Reactivity of a Monoclonal Antibody With Tissues and Tumors From the Human Breast

Immunohistochemical Localization of a New Antigen and Clinicopathologic Correlations

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The reactions of a monoclonal antibody to the MCF7 breast cancer cell line were immunohistochemically studied on a variety of breast tumors, primary and metastatic, on mammary epithelium and on nonneoplastic breast lesions. A high proportion of positive reactions was observed in ductal, lobular, and tubular carcinomas as well as in mammary Paget's disease. Mucinous, medullary, and papillary carcinomas showed a low incidence of reactivity. Carcinomas with metaplasia, carcinoids, and nonepithelial breast tumors were unreactive with the antibody. Positive immunostaining was documented also in nodal and extranodal metastatic lesions. The staining of nodal metastases was correlated with the positive reaction of the primary tumor. Reactivity was widely distributed in normal breast epithelial cells and in benign breast lesions. Staining of nonneoplastic mammary epithelia was associated with reactivity of adjacent neoplastic tissues. Staining differences between nonneoplastic and neoplastic mammary tissues were related to the intensity and cytologic distribution of the labeling. Heterogeneous reactivity of morphologically similar cells was documented in nonneoplastic and neoplastic breast epithelial cells as well as in nodal and extranodal breast carcinoma metastases. Immunohistologically detectable antigen was not correlated with prognostic factors such as histologic grade or nodal status. A retrospective study of T1NO cases failed to substantiate any prognostic value for the reactivity of primary breast tumors with this monoclonal antibody. (Am J Pathol 1984, 115: 47–56)

A VARIETY of monoclonal antibodies (MAbs) have been recently raised against human mammary carcinoma[1–12]; therefore, the role of these reagents in the immunohistologic characterization of breast tumors needs to be investigated.[13] We recently reported the production and characterization of a MAb, designated MBr1,[12] which was prepared against the human breast cancer cell line MCF7 and was shown to identify a low-molecular-weight glycolipidic antigen.[14] The range of immunohistologic reactivity of MBr1[15] included mammary epithelium, mammary carcinomas, several nonneoplastic epithelial cell types, and a variety of adenocarcinomas and squamous carcinomas.

The aim of the present study was to analyze the immunohistologic distribution of the MBr1-defined antigen in several histotypes of breast tumors, in metastatic breast carcinoma, in normal mammary epithelium and in nonneoplastic breast lesions. The findings were also correlated with clinicopathologic

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data, such as histologic grading, nodal status, age, and prognosis.

Materials and Methods

MAbs

Murine monoclonal IgM MBr1 was raised against the membrane fraction of the MCF7 breast cancer cell line as previously reported.[12] The antibody stock used was derived from a pool of murine MBr1 ascites, partially purified by ammonium sulfate precipitation, and diluted, after checkerboard titrations, to a final protein concentration of 3 μg/ml. The reactivity at this dilution was comparable to that of purified MBr1 diluted to 1.5 μg/ml. Murine monoclonal IgM B3, substituted with MBr1 in controls, was reactive with murine lymphomas.[16]

Immunohistochemistry

Owing to its reported sensitivity,[17] we used a slightly modified avidin-biotin complex[18] immunoperoxidase technique as previously described.[15] Controls consisted of substitution with fetal calf serum or an unrelated murine IgM, at a normalized concentration, for MBr1.

Tissues

The tissues studied had been routinely fixed in Bouin's and embedded in paraffin. Previous studies revealed optimal preservation of the immunohistochemical reactivity under these conditions.[15] Sections were cut at 5 μ, placed on albumin-coated slides, and heated overnight at 37 C. In most cases, more than one section from different areas of the tumor, as well as sections from adjacent nonneoplastic breast, were examined.

Histologic Studies

Slides, pathology records, and patients' charts were studied. Special stains, including periodic acid–Schiff, Alcian blue, and the Grimelius argyrophylic stain, were used in specific cases for substantiation of the pathologic diagnosis. The slides stained with MBr1 were evaluated for the number of positive cells, intensity of the staining, and cytologic distribution of the staining. Staining patterns were classified and recorded as follows: *a*) staining at the luminal membranes (apical staining); *b*) staining of intraluminal secretion products; *c*) cytoplasmic staining; *d*) staining of the intracytoplasmic lumens (ICL); and *e*) staining of the outer cell membranes in tight clusters of tumor cells (marginal staining).

Prognostic Study

The primary breast tumors used to evaluate the prognostic significance of their immunohistologic reactivity with MBr1 were cases entered from 1973 to 1980 in a randomized controlled follow-up trial, which compared radical mastectomy with quadrantectomy, axillary dissection, and radiotherapy in T1NO patients. The protocol of this trial is described elsewhere.[19] Cases with positive axillary lymph nodes were treated with adjuvant radiotherapy (from 1973 to 1976) or adjuvant chemotherapy according to the CMF regimen (from 1976 to 1980). All patients were followed in the outpatient clinic, and recurrence-free intervals and overall survival were documented. The main data were recorded in an automated data system and updated monthly. Actuarial curves revealed no differences in disease-free and overall survival rates that could be related to different treatment modalities.[19] The tissues from this series of cases were fixed as previously described. Many of the tumors were small, and therefore sampling artifacts were reduced. However, when neoplastic tissue was distributed in more than one block, sections from all available paraffin blocks were examined.

Results

Breast Carcinomas

The immunoreactivity in different histotypes of breast carcinoma is summarized in Table 1. Immunohistologically detectable antigen was comparable in ductal (Figure 1a), lobular (Figure 1b), mixed ductal and lobular histotypes, as well as in mammary Paget's disease. The incidence of staining appeared to be slightly higher in tubular carcinomas. Mucinous, medullary, and papillary varieties revealed a small proportion of positive reactions. Three carcinomas with metaplasia (chondroid, spindle cell, and squamous) and two carcinoids of the breast were unreactive with the antibody. The immunologic staining reactions of tumor cells were markedly heterogeneous. With regard to the number of positive cells in tumor sections, the cases studied could be divided into four groups: 1) unreactive (−); 2) focally reactive (+, ie, with small clusters of cells or isolated cells positive in a few high-power fields); 3) reactive (++, ie, with a measurable percentage, up to 50%, of positive neoplastic cells); 4) diffusely reactive (+++, ie, with more than 50% positive neoplastic cells). A

Table 1—Reactions of MBr1 on Primary Breast Carcinomas

	− (%)	+ (%)	+ + (%)	+ + + + (%)	Positive/total (%)
Ductal	17 (34)	8 (16)	7 (14)	18 (36)	33/50 (66)
Lobular	8 (28)	4 (14)	5 (17)	12 (41)	21/29 (72)
Mixed ductal and lobular	2 (25)	1 (12)	2 (25)	3 (38)	6/8 (75)
Tubular	1 (20)	0 (0)	0 (0)	4 (80)	4/5 (80)
Paget's disease	5 (31)	4 (25)	4 (25)	3 (19)	11/16 (69)
Mucinous	3 (50)	3 (50)	0 (0)	0 (0)	3/6 (50)
Medullary	4 (80)	1 (20)	0 (0)	0 (0)	1/5 (20)
Papillary	2 (67)	1 (33)	0 (0)	0 (0)	1/3 (33)
Carcinoma with metaplasia	3 (100)	0 (0)	0 (0)	0 (0)	0/3 (0)
Carcinoid	2 (100)	0 (0)	0 (0)	0 (0)	0/2 (0)

"patchwork" heterogeneity pattern (Figure 1b), as described by Nuti et al[20] and Horan Hand et al,[21] was most frequently observed. Neoplastic emboli within mammary veins or lymphatics revealed a similar pattern of heterogeneity (Figure 1c). However, in a few cases MBr1 reactivity appeared concentrated in well-defined areas of tumor, whereas the adjacent neoplastic tissue was either unreactive or focally reactive.

The cytologic distribution of the labeling revealed a spectrum of variations related to different tumor types or to areas with different differentiation within the same neoplastic lesion. In well-differentiated ductal carcinomas, or in areas where the tumor formed glandular structures, apical and secretion product staining predominated (Figures 2a and b). In moderately to poorly differentiated ductal carcinomas, and in lobular carcinomas, circumferential membrane and cytoplasmic staining were prominent (Figures 2c and d). A marginal pattern of labeling was often seen in tight clusters of ductal carcinoma cells infiltrating lymphatic spaces. In lobular carcinomas, ICLs were well defined by a dark diaminobenzidine reaction product (Figures 2e and f). Malignant cells of mammary Paget's disease had cytoplasmic, membrane and ICL staining (Figure 2g). Such staining was strong and consistent and could be easily differentiated from the occasional staining of epidermal keratinocytes, some of which reacted with MBr1, particularly in cases with inflammatory changes.[15] In medullary carcinomas, small clusters of tumor cells only had intense cytoplasmic staining, whereas the bulk of the tumor was unreactive. In mucinous carcinomas, a few nests of tumor cells, surrounded by epithelial mucin, had marginal labeling associated with focal staining of the adjacent mucin. Focal apical reactivity was detected in one out of three papillary carcinomas tested.

The relationship between MBr1 staining and histologic grading[22] in ductal carcinomas (Table 2) revealed high incidences of reactive (+ +) and diffusely reactive (+ + +) cases and a low incidence of focally reactive (+) cases in Grade 1 tumors. Such differences were not statistically significant when evaluated with the conventional chi-square test but were consistent

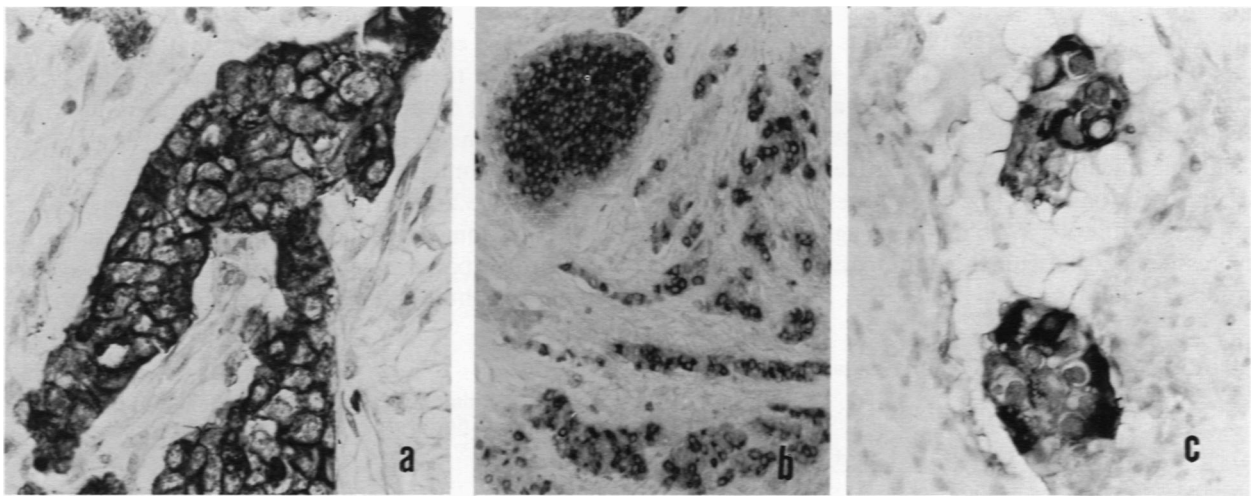

Figure 1—Reactions of MBr1 on breast carcinomas. a—Membrane and cytoplasmic staining in an infiltrating ductal carcinoma. (ABC IP, ×1050) b—Patchwork staining heterogeneity in an infiltrating lobular carcinoma: notice the strongly positive cells adjacent to unreactive cells. (ABC IP, ×400) c—Patchwork heterogeneity of staining in neoplastic emboli within a mammary lymphatic vessel. (ABC IP, ×1320)

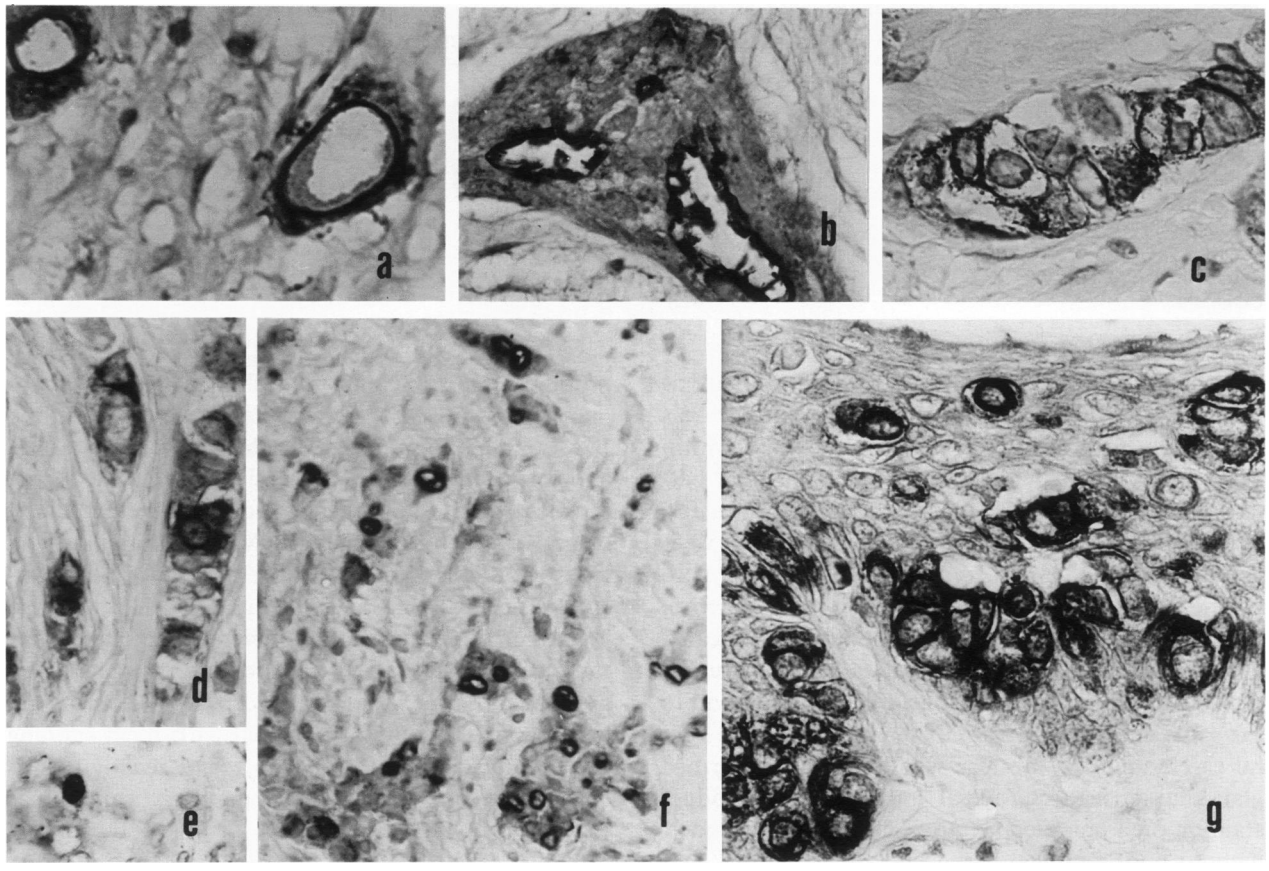

Figure 2—Different distributions of MBr1 reactivity in infiltrating breast carcinomas. **a**—Apical reaction in a well-differentiated ductal carcinoma. (ABC IP, × 1650) **b**—Labeling restricted to the apical cell membranes along the neoplastic lumens of a ductal carcinoma. (ABC IP, × 1650) **c**—Cell membrane reaction in a ductal carcinoma. (ABC IP, × 1650) **d**—Heterogeneous cytoplasmic staining in a lobular carcinoma. (ABC IP, × 1650) **e, f**—Labeling restricted to ICL in a lobular carcinoma (ABC IP, **e**, × 2050; **f**, × 1030) **g**—Cytoplasmic and membrane reaction of malignant cells in Paget's disease of the nipple. (ABC IP, × 1650)

with the higher incidence of reactivity in tubular carcinomas (Table 1). The correlation between MBr1 staining and nodal status (Table 3) revealed a high proportion of focally reactive cases (+) and a low proportion of diffusely reactive (+++) cases in tumors from patients with more than three positive axillary lymph nodes. The differences were not statistically significant by the chi-square test but were consistent with the trend toward a more diffuse staining of Grade 1 tumors. With regard to age, MBr1 antigen expression was documented in patients of all age groups (Table 4).

Reactions on areas of intraductal and in situ lobular carcinoma are shown in Table 5. Correlation of the staining of foci of noninvasive carcinoma with that of the corresponding infiltrating tumor revealed concordant reactivity in 30 of 33 cases studied (91%). In 20 of 33 cases (61%), both noninvasive and invasive components reacted positively; in 10 cases (30%), both were unreactive; and in 3 cases (9%), only the noninvasive and invasive components reacted differently. In some specimens, particularly those with a cribriform pattern, labeling was associated with luminal membranes and secretion product (Figure 3a).

Table 2—MBr1 Staining of Primary Ductal Carcinomas and Histologic Grading

	− (%)	+ (%)	++ (%)	+++ (%)	Positive/ total (%)
Grade 1	2 (22)	0 (0)	2 (22)	5 (56)	7/9 (78)
Grade 2	12 (44)	3 (11)	4 (15)	8 (30)	15/27 (56)
Grade 3	3 (21)	5 (36)	1 (7)	5 (36)	11/14 (79)
Total					33/50 (66)

Table 3—Nodal Status and MBr1 Staining of Primary Breast Carcinomas (Infiltrating Ductal and Lobular)

Positive nodes	− (%)	+ (%)	++ (%)	+++ (%)	Positive/ total (%)
−	11 (29)	5 (13)	5 (13)	17 (45)	27/38 (71)
1-3	4 (40)	1 (10)	2 (20)	3 (30)	6/10 (60)
>3	5 (31)	6 (38)	2 (12)	3 (19)	11/16 (69)

Table 4—MBr1 Staining and Age in Invasive Ductal and Lobular Carcinomas

Age	− (%)	+ (%)	+ + (%)	+ + + (%)	Positive/ total (%)
≤35	2 (40)	2 (40)	0 (0)	1 (20)	3/5 (60)
35–50	11 (28)	5 (13)	5 (13)	18 (46)	28/39 (72)
>50	9 (32)	7 (25)	4 (14)	8 (29)	19/28 (68)

In solid growth patterns, staining was also cytoplasmic and circumferentially membranous. There was an intense reactivity of apical buds that projected into the lumen in several cases. Reactivity of ICL was occasionally seen in intraductal carcinomas and was more prominent in *in situ* lobular lesions (Figure 3b).

Prognostic Study

Primary breast tumors from 240 T1NO patients (114 T1N- and 129 T1N+) were examined and divided into three subsets according to their MBr1 reactivity: unreactive (−), focally reactive (+), and reactive (+ +/+ + +). The proportions of these three subsets were comparable in the N− and N+ groups (Table 6). The differences were not statistically significant by the chi-square test. The incidences of disease relapse (distant metastases and/or local recurrences), separately considered for the N− and N+ groups, were similar in the MBr1 reactivity subsets (Table 6). Differences in the incidence of disease relapse, when one considers the unreactive subset versus the focally reactive and reactive subsets combined (ie, 9/58, or 16%, versus 10/71, or 14%, for the N− group and 10/39, or 20%, versus 13/52, or 25%, for the N+ group), or the unreactive and focally reactive subsets combined versus the reactive subset (ie, 14/78, or 18%, versus 5/50, or 10%, for the N− group and 17/58, or 29%, versus 6/33, or 15%, for the N+ group), were also not statistically significant by the chi-square test.

Metastatic Breast Carcinomas

The reactivity of metastatic cells in axillary lymph nodes and in bone marrow and peritoneal biopsies is shown in Tables 7 and 8, respectively. Sampling bias might have contributed to the lower percentage of positive cases, particularly in biopsies, where a limited amount of tissue, with small tumor clumps or isolated tumor cells, was available for study. Nonetheless, a somewhat lower incidence of MBr1 staining in metastatic lesions was suggested by the correlation of the immunoreactivity of nodal metastases with that of primary tumors in cases with adequately sampled lesions (Table 7). The data revealed concordant reactivity in 34 of 40 cases (85%). In 6 cases (15%), the metastases were immunonegative, whereas primary tumors were immunopositive for the antigen. In no case with an immunoreactive metastasis was the primary tumor negative. Metastatic cells in lymph nodes had a degree of heterogeneity in their reactions that was comparable to that of the primary tumor. Even in diffusely reactive cases, foci of unreactive tumor cells were present.

Patterns of labeling distribution included circumferential membrane (Figure 4a), cytoplasmic (Figure 4b), and, in metastatic lobular carcinomas, ICL staining (Figure 4c). Marginal staining was focally observed in tight clumps of malignant cells within lymphatics adjacent to metastatic lymph nodes or in nodal sinuses.

Heterogeneity of reactivity was also documented in peritoneal and bone marrow metastases (Table 8). The labeling of metastatic cells lining the peritoneal cavity was frequently concentrated at the apical cell membranes but shifted to the entire circumference of the cell membrane in free-floating cells detached from the adjacent tumor (Figure 5). The labeling of cancer cells within mesenteric adipose tissues or in bone marrow was homogeneously distributed on the cell membrane and the cytoplasm (Figure 5b).

Nonneoplastic Breast Tissues

The reactivity of nonneoplastic mammary epithelium sampled in carcinoma mastectomy specimens was evaluated in 80 cases and correlated with the staining of the corresponding tumor in 60 cases. Nonneoplastic normal breast epithelium was reactive in 66 of 80 cases (82%). There was heterogeneity in the number of ducts and ductules staining in different cases: in about 50% of the cases, reactivity was restricted to a few ductules, in the other 50% it was

Table 5—Reactions of MBr1 on Areas of Noninvasive Breast Carcinoma

	− (%)	+ (%)	+ + (%)	+ + + (%)	Positive/total (%)
In situ lobular	3 (43)	1 (14)	1 (14)	2 (29)	4/7 (57)
Intraductal, solid and cribriform	8 (33)	4 (17)	1 (4)	11 (46)	16/24 (67)
Intraductal, papillary	2 (100)	0 (0)	0 (0)	0 (0)	0/2 (0)

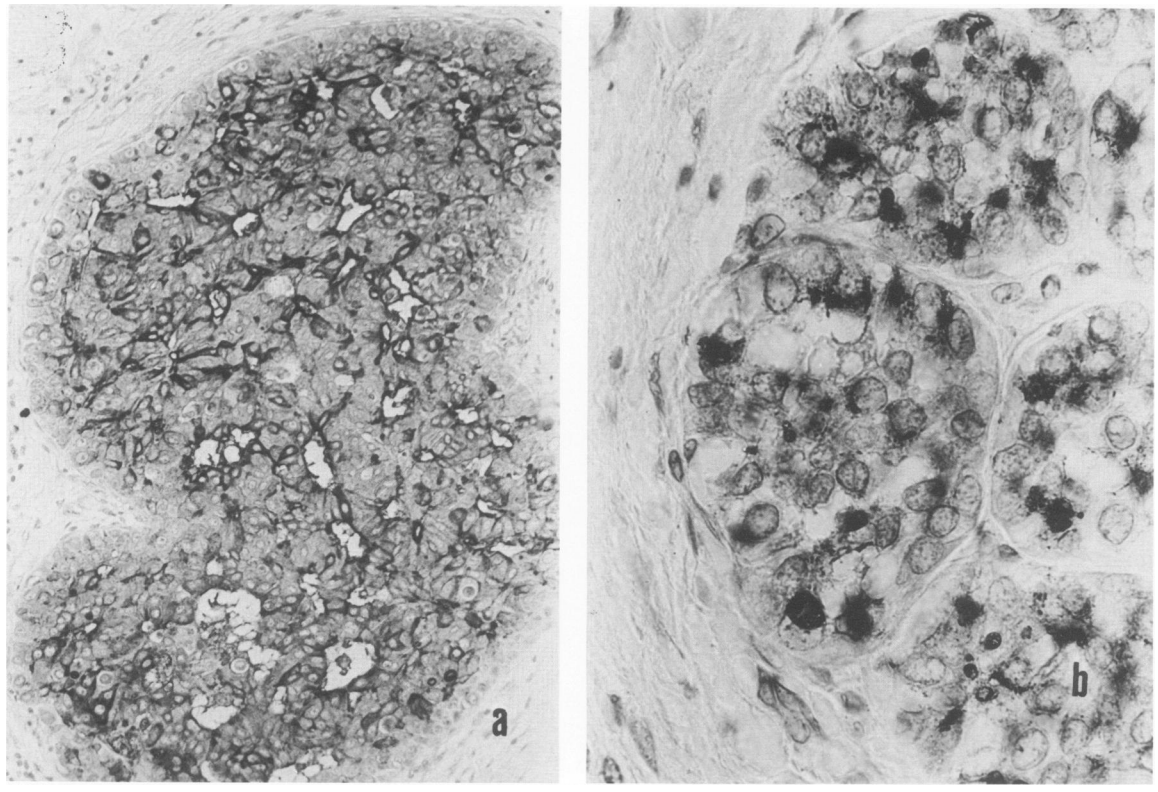

Figure 3—Different distributions of MBr reactivity in noninvasive breast carcinoma cells. **a**—Predominantly apical staining in an area of intraductal carcinoma with cribriform pattern. (ABC IP, ×660) **b**—Reactivity of ICL in an area of *in situ* lobular carcinoma. (ABC IP, ×2050)

more homogeneously distributed in the mammary glandular system. In contrast with the heterogeneous reactions observed in different ductal or ductular structures, there was very little heterogeneity among adjacent, nonneoplastic cells that lined the same glandular lumen (Figures 6a and b). The correlation between the staining of the mammary epithelium and that of the corresponding invasive carcinoma revealed that there was a high degree of concordance of antigen expression (in 55 of 60 cases, 92%) between the two. In 45 of 60 cases (75%), mammary epithelium and carcinoma cells reacted positively, whereas in 10 of 60 cases (17%) both were unreactive. In 5 cases only (8%), nonneoplastic epithelia reacted, whereas tumors were unreactive, but in no case was there staining of cancer cells when the nonneoplastic epithelium was unreactive.

Labeling of nonneoplastic epithelia was predominately located at the apical membranes and in the secretion products (Figure 6a), although differences were observed in some lobular epithelia, where a more cytoplasmic distribution was documented (Figure 6b). Reactivity of apical buds was often detected in ductal epithelia. The staining of the attenuated mammary epithelium that lined cysts in areas with fibrocystic changes was common, whereas the staining of cysts with apocrine metaplasia was focal and rare.

With regard to the labeling of breast tissues with lactational changes, foci of strong reactivity, which

Table 6—Disease Relapse (Distant Metastases and/or Local Recurrences) and MBr1 Reactivity in T1NO Primary Breast Carcinomas

		T1N−				T1N+		
Reaction	Patients (%)	NE*	%	Relapse (%)	Patients (%)	NE	%	Relapse (%)
−	58 (45)	49	84	9 (16)	49 (43)	39	80	10 (20)
+	21 (16)	16	76	5 (24)	26 (23)	19	73	7 (27)
+ +/+ + +	50 (39)	45	90	5 (10)	39 (34)	33	85	6 (15)

* No event.

Table 7—MBr1 Reactivity of Nodal Metastases and of Primary Breast Carcinomas (Metastasis/Primary): Concordant Reaction in 34/40 Cases (85%)

−/− (%)	−/+ (%)	+/− (%)	+/+ (%)	Positive/ total (%)
11 (27)	6 (15)	0 (0)	23 (58)	23/40 (58)

were restricted to individual lobular units, were detected in the two specimens tested (from breast cancer patients in the third trimester of pregnancy). Cytoplasmic and secretion product staining appeared more prominent than in resting breast (Figure 6c).

The reactions of MBr1 on breast tissues from patients with benign breast diseases is shown in Table 9. Labeling was localized at the apical membranes in fibroadenomatoid mammary dysplasia and, focally, in sclerosing adenosis. Reactivity was seen in the epithelial component of fibroadenomas and in gynecomastia. Apical and secretion product staining predominated, but cytoplasmic reactivity was also present. An intraductal papilloma and a specimen with juvenile hyperplasia (from a 12-year-old) were unreactive.

Nonepithelial Tumors of the Breast

Malignant nonepithelial tumors of the breast were unreactive with MBr1 (Table 10). In contrast, the mammary epithelium adjacent to, or entrapped within, these lesions, or the benign epithelial component of malignant phyllodes tumors, was positive. Staining of the apical cell membranes and apical buds, similar to that of normal mammary ducts, was often seen in such benign epithelia.

Discussion

The studies reported here demonstrate MBr1 staining in normal and lactational breast epithelium, as well as in primary and metastatic breast carcinomas. Nonepithelial breast tumors were unreactive. Benign breast lesions within the morphologic spectrum of fibrocystic disease and the epithelial component of fibroadenomas were found to be reactive. Gynecomastia was strongly positive. With regard to its overall reactivity with different histologic types of breast carcinoma, MBr1 appeared to discriminate a group of tumors with a high positivity incidence (ie, tubular, ductal, lobular, and mixed ductal and lobular types), a group with a low positivity incidence (ie, mucinous, medullary and papillary carcinomas), and an apparently unreactive group (ie, carcinomas with metaplasia, breast carcinoids). Heterogeneity of staining was seen in primary tumors, intravascular tumor emboli, and nodal and distant metastases. Ductal, tubular, lobular, and Paget's varieties included unreactive, focally antigen-positive, and diffusely antigen-positive tumor subsets, whereas mucinous, medullary, and papillary tumors were found to be either antigen-negative or focally positive. The heterogeneity of nonneoplastic mammary epithelium, either resting or with lactational changes, differed from that of carcinomas, in that staining variations usually occurred between adjacent histologic units (ie, ductules or lobules), rather than between adjacent cells. The heterogeneity of staining revealed by MBr1 was consistent with findings reported for different polyclonal serums[23,24] and MAbs[1,7,8,20] to breast epithelium or breast carcinoma. The nature and implications of heterogeneous antibody binding to cancer cells have been discussed in detail by Horan Hand et al.[21]

Differences in the cytodistribution of the reactivity could be correlated with specific histologies. Immunoreactivity predominated at the apical cell membranes in microscopically normal breast epithelium and in epithelia with fibrocystic changes. Tubular and well-differentiated ductal carcinomas were similar in that apical staining was largely dominant. In contrast, poorly differentiated carcinomas were characterized by circumferential membrane and cytoplasmic staining. Reactivity of ICL was especially prominent in lobular carcinomas, both *in situ* and infiltrating. Results consistent with such findings were reported for polyclonal serums to T antigen,[25] for lectins,[25-27] polyclonal serums,[28-30] and MAbs[4,7,8] to human milk fat globule membrane (MFGM) antigens, and for MAbs to other breast-carcinoma-associated antigens.[10,11] Finally, it should be pointed out that lectin binding carbohydrate chains were demonstrated in ICLs of lobular carcinomas.[31]

Positive staining was detected in patients from the premenopausal and postmenopausal age groups. There were no statistically significant associations between MBr1 reactivity of primary tumors and histo-

Table 8—Reactions of MBr1 on Distant Breast Carcinoma Metastases

	− (%)	+ (%)	+ + (%)	+ + + (%)	Positive/total (%)
Peritoneum	2 (50)	0 (0)	0 (0)	2 (50)	2/4 (50)
Bone marrow	6 (46)	1 (8)	5 (38)	1 (8)	7/13 (54)

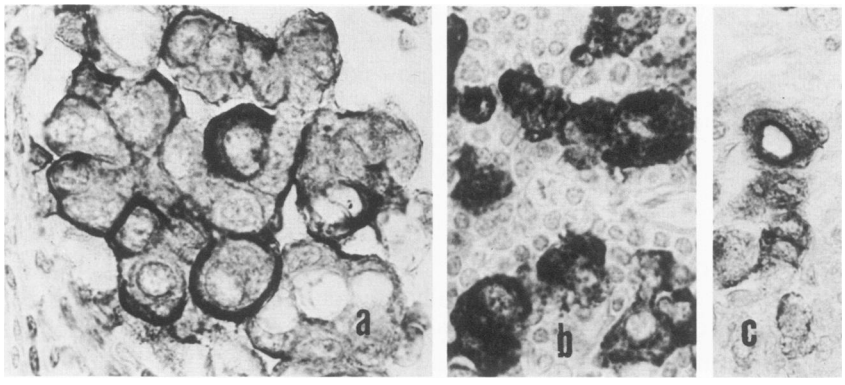

Figure 4 — Different distributions of MBr1 reactivity in breast carcinoma nodal metastases: cell membrane (**a**), cytoplasmic (**b**), and ICL (**c**) staining. (ABC IP, × 1650)

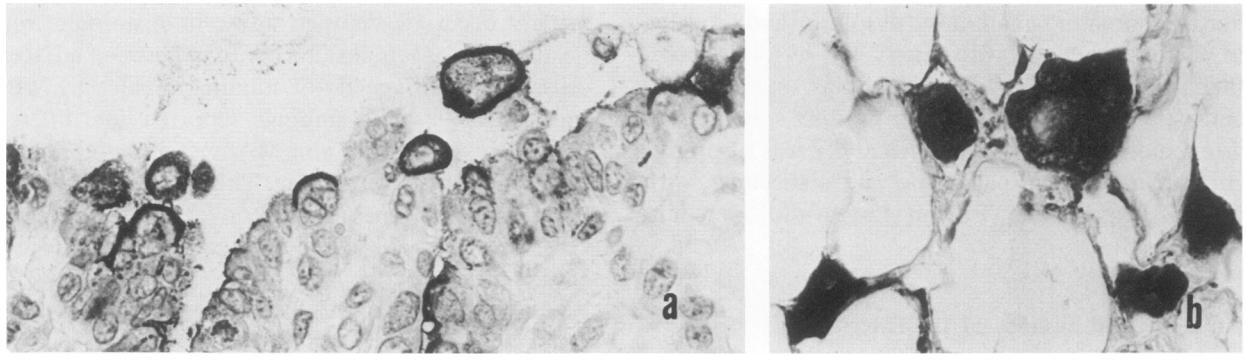

Figure 5 — Reactions of MBr1 on distant breast carcinoma metastases. **a** — Metastatic breast carcinoma in a peritoneal biopsy: notice apical membrane reactions of tumor cells adherent to substrate and circumferential membrane staining of free-floating tumor cells. (ABC IP, × 1650) **b** — Strong cytoplasmic reaction of breast cancer cells metastatic in intertrabecular marrow space occupied by adipose tissue. (Bone marrow biopsy, ABC IP, × 1650)

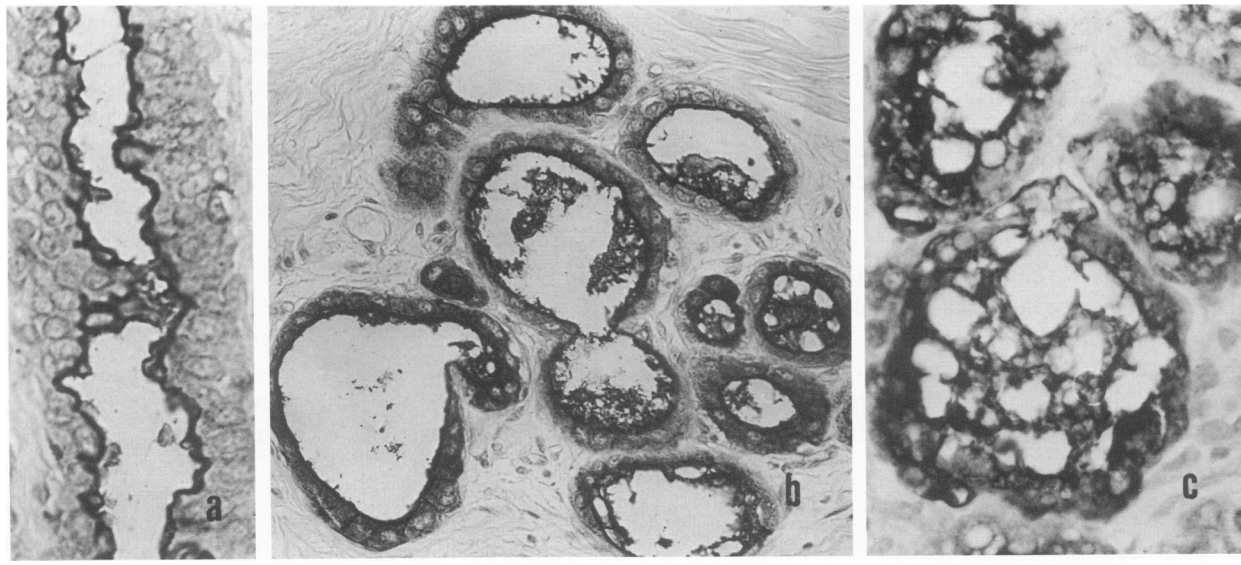

Figure 6 — Reactions of MBr1 on nonneoplastic breast epithelia. **a** — Apical labeling in a normal breast ductule. (ABC IP, × 1650) **b** — Apical and cytoplasmic staining in a mammary lobule. (ABC IP, × 830) **c** — Secretion product, apical and cytoplasmic reaction in mammary acini with lactational changes. (ABC IP, × 1650)

Table 9—Reactions of MBr1 on Benign Breast Lesions

	–	+	+ +	+ + +	Positive/total
Fibroadenoma	3	3	1	1	5/8
Sclerosing adenosis	4	1	0	0	1/5
Fibroadenomatoid dysplasia	0	1	0	0	1/1
Gynecomastia	0	0	0	1	1/1
Intraductal papilloma	1	0	0	0	0/1
Juvenile hyperplasia	1	0	0	0	0/1

logic grading or nodal status. However, trends were noted toward a more homogeneously diffuse reactivity of Grade 1 ductal carcinomas and toward higher proportions of focally reactive and unreactive tumors in patients with more than three positive axillary nodes. With regard to potential prognostic implications, we failed to substantiate any value for the MBr1 staining of primary tumors in the prediction of the clinical course of T1NO patients. There appeared to be relationships between the reactivities of nonneoplastic epithelium, noninvasive carcinoma, and invasive carcinoma. Similarly, the MBr1 reactivity of primary tumors was correlated to that of nodal metastases. Staining of nodal metastases was never documented when the primary lesion was MBr1 negative. However, some metastases of reactive primary carcinomas were unreactive.

The specificities of staining obtained with MBr1 in breast tissues and tumors were comparable to those reported for other MAbs to antigens shared by breast epithelium and breast carcinoma. Foster et al[7,8] reported the reactivities of four MAbs to MFGM. Antibody LICR-LON/M8 appeared similar to MBr1, in that staining was related to cell polarity in nonneoplastic epithelium and carcinomas. Its spectrum of reactivity included ductal, lobular, mucinous, and medullary carcinomas. Antibody LICR-LON/M18 differed from MBr1 in its low incidence of reactivity with breast carcinomas and in that staining was predominantly cytoplasmic. Antibodies LICR-LON/M3 and LICR-LON/M24 could be readily distinguished from MBr1 in that they were reported to react with interepithelial cell membranes in nonneoplastic breast epithelium. The two MAbs to MFGM reported by Arklie et al[4] appeared similar to MBr1 in their immunohistologic reactivity with breast tissues and tumors. In contrast, three MAbs against an estrogen-induced protein[9] were distinct from MBr1 in that they did not react with fibroadenomas, gynecomastia, or lactating breast. Other MAbs against breast-carcinoma-associated antigens[1,20,32] clearly differed from MBr1 in that they showed limited immunohistochemical binding to nonneoplastic mammary epithelium and a more restricted staining of mammary carcinomas.

The potential staining of poorly differentiated breast carcinomas, the lack of reactivity of nonepithelial breast tumors, the labeling of metastatic mammary carcinomas, and the possibility of using conventionally paraffin-embedded material suggest a potential diagnostic role of MBr1 in tumor histopathology. With regard to such an application, it should be pointed out that the immunohistologic reactivity of MBr1 was previously shown[15] to be widely distributed in epithelial tissues other than breast and in nonbreast carcinomas. Therefore, MBr1 staining in metastatic lesions may not be helpful in determining their primary site, but it can be useful in confirming an epithelial origin. The differential binding of MBr1 to primary breast tumors could also be exploited for a more precise histotyping of such lesions. In view of its lack of reactivity with benign mesothelial cells,[15] we successfully applied MBr1 to the immunocytochemical screening of carcinoma cells in serous effusions from breast cancer patients.[33] In addition, we are currently studying the ability of MBr1 to detect epithelial micrometastases of isolated carcinoma cells in bone marrow biopsy specimens from breast cancer patients.

Table 10—Reactions of MBr1 on Nonepithelial Breast Tumors and Adjacent Breast Epithelia (Positive/total)

	Tumor	Mammary epithelium
Malignant fibrous histiocytoma	0/2	2/2
Malignant phyllodes tumor	0/3	2/3
Angiosarcoma	0/1	NT*
Lymphoma (immunoblastic)	0/1	1/1

* Not tested.

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