populations and the possible occurrence of contaminating antibodies in these reagents has questioned the validity of these studies. These limitations have been overcome by the development of MoAb3 which are highly specific and homogeneous. Using the hybridoma technique, we have developed MoAb to human histocompatibility antigens (26, 28) and to membrane-bound and cytoplasmic antigens which are expressed by human melanoma cells but are not detectable in melanocytes (17, 18, 36). The latter antibodies have been found to react also with other types of tumors and will be referred to as antibodies to tumor-associated antigens.

In this study, we have analyzed surgically removed and cultured human breast carcinoma cells with MoAb to histocompatibility antigens and to tumor-associated antigens, since these tumor cells may acquire tumor-associated antigens (1, 4, 6, 15, 16, 20, 21, 29, 31, 32, 38) and may change in the expression of histocompatibility antigens (4, 34).

MATERIALS AND METHODS

Human Tissues and Cell Lines. Normal mammary gland tissue was obtained from 4 patients at various stages of the menstrual cycle and from a 5-months-pregnant patient. Breast tumor tissues and axillary lymph node metastasis were obtained from patients (age range, 29 to 78 years) undergoing surgical treatment at the Regina Elena Cancer Institute. None of the patients had received chemo- or radiotherapy prior to surgery. Tissue specimens upon removal were divided into 2 parts. One was fixed in formaldehyde and processed for routine histological examination. The other was snap-frozen in liquid nitrogen; 4-\( \mu \)m-thick cryostat sections were obtained on the same day and were fixed in cold absolute acetone for 10 min. This fixative was preferred to others because it retained the most detail after fluorescent staining. Sections were either immediately used as a substrate in an IIF test or stored at -20\(^{\circ}\), storage for up to 6 months under these conditions did not alter the reactivity of tissue substrates in IIF.

The breast carcinoma cell lines ALAB 496, BT-20, Hs 578T, and Hs 905T were obtained from Dr. W. Nelson-Rees at the Naval Biomedical Research Laboratory, Oakland, Calif. The breast adenocarcinoma T 47D was kindly provided by Dr. S. Spiegelman at this Institution. The B-lymphoid cell line Raji, the T-lymphoid cell line 1301, and the melanoma cell lines Colo 38 and M21 are perpetuated in Roswell Park Memorial Institute Medium 1640 added with 10% calf serum.

MoAb to Human Histocompatibility Antigens and to Membrane-bound and Cytoplasmic Tumor-associated Antigens. The MoAb NAMB-1 reacts with free and HLA-A,B,C-associated human \( \beta_2 \)-m (23), the MoAb Q6/64 reacts with a monomorphic determinant expressed on the heavy chain of HLA-A,B,C antigens (27), and the MoAb Q2/70, Q5/13 recognize monomorphic determinants of HLA-DR antigens (26). The MoAb 345, 1345 reacts with a \( M \), 85,000 membrane-bound glycoprotein linked to a \( M \), 30,000 polypeptide by disulfide bridges (17). The MoAb 376.96S recognizes a \( M \), 94,000 membrane-bound glycoprotein (18). The MoAb 225.28S reacts with a membrane-bound \( M \), 280,000 glycoprotein noncovalently associated with a glycoprotein with the molecular weight higher than 440,000. The MoAb 465.12S recognizes a cytoplasmic antigen which comprises a \( M \), 94,000, a \( M \), 75,000, a \( M \), 70,000, and a \( M \), 25,000 glycoprotein (36). The MoAb W6/32, which identifies a framework determinant of HLA-A,B,C antigens (24), was purchased from Accurate Chemical and Scientific Co. (Hicksville, N. Y.).

Conventional Antisera. Fluorescein-labeled rabbit anti-mouse IgG antibody (1 volume; Cappel Laboratories, Cochranville, Pa.) was absorbed with 1 volume of packed AB, Rh-positive human RBC and 1 volume of human plasma which had been insolubilized with glutaraldehyde (2). The absorbed xenoantiserum was used in IIF at a protein concentration of 1 mg/ml and with a fluorescein:protein ratio of 3.

Serological Tests. IIF on 4-\( \mu \)m-thick acetone-fixed cryostat sections and SPA binding assay in a 96-well microtitrator plate were performed as described (22, 23). When MoAb belong to the IgG subclass, which does not bind SPA, target cells sensitized with the MoAb were reacted with a rabbit anti-mouse immunoglobulin antiserum before being tested with SPA.

RESULTS

IIF Staining of Normal Mammary Gland Tissue with a Panel of MoAb. The reactivity of the panel of MoAb with normal human mammary tissue was extensively investigated in order to establish the appropriate conditions for IIF and SPA tests on primary breast tumors. The results are summarized in Table I. Most MoAb reacted with normal mammary epithelial cells. It is important to note that, with the exception of MoAb 47D, which recognizes an antigen expressed only by melanoma lines, all MoAb tested were reactive with normal mammary gland but failed to react with myoepithelial cells (Table I).

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3 The abbreviations used are: MoAb, monoclonal antibody; IIF, indirect immunofluorescence; \( \beta_2 \)-m, \( \beta_2 \)-microglobulin; SPA, 125I-protein A.
mammary tissue was determined on biopsies from 4 patients at different times of the menstrual cycle and from a 5-months-pregnant patient. Antibodies were not tested with functioning lactating breast and with normal mammary tissue in culture, since the former tissue was not available, and attempts to culture normal mammary tissue were unsuccessful. The anti-HLA-A,B,C MoAb W6/32 and the anti-β2-μ MoAb NAMB-1 (Fig. 1, a and b) caused bright and homogenous fluorescence of ductal epithelial cell plasma membrane. Staining with the latter MoAb was more intense than that with the former one. Staining with both MoAb did not show significant differences with tissue specimens obtained from donors at various stages of their menstrual cycle. Anti-HLA-DR MoAb Q5/13 reacted weakly with mammary epithelial cells. The intensity of the stain varied markedly among tissue specimens from the 4 donors and among cells within each tissue section (Fig. 1c). A lower degree of heterogeneity was found among cells in tissue sections obtained from a 5-months-pregnant patient.

The MoAb 345.134S to a Mr 85,000 glycoprotein; the MoAb 376.96S to a Mr 94,000 glycoprotein; and the MoAb 225.28S to a Mr 280,000 glycoprotein did not stain normal mammary gland tissues. The MoAb 465.12S to a cytoplasmic antigen stained the cytoplasm of epithelial cells of the apical portion of mammary glands; the staining has a very fine granular distribution and is generally weak (Fig. 1d), although some nests of cells show a stronger positivity. Similar patterns of staining were found with normal mammary tissues sampled from biopsies of breast carcinoma.

IIF Staining of Breast Tumors with a Panel of MoAb. Results of testing histological types of breast carcinomas with the panel of MoAb are summarized in Table 1, and representative patterns of IIF staining are shown in Figs. 2 to 5. The following points are noteworthy: (a) there is significant heterogeneity in the pattern of reaction of the tumor lesions with most of the MoAb; (b) the intensity of staining of the large majority of the tumors tested with the anti-HLA-A,B,C MoAb W6/32 appears to be either reduced or increased; (c) the reactivity of the anti-β2-μ MoAb NAMB-1 paralleled that of normal breast in the majority of cases studied. Comparison of the reactivity of the anti-HLA-A,B,C and β2-μ MoAb showed discordance in about 45% of the cases tested. This discordance in reactivity was also observed when the staining with the anti-β2-μ MoAb NAMB-1 was compared with that obtained with the MoAb Q6/64 directed to a different determinant on the heavy chain of HLA-A,B,C antigens; (d) the anti-HLA-DR MoAb Q5/13 showed a reduced reactivity with most of the cases tested. This finding does not appear to reflect a change in the expression of the determinant identified by the MoAb Q5/13, since similar staining patterns were obtained with the MoAb Q2/70 and Q5/6 to distinct determinants of HLA-DR antigens; (e) MoAb 376.96S stained only 9 of the 32 breast tumors tested. The fluorescence was generally weak and highly variable in inten-
sity among nests of cells within each lesion; (f) MoAb 345.134S stained <50% of the carcinomas tested; (g) MoAb 225.28S did not stain any of the tumors tested; (h) MoAb 465.12S displayed a normal or increased reactivity (Fig. 5) with 42 and 40% of the cases tested, respectively. In 18% of the tumors tested, no cytoplasmic stain could be detected.

**DISCUSSION**

Analysis of surgically removed breast carcinoma tissues with a panel of MoAb to membrane-bound and cytoplasmic antigens has shown that malignant transformation of human mammary cells may be associated with changes in the expression of histocompatibility antigens and with the appearance of antigens undetectable in normal mammary cells. Other investigators using conventional antisera (16, 21, 31, 38), MoAb (5, 29), and cell-mediated immunity assays (1, 4, 15, 32) have identified antigens associated with human mammary tumors. The relationship between the antigens defined by our MoAb and those identified with human MoAb (29), murine MoAb (33), and

**Table 2**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tumor type</th>
<th>HLA-A,B,C</th>
<th>β2m</th>
<th>HLA-DR</th>
<th>M, 85,000 glycoprotein</th>
<th>M, 94,000 glycoprotein</th>
<th>Cytoplasmic MAA</th>
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<td>1</td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>Ductal infiltrating</td>
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<td>=</td>
<td>=</td>
<td>ND</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>Ductal infiltrating</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Ductal infiltrating</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Ductal infiltrating</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>ND</td>
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<td></td>
</tr>
<tr>
<td>6</td>
<td>Ductal infiltrating</td>
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<td>=</td>
<td>ND</td>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>Ductal infiltrating</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>ND</td>
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<td></td>
</tr>
<tr>
<td>8</td>
<td>Ductal infiltrating</td>
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<td>=</td>
<td>=</td>
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<td></td>
</tr>
<tr>
<td>9</td>
<td>Ductal infiltrating</td>
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<tr>
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<tr>
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<td>=</td>
<td>ND</td>
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<td></td>
</tr>
</tbody>
</table>

In primary and metastatic lesions: =, similar degree of staining; ↑, increased degree of staining; ↓, decreased degree of staining; ND, not determined.

**Table 3**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Anti-HLA- A,B,C</th>
<th>Anti-β2m</th>
<th>Anti-HLA- DR (Q5/13)</th>
<th>M, 85,000 glycoprotein</th>
<th>M, 95,000 glycoprotein</th>
<th>Anti-M, 85,000 glycoprotein (345.13S)</th>
<th>Anti-M, 85,000 glycoprotein (225.28S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast carcinoma</td>
<td>ALAB 496</td>
<td>2,000</td>
<td>ND</td>
<td>0</td>
<td>5,700</td>
<td>6,000</td>
<td>0</td>
</tr>
<tr>
<td>BT-20</td>
<td>2,000</td>
<td>5,100</td>
<td>0</td>
<td>7,000</td>
<td>2,500</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HS 905T</td>
<td>100</td>
<td>ND</td>
<td>0</td>
<td>1,000</td>
<td>1,000</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HS 578T</td>
<td>7,000</td>
<td>7,800</td>
<td>400</td>
<td>3,200</td>
<td>2,800</td>
<td>0</td>
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<tr>
<td>T 470</td>
<td>17,800</td>
<td>14,800</td>
<td>13,000</td>
<td>14,250</td>
<td>13,900</td>
<td>2,300</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>Colo 38</td>
<td>30,000</td>
<td>31,000</td>
<td>3,000</td>
<td>9,400</td>
<td>6,400</td>
<td>31,000</td>
</tr>
<tr>
<td>M21</td>
<td>25,000</td>
<td>26,000</td>
<td>1,000</td>
<td>10,000</td>
<td>2,300</td>
<td>41,000</td>
<td></td>
</tr>
<tr>
<td>Lymphoid</td>
<td>1301</td>
<td>12,000</td>
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<td>0</td>
<td>4,700</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Raji</td>
<td>5,000</td>
<td>6,400</td>
<td>8,000</td>
<td>5,400</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

ND, not determined.
cell-mediated immunity assays (1, 4, 15, 31) cannot be established because of lack of information about the molecular profile of the corresponding antigens. On the other hand, the antigens that we have identified are distinct from those defined by Colcher et al. (6) with monoclonal xenon antibodies, since the latter do not react with melanoma cells. Finally, our antigens have a molecular structure different from those of the antigens described by Leung et al. (20) and Mesa-Tejada et al. (21).

In agreement with data in the literature (35), our study has shown a high degree of antigenic heterogeneity in surgically removed breast cancer tissues as well as differences between primary tumors and autologous metastases. This finding does not appear to be a technical artifact, since related studies have shown a high agreement of the intensity of IIF staining with the level of antigen detected with a double determinant immunohasasay. The relationship of the antigenic heterogeneity to the clinical heterogeneity of human breast cancers (11) cannot be determined at this time, since we do not have sufficient information on the clinical course of the disease in the patients investigated. The biological relevance of our data is suggested by the role of HLA-A, B, C antigens in immune T-cell killing (33), by the correlation in animal model systems between expression of antigenic determinants recognized by MoAb and metastasizing properties of tumor cells (30), and by the influence of the level of H-2 antigens on the growth of tumor cells (10) and on their metastatic properties (9).

The discordant patterns of reactions of the anti-β2-~/MoAb NAMB-1 and of the anti-HLA-A, B, C heavy-chain MoAb W6/32 are of interest, since these structures represent the 2 subunits of the HLA-A, B, C complex (14). The similar reactivity of the 2 MoAb with HLA-A, B, C antigens bearing cells of different histological type (endothelial cells) in the tumor sections argues against difference in affinity as a mechanism to explain this finding. Furthermore, differences in the reactivity of the anti-mouse immunoglobulin antiserum with the 2 MoAb are not likely to cause the differential reactivity, since the activity of one antibody is not uniformly higher than that of the other one with all the targets tested, and the patterns of staining were repeated with a third monoclonal antibody. The reactivity of the anti-β2-~/MoAb NAMB-1 was higher than that of the anti-HLA-A, B, C MoAb W6/32 with normal mammary epithelial cells suggesting that β2-~ is expressed in larger amounts than is the heavy chain of HLA-A, B, C antigens. We do not know whether the excess β2-~ is expressed on the cell membrane in a free form or in association with other components. β2-~ has been reported to be associated with T-cell surface antigens (7) and some tumor-associated antigens (for review, see Ref. 8). Changes in the ratio between β2-~ and heavy chain of HLA-A, B, C antigens on breast carcinoma cells are likely to reflect variations in the synthesis rate and/or catabolism of free β2-~, since the free HLA-A, B, C antigen heavy chain has been reported not to be expressed on the cell membrane (8), and the determinant identified by the MoAb W6/32 is expressed only when the heavy chain is associated with β2-~ (24).

Normal mammary tissues from various donors varied in the expression of HLA-DR antigens. A mechanism for this observation is suggested by the finding in mice and guinea pigs that the expression of la-like antigens on mammary gland epithelial cells is under hormonal control (19). Changes in the expression of HLA-DR antigens on cells which undergo malignant transformation have been detected in various types of tumors of non-lymphoid origin (for review, see Ref. 25), but they have manifested themselves as unexpected appearances of HLA-DR antigens. To the best of our knowledge, breast carcinomas represent the first type of human tumors with loss of HLA-DR antigens associated with malignant transformation of cells. Medullary and tubular carcinomas are the only type of breast carcinomas which express HLA-DR antigens. The infiltration of the former tumors with lymphocytes raises the possibility that tumor cells may absorb HLA-DR antigens shed from lymphocytes, since exchange of la-like antigens has been reported to occur among cells (37). We consider this mechanism unlikely, although we cannot exclude it, since HLA-DR antigens are not detectable in cases of ductal breast carcinomas with lymphocyte infiltrates and in other tumors (e.g., Warthin’s tumors) with a massive presence of lymphoid cells (22). The expression of HLA-DR antigens by medullary breast carcinomas is intriguing since this type of breast carcinoma has the most favorable prognosis (12), and la-like antigens have been shown to influence the immunogenicity of tumor-specific antigens in animal systems (13). If not coincidental, this association may reflect a possible role of immunological events in the clinical course of breast carcinoma.

Whether the antibodies that we have used can be useful to develop immune diagnostic and immune therapeutic approaches to breast carcinomas cannot be established at present, since we have not yet tested them with benign and inflammatory lesions of mammary tissues. The cytoplasmic antigen identified by the MoAb 465.12S is expressed by normal mammary epithelial cells and in primary and metastatic breast carcinomas but is not detectable in lymphoid cells. Studies are in progress to determine the usefulness of the MoAb 465.12S in conjunction with conventional histopathology to score metastasized axillary lymph nodes, the number of which has been reported to correlate with tumor reoccurrences (38).

ACKNOWLEDGMENTS

The authors would like to thank Ellen Schmeding and C. Gama for their expert secretarial assistance.

REFERENCES


Fig. 2. IIF staining of frozen sections of infiltrating ductal carcinoma with anti-HLA-A,B,C MoAb W6/32 (a) and of medullary carcinoma with anti-β2-µ MoAb NAMB-1 (b). Level of expression of HLA-A,B,C antigens and of β2-µ in the 2 carcinomas tested is comparable to that found in normal mammary gland epithelium. a, × 1000; b, × 1000.
Fig. 3. IIF staining of frozen sections of a medullary breast carcinoma (a) and of a tubular breast carcinoma (c) with the MoAb Q5/13 to HLA-DR antigens. Phase-contrast observation (b) of the same section shown in a indicates that the fluorescent staining is associated with neoplastic cells. a, ×1000; b, ×1000; c, ×640.
Fig. 4. IIF staining of frozen sections of a medullary breast carcinoma with the MoAb 376.96S to a Mr 94,000 glycoprotein (a) and of an infiltrating ductal carcinoma with the MoAb 345.134S to a Mr 85,000 glycoprotein (b). a, × 1000; b, × 1000.
Fig. 5. IIF staining of frozen sections of a medullary (a) and a papillary (b) breast carcinoma with the MoAb 465.12S to a cytoplasmic antigen. The level of this antigen in the 2 carcinomas tested is higher than in normal mammary epithelial cells. a, × 400; b, × 480.
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