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Report

Class distribution of immunoglobulin-containing plasma cells in the stroma of medullary carcinoma of breast

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Summary

A class distribution of plasma cells associated with the stroma in twenty-eight cases of medullary carcinoma of the breast was investigated by an unlabeled immunoperoxidase method. The stroma of the medullary carcinomas tested was found to contain predominantly IgG plasma cells except in two cases. Stroma of the other types of breast carcinoma, including ten cases of papillo-tubular carcinoma, five cases of scirrhous carcinoma, and six cases of medullary tubular carcinoma, contained predominantly IgG plasma cells, although few plasma cells were associated with carcinoma tissues in the latter group. Plasma cells associated with control specimens, including normal breast, fibroadenoma, cystic disease, and intraductal papilloma, were found to be predominantly of IgA type. Few carcinomatous epithelial cells contained secretory components in the cytoplasm, while a number of cells positive for secretory components were observed in acinar and ductular epithelia of normal breast tissues and in benign proliferative lesions of the breast. It is suggested that the lymphoid cells infiltrating the stroma of medullary carcinoma represent a sign of host immune response against the carcinoma cells which is related to the well-known favorable prognosis associated with this tumor.

Introduction

Epidemiological studies have revealed that the incidence of breast cancer in Japanese women is only about one-sixth of that of Caucasian women of corresponding ages, and survival rates for patients with breast cancer are also higher in Japan than in the United States (1). Comparative histological studies on breast cancer between these two racial groups have shown that a medullary histologic type is commoner and lymphoplasmacytic infiltration in the cancer tissue is more marked in Japanese cases than in American cases (2–6).

It is well known that the mammary gland is a component of the secretory immune system. Differences in immunoglobulin concentrations between benign and malignant breast lesions have been attracting attention from many workers. Recently, McCarty et al. (7) reported that an association of IgA and IgM with benign breast lesions was in significant contrast to the association of IgG with malignant lesions in direct immunofluorescent studies of different types of breast lesions, although they did not refer to the relationship between histological types of breast cancer and immunoglobulin

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classes. Hsu et al. (8) reported that plasma cell-rich stroma of medullary carcinoma contained predominantly IgA plasma cells, suggesting the persistence of normal epithelio-stromal relationships in this type of breast cancer. In attempting to find possible reasons for the difference between the two races in the nature of breast cancer, we tried to reassess the class distribution of Ig-containing plasma cells in medullary carcinoma in comparison with other types of breast carcinomas as well as benign lesions in Japanese cases.

Materials and methods

Tissues

Paraffin blocks of biopsy specimens of breast cancers were collected from the Division of Pathology, Clinical Laboratory of the Nagoya University Hospital, the Aichi Cancer Center-Hospital, and Nagoya National Hospital. These included twenty-eight cases of medullary carcinoma, ten cases of papillotubular carcinoma, five cases of scirrhous carcinoma, and six cases of medullary tubular carcinoma. Normal breast tissues from nine autopsy cases, six cases of fibroadenoma, and four biopsy cases of cystic disease used as controls were also obtained from Nagoya University Hospital. All cases of medullary carcinoma presented the histologic characteristic proposed by Ridolfi et al. (9) for typical medullary carcinoma. The histological type also fulfilled the criteria standardized by W.H.O. (10). Mean diameters (the greatest and shortest diameter and thickness) of individual cases of this type ranged from 1 cm to 6 cm as measured in the fresh resected specimens, and the average tumor size of all these cases was 3.0 cm. None of these cases had axillary metastases or skin involvement at the time of surgery. The other types of breast carcinoma were selected, based on similar criteria as adopted by Hsu et al: 1) tumor size of approximately 3 cm in diameter, 2) absence of axillary node metastasis or skin involvement at the time of surgery, and 3) the presence of at least five plasma cells per high power field (objective lens: × 40) in the stroma. Patient ages and tumor sizes of the cases used are summarized in Table 1. According to the protocols for the preparation of light microscopic specimens in each institution, the specimens used in this investigation were all fixed with phosphate-buffered 10% formalin. Serial sections 5 μm thick were made from the paraffin blocks.

Immunoperoxidase staining

The peroxidase-antiperoxidase (PAP) method (11) was used in this investigation. Rabbit antisera (IgG fraction) specifically directed against heavy chains of human IgG, IgM, and IgA (Hoechst, West Germany) were used as the primary antisera. A goat antiserum to rabbit IgG was made in our laboratory by immunizing a goat with purified rabbit IgG, and rabbit antiperoxidase-peroxidase complex was purchased from Dako Co. Ltd. U.S.A. Rabbit antiserum against human secretory component (SC) was purchased from Dako (U.S.A.). The antiserum was absorbed with immobilized human serum to eliminate the antibody activity to IgA and diluted with phosphate-buffered saline (PBS), pH 7.2, at 1:200 before use.

Immunohistochemical staining was performed according to the methods recommended by Jacobson et al. (12). Before staining, the deparaffinized sections were first immersed in 0.3% hydrogen peroxide solution in absolute methanol for 30 minutes to block the endogenous peroxidase activity, followed by treatment with normal, nonimmune goat serum (diluted at 1:10) for ten minutes to reduce nonspecific background staining. Then the sections were covered with one of the primary antisera (diluted at 1:400) and incubated. After being washed extensively in phosphate-buffered saline, the sections were similarly incubated in the goat antiserum against rabbit IgG (diluted at 1:30), followed by incubation with rabbit antiperoxidase-peroxidase complex (diluted at 1:100).

Each step of the incubation with the antisera lasted 30 minutes at 37° C, followed by washing with PBS for 30 min. After being stained with diaminobenzidine (DAB) (DOTITE) solution to localize the peroxidase sites, the sections were rinsed in water and counterstained with hematoxylin.

Phosphate-buffered saline or nonimmune sera (normal rabbit and goat sera) were used as substitutes in each step to test the nonspecific binding of antisera, and the results were consistently negative.

Plasma cell counting

Ig-containing cells in breast tissues were enumerated by a method similar to that used by Hsu et al. (8). In short, the class distribution of the Ig-containing cells in 49 cases of breast carcinoma was determined by examination of 50 random high power fields (HPF) (objective × 40) of each serial section stained for individual heavy chains. The number of positively stained cells was scored. The class distribution of the Ig-containing cells in fibroadenoma, mammary dysplasia, and intraductal papilloma was also determined in much the same way as above.

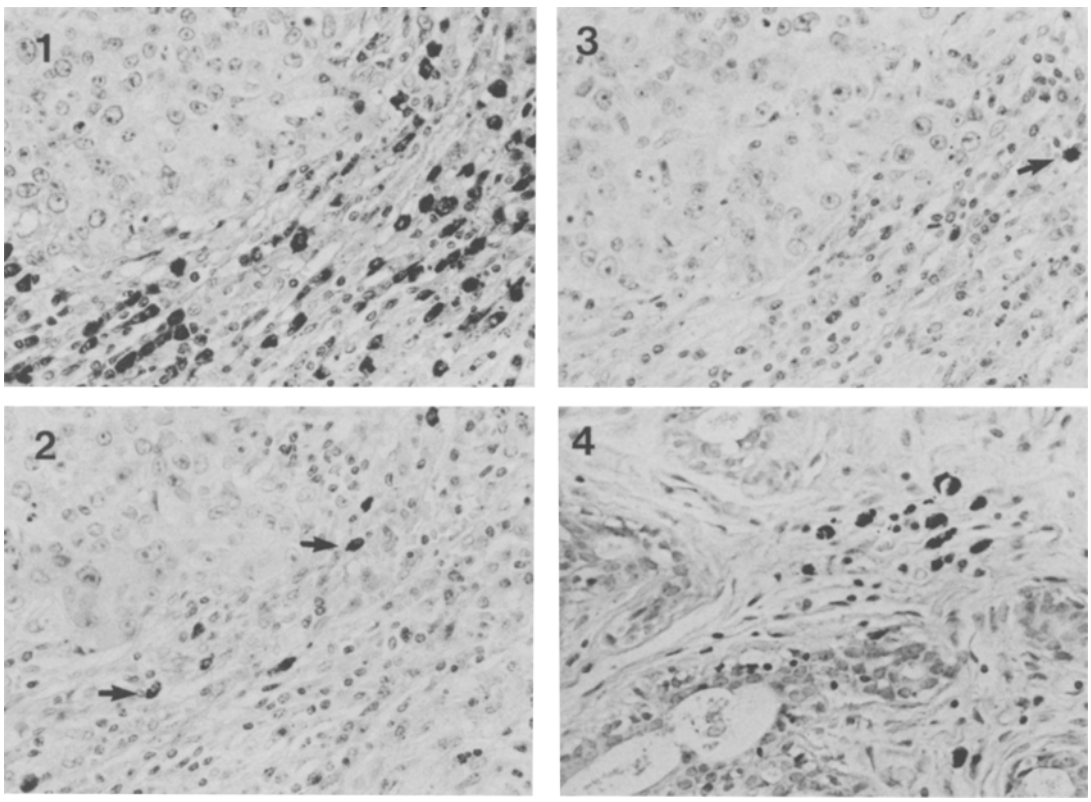
In normal breast tissue, in which the plasma cells tend to localize near the acinar or ductal epithelium, the Ig-containing cells were counted on adjoining sections from the same lobules. For each

Table 1. Summary of immunoperoxidase staining of human breast tissues, and benign and malignant breast lesions.

	Number of cases	Average age	Average tumor size (cm)	Percentage distribution of plasma cells		
				IgA	IgG	IgM
Normal Breast	9	36		81 ± 11.0[1] (60–94)[2]	12 ± 7.5 (3–27)	7 ± 8.8 (0–30)
Fibroadenoma	6	40		89 ± 6.4 (79–96)	9 ± 5.9 (3–20)	2 ± 2.1 (0–6)
Mammary Dysplasia	4	40		92 ± 4.5 (85–97)	5 ± 3.6 (1–10)	3 ± 2.0 (1–5)
Intraductal Papilloma						
IgA dom.	4	40		72 ± 18.7 (52–92)	25 ± 21.2 (1–48)	3 ± 3.5 (0–9)
IgG dom.	1	59		38	61	1
Medullary Carcinoma						
IgA dom.	2	44	1.4	48 ± 2.4 (46–50)	38 ± 9.3 (29–48)	14 ± 11.5 (2–2.5)
IgG dom.	26	48	3.2	11 ± 9.0 (0–39)	84 ± 10.7 (57–99)	5 ± 5.8 (0–24)
Invasive Duct Carcinoma						
Papillotubular Type						
IgA dom.	4	47	2.7	76 ± 8.7 (63–87)	22 ± 9.2 (10–34)	2 ± 0.8 (1–3)
IgG dom.	6	66	2.5	16 ± 7.8 (4–28)	83 ± 8.3 (70–96)	1 ± 0.9 (0–2)
Medullary Tubular Type						
IgA dom.	2	59	2.2	68 ± 4.3 (64–73)	31 ± 3.3 (27–34)	1 ± 1.0 (0–2)
IgG dom.	4	47	2.9	8 ± 3.0 (5–13)	84 ± 13.1 (62–95)	8 ± 13.1 (0–30)
Scirrhous Type						
IgA dom.	1	41	2.5	93	7	0
IgG dom.	4	52	3.9	16 ± 13.3 (1–38)	82 ± 14.9 (58–99)	2 ± 1.7 (0–4)

[1] Mean S.D.

[2] Range of distribution



Figs. 1–3. Immunoperoxidase staining of serial sections of a medullary carcinoma of the human breast with antisera to immunoglobulin. All sections were counterstained with hematoxylin. 320 ×. Fig. 1 shows abundant IgG-containing cells surrounding the tumor alveoli. Fig. 2 shows small numbers of IgA-containing cells (arrows). Fig. 3 shows few IgM-containing cells (arrow).

Fig. 4. Immunoperoxidase staining of normal human breast tissue with antiserum to IgA. Most lymphoid cells in the connective tissue were heavily stained. 320 ×.

section, the total number of Ig-containing plasma cells was calculated by counting those in ten lobules.

Ig class distribution of plasma cells was determined as a percentage of the total stained plasma cells in each case (for instance, numbers of IgG plasma cells/summation of IgG + IgA + IgM plasma cells in 3 serial sections × 100 for % IgG plasma cell). The results were expressed as mean percentages.

Results

The class distribution of plasma cells are summarized in Table 1.

Carcinomas

Medullary carcinoma

In twenty-six cases out of twenty-eight, the plasma cell population was predominantly of IgG cells (Figs 1–3), and the average proportion of IgA, IgG, and IgM plasma cells was 11, 84, and 5, respectively. In only two cases did the plasma cells in medullary carcinoma show a class distribution in favor of IgA cells, and the ratio of IgA, IgG, and IgM plasma cells was 48:38:4.

Medullary tubular carcinoma

IgG plasma cells increased in population in this type. In four out of six cases, the plasma cell class was predominantly IgG cells, and the average proportion of IgA, IgG, and IgM was 8:84:8. In the

remaining two cases the plasma cells showed a class distribution in favor of IgA cells; the average proportion of IgA, IgG and IgM, was 68:31:1.

Papillotubular carcinoma

In six out of ten cases, the plasma cell class distribution was predominantly IgG cell, and the IgA, IgG, and IgM plasma cell ratio was 16:83:1.

In the remaining four cases, the plasma cells showed a class distribution in favor of IgA cells; the proportion of IgA, IgG, and IgM plasma cells, was 76:22:2.

Scirrhous carcinoma

This type showed an increase in IgG plasma cell population. In four out of five cases, the plasma cell class distribution was predominantly IgG cell; the proportion of IgA, IgG, and IgM plasma cells was 16:82:2. In the one exceptional case, the plasma cells in scirrhous carcinoma showed a class distribution in favor of IgA cells (93:7:0).

Benign breast lesions

Fibroadenoma

The plasma cells in all cases of fibroadenoma examined were predominantly IgA cells (IgA 89, IgG 9, IgM 2).

Mammary dysplasia

The plasma cells in this type of breast lesion were predominantly IgA cells (IgA 92, IgG 5, IgM 3).

Intraductal papilloma

The plasma cells in intraductal papilloma were predominantly IgA cells. The average proportion of IgA, IgG, and IgM plasma cells was 72:25:3. There was one exceptional case in which the plasma cells showed a class distribution in favor of IgG cells (38:61:1).

Normal breast tissues

The plasma cells in normal breast were predominantly IgA cells (IgA 81, IgG 12, IgM 7) (Fig. 4).

Fig. 5 shows the relationship between percentages of IgG and IgA plasma cells for all cases examined (IgM plasma cells excluded). Most of the cancer cells are seen grouped toward the upper left corner of the figure, indicating IgG plasma cell predominance.

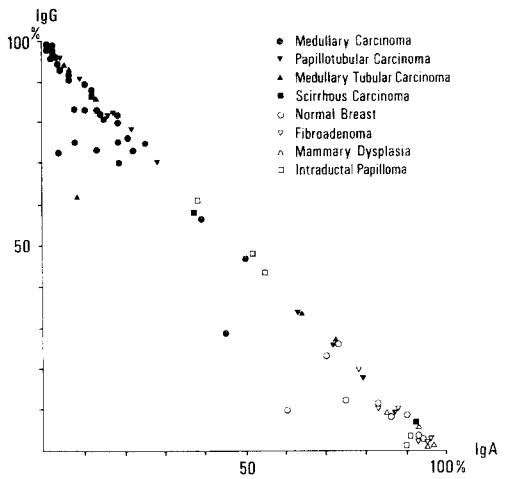


Fig. 5. Relationship between percentage of IgG and IgA plasma cells for all cases examined (IgM excluded). Most of the cancer cases are seen grouped toward the upper left corner of the figure, indicating IgG plasma cell predominance.

Distribution of secretory components (SC) in normal and diseased breast

Few SC-containing carcinoma cells were identified in any of the breast cancer specimens tested in this investigation. However, a number of SC-positive cells were observed in the acinar or ductular epithelia of normal breast as well as in those of dysplasia, intraductal papilloma, and fibroadenoma, although less frequently in the latter two.

Discussion

This study revealed that the vast majority of cases of medullary carcinoma of the breast in Japanese women contained predominantly IgG plasma cells in the stroma and few SC-positive cells in cancerous epithelia. In invasive duct carcinoma (including papillary tubular, medullary tubular, and scirrhous types) there were more cases with IgG plasma cell predominance than with IgA plasma cell predominance, although the difference in inci-

dence was not statistically significant.

Our results apparently disagree with those reported by Hsu et al. (8) with regard to medullary carcinoma, and agree well with those reported by McCarty et al. (7). Our results also agree with those reported by Roberts et al. (13), in which breast cancer tissues with a marked plasma cell infiltration contained significantly higher total immunoglobulin and IgG levels in tissue extracts than did those with minimal infiltration. The differences in histological criteria of medullary carcinoma seem to be negligible, because we followed exactly those proposed by Ridolfi et al. (9). Since it is often difficult to distinguish the atypical medullary carcinoma with decreased lymphoid cell infiltration from the medullary tubular carcinoma with scanty glandular structures, the cases of medullary carcinoma used in this investigation were restricted only to those with marked lymphoid cell infiltration. Our results on the class distributions of Ig-containing cells and the numbers of SC-positive epithelial cells in the controls, including normal mammary gland as well as benign mammary lesions, were almost identical with those reported by others (8, 14). From this we infer that the immunohistochemical procedures employed in this investigation were reliable. One possible explanation for the disagreement between our results and those of Hsu et al. is that the comparatively larger number of cases of medullary carcinoma used in the present investigation may influence the statistical results of class distribution of Ig-containing cells. Another possibility is that the IgA plasma cell predominance may occur at the early developmental stages of medullary carcinoma, because the two cases observed with IgA plasma cell predominance were significantly smaller in size than those with IgG plasma cell predominance.

It is well accepted that patients having medullary carcinoma with marked lymphoid cell infiltration have better prognosis than those with the other types of invasive breast carcinoma (9, 10, 15). Based on the results of immunohistochemical investigations showing that lymphoid cells infiltrating in breast cancer tissues were predominantly of T-cells, Shinohara et al. (16) suggested that the T-cells represented the host resistance against cancer and the intensity of the T-cell infiltration correlated with the clinical prognosis of the patients. Hsu et al. (8) speculated that the persistence of SC in medullary carcinoma cells together with IgA plasma cell predominance in the stroma represented better functional differentiation of the carcinoma type as compared with the other types of invasive breast carcinoma, and that this accounted for the more favorable prognosis. Although cellular factors are thought to play a key role in cancer immunity, our results may provide further evidence that the good prognosis of medullary carcinoma is related to antigenicity high enough to evoke the host immune response. The low antigenicity of the other types of invasive breast carcinoma is further suggested by the fact that lymphoid cells infiltrating the stroma were far fewer in number than those infiltrating the stroma of medullary carcinoma, and by the higher incidence of cases with IgA plasma cell predominance in the other.

Besides differences in some nutritional factors, certain immunological factors have been suggested to relate to the differing geographical or racial risks of breast cancers (17, 18). The incidence of medullary carcinoma is statistically higher in Japanese than in Caucasians (2, 3, 4, 6). Nevertheless, because of the low incidence of this carcinoma relative to the other types of breast cancer, it is unlikely that this influences the racial or geographical difference. The above discrepancy in the Ig-class distribution in the stroma of medullary carcinoma between Japanese and American cases may be attributable to some differences in immunological capability toward breast carcinoma between the two racial groups.

Teramoto et al. (19) reported that hybridoma cells made by fusion of axillary lymph node cells from breast cancer patients with murine myeloma cells liberated a human monoclonal antibody which reacted intensely with breast cancer cells, weakly with benign breast lesions, and not at all with normal mammary glands. Although it is not known from this investigation whether IgG plasma cells infiltrating carcinoma tissues actually produced antibodies directed against the carcinoma cells, the regional lymph node cells of medullary carcinoma

may be better resources for the production of monoclonal antibodies to breast carcinoma cells.

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