Characterization of A New Breast Cancer-Associated Antigen and Its Relationship to MUC1 and TAG-72 Antigens

YUKO HARADA¹, NORIAKI OHUCHI¹, TAKASHI MASUKO², YOSHIHITO FUNAKI¹, SHOZO MORI¹, SUSUMU SATOMI¹ and YOSHIYUKI HASHIMOTO³

¹The Second Department of Surgery, Tohoku University School of Medicine, ²Department of Molecular Biology, Pharmaceutical Institute, Tohoku University, and ³Department of Hygienic Chemistry, Pharmaceutical Institute, Tohoku University, Sendai 980-77

HARADA, Y., OHUCHI, N., MASUKO, T., FUNAKI, Y., MORI, S., SATOMI, S. and HASHIMOTO, Y. Characterization of A New Breast Cancer-Associated Antigen and Its Relationship to MUC1 and TAG-72 Antigens. Tohoku J. Exp. Med., 1996, 180 (3), 273-288. —— We have characterized a new tumor-associated antigen defined by monoclonal antibody (Mab) generated against HMA-1 breast cancer cell line. Mab AM-1 was selected based on its preferential reactivity to breast cancer cells versus to normal or benign epithelial cells by immunofluorescence and immunohistochemical assays of cultured, or fresh specimens. AM-1 demonstrated strong reactivity to breast cancer cell lines including HMA-1, YMB-1-E, YMB-1 and MDA-MB-231 in flow cytometry. In immunoprecipitation, AM-1 recognized high molecular weight components of 160-210 kDa and >370 kDa. Reactivity with HMA-1 cells was diminished markedly when treated by heat, protease or periodate, suggesting that the antigenic epitope is composed with carbohydrates and peptides. Enzyme digestion of precipitated antigens demonstrated that the antigen contains O-linked and N-linked carbohydrates with neuraminic acid structures. Furthermore, binding inhibition and sandwich ELISA assays using MAb reactive with known breast cancer-associated antigens and synthetic MUC1 core peptide (PDTRPAPGSTAPPAGVTSAPDTR) demonstrated that the antigen is distinct from CEA, TAG-72 or MUC1, while the antigen conjoins with MUC1 and TAG-72 as a trimmer form in HMA-1 cells. These results suggest that AM-1 recognizes a novel glycoprotein which is abundant in breast cancer, and may be utilized in the management of breast cancer patients. —— breast cancer; MUC1; monoclonal antibody; tumor-associated antigen

Monoclonal antibodies (MAbs) which identify tumor-associated antigens (TAAs) have been generated and some of them are now utilized in immunohistological or serological diagnosis of human epithelial malignancies. In our labo-
ratories, MAbs to TAA s on various human cancers or oncogene products have been generated and the applications of MAbs have been endeavored (Masuko et al. 1984, 1989). Among TAA s, mucin is one of the most important and valuable antigen. The cDNA coding several core proteins of mucin have recently been cloned and their structures have been extensively analyzed (Duhig et al. 1988).

The first characterized human mucin gene, designated MUC1, was previously described as EMA (Heyderman et al. 1979), PAS-O (Shimizu and Yamauchi 1982), NPG (Ceriani et al. 1983), MAM-6 (Hilkenes et al. 1984), PUM (Swallow et al. 1987), PEM (Duhig et al. 1988), or Episialin (Ligtenberg et al. 1990). MUC1 is a highly glycosylated, high molecular weight (> 400 kDa) glycoprotein, which occurs in human breast milk and is highly expressed in adenocarcinomas of the breast, colon, ovary and other organs. The cDNA clones of MUC1 revealed the protein containing tandem repeats of 20 amino acids (PDTRPAPGSTAPPAAHG-VTSA) and the corresponding gene is located on chromosome 1q21-24 (Swallow et al. 1987; Duhig et al. 1988). Only little is known, however, about the biological functions of MUC1. Recent reports have indicated that DF3 antigen, which contains MUC1 core peptide, may inhibit adhesion of eosinophile to antibody-coated targets (Hayes et al. 1990), or alter cellular aggregation (Ligtenberg et al. 1992). Many MAbs recognizing MUC1 have been generated (Croghan et al. 1983; Price et al. 1990; Devine et al. 1991; Perry et al. 1992) and some are clinically utilized as immunodiagnostic adjuncts such as CA15-3 (Hayes et al. 1986), DU-PAN-2 (Lan et al. 1987) and BCA225 (Mesa-Tejada et al. 1988).

Another breast cancer-associated mucin is TAG-72 (Johnson et al. 1986), which is also high molecular weight (> 1,000 kDa) glycoprotein, containing siaayl Tn antigen. This antigen is highly expressed in the breast, ovary, lung, colon and gastric adenocarcinomas (Thor et al. 1986; Ohuchi et al. 1986). MAbs B72.3 and CC49 recognizing TAG-72 antigen have been applied to serological diagnosis of the breast, ovary and gastrointestinal malignancies in radioimmunoassay, CA72-4 (Ohuchi et al. 1989a; 1990). These antigens are thus valuable as tumor markers in diagnosis of breast cancer patients. In the light of the degree of antigenic heterogeneity observed in most human carcinomas (Ohuchi et al. 1987; Ho et al. 1993), however, the use of mixtures of MAbs reactive with different antigen is essential for the application of MAbs as immunological adjuncts in the management of breast cancer (Ohuchi et al. 1989b). This study was initiated to develop new antibodies which might recognize antigens distinct from those previously defined by other MAbs.

We have generated two murine MAbs, AM-1 and AM-2, against a human breast cancer cell line, HMA-1 (Matano et al. 1991). HMA-1 has been shown to express various TAA s such as MUC1, TAG-72, siaayl Lex and c-erbB-2 protooncogene product (Ohuchi et al. 1995). We describe here that AM-1 and AM-2 recognize high molecular weight glycoprotein which is distinct from MUC1, TAG-72 and CEA. We also describe characterization of the antigen and discuss
the possible clinical application of the MAbs.

**Materials and Methods**

**HMA-1 cell line**

HMA-1 cell line was established from ascitic effusion from a 48 year old premenopausal female patient with breast cancer (Matano et al. 1991). Cytology of the ascitic fluids revealed adenocarcinoma which showed same histopathologic characteristic as the primary carcinoma of breast. HMA-1 was shown to be an epithelial cell line with intracytoplasmic vacuoles, microvilli, desmosomes and tonofibrils in accordance with human breast cancer. This cell line was positive for estrogen receptor. HMA-1 had a good cell growth ability with a doubling time of 46 hr, and was maintained by culture in RPMI-1640 medium containing 10% fetal calf serum (FCS).

**Monoclonal antibodies**

MAbs HMFG-1, HMFG-2 (Taylor-Papadimitriou et al. 1981), and SM-3 (Burchell et al. 1987, 1989), in culture supernatant were kindly offered by Drs. J. Taylor-Papadimitriou and J. Burchell, Imperial Cancer Research Fund, UK. MAbs DF3 (Kufe et al. 1984) and B72.3 (Johnson et al. 1986) were kind gifts from Dr. J. Schlom, National Cancer Institute, National Institutes of Health, USA. MAb MUSE11 in culture supernatant (Ban et al. 1989) was a kind gift from Drs. A. Yachi and K. Imai, Sapporo Medical College, Japan. MAb 4C8 was obtained from MBL, Nagoya, Japan.

**Production of hybridomas**

Male Balb/c mice were immunized with HMA-1 cells (3 × 10⁶) dissolved in cultured supernatant by three intraperitoneal and one intravenous injections of the cells. Cell fusion between the immunized spleen cells and the P3X63Ag 8.653 mouse myeloma cells was performed 3 days after the final booster injection. Selected hybridomas were cloned by limiting dilution method. Two IgM MAbs, AM-1 and AM-2, were selected because of their preferential reactivity with breast cancer cells.

**Flow cytometry**

Human cell lines originated from various tissues were examined to determine the reactivity of MAbs, AM-1 and AM-2. All cell lines (2 × 10⁶ cells) were incubated with MAb AM-1 or AM-2 at 10 μg/ml for 30 min on ice. After washing in PBS three times, samples were labeled with fluorescein isothiocyanate conjugated rabbit antimouse immunoglobulin (Dako, Kyoto). Surface immunofluorescence intensity of individual cells was determined using a FACScan (Becton Dickinson, Sunnyvale, CA, USA).
Immunoperoxidase staining

Normal tissues other than the breast were obtained at autopsy, while all breast tissues were obtained from surgical specimens. Formalin-fixed, paraffin-embedded tissues were cut at 5 μm in thickness. The sections were stained with MAb AM-1 or AM-2 using an avidin biotin immunoperoxidase complex system. In brief, endogenous peroxidase was inhibited by immersing sections in 0.3% H₂O₂ for 15 min, then diluted normal horse serum (×10) was dispensed to each section for blocking of nonspecific binding of staining reagents. After 30 min incubation, the sections were treated with culture supernatant of AM-1 (×2) or AM-2 (×5) for 60 min at 4°C, then incubated with biotinylated anti-mouse IgG (Dako) for 30 min. After a PBS wash, the slides were treated with avidin dehydrogenase and biotinylated horseradish peroxidase H complex (Vector Lab., Burlingame, CA, US) for 30 min. After another rinse in PBS, the sections were finally incubated with diaminobenzidine (Sigma, St. Louis, MO, USA) in 0.03% H₂O₂ for 5 min and then counterstained with hematoxylin.

Enzymatic and chemical treatments

In order to analyze antigenic determinants recognized by the MAbs, HMA-1 cells were pretreated with heat (100°C, 5 min), pronase (500 μg/ml, Sigma) or NaIO₄ (5 mM/ml, Sigma) before incubation of the MAbs with the cells. Following 3 washes in PBS, the reactivity of MAbs with HMA-1 cells were determined by flow cytometry.

Immunoprecipitation with enzymatic digestion

Surface antigens of HMA-1 cells were labeled with ¹²⁵I by glucose oxidase-lactoperoxidase method as described by Mitchell and colleagues (1981). The ¹²⁵I-labeled cells were dissolved in extraction buffer containing 0.5% Nonidet P-40 (Sigma), 0.01% Thimerosal (Sigma), Antipain (50 μg/ml, Wako, Osaka), Aprotinin (20 μg/ml, Wako), Leupepsin (50 μg/ml, Wako) and phenylmethylsulfonyl fluoride (1 mM, Wako) in TBS buffer (0.15M NaCl and 0.05M Tris, Wako). The supernatant of cell lysate was precleaned by 4 hr incubation with normal mouse serum binding gel. The residual antigenic components were immunoprecipitated with purified MAb AM-1 or AM-2 bound to FMP activated-cellulofine (Seikagaku Co., Tokyo). The immunoprecipitated gels were quartered and subjected to enzymatic treatments for 3 hr at 37°C using neuraminidase (0.1 U/ml, Seikagaku Co.), O-glycanase (25 mU/ml, Seikagaku Co.) or N-glycanase (10 U/ml, Seikagaku Co.). After washing with PBS 5 times, the antigenic components were extracted from the immune complex by heating with 2% SDS solution with 5% 2-mercaptoethanol. Then, the precipitates and standards for molecular weight determination were subjected to 6% SDS poly-acrylamide gel electrophoresis.
Sandwich enzyme linked immunosorbent assay (ELISA)

Sandwich enzyme-linked immunosorbent assay (ELISA) was performed using MAbs DF3, B72.3, 4C8 (anti-CEA), AM-1 and AM-2. Aliquot (50 µl) of antibodies (20 µl/ml) was passively absorbed in duplicate on 96 well plates (Sumitomo Bakerite, Tokyo) overnight at 4°C. After washing once with PBS, each well was treated with PBS containing 1% Bovine Serum Albumin (BSA) overnight at 4°C to block nonspecific binding of the staining reagents. Cell lysate of HMA-1 (50 µl) equivalent to 1 x 10^7 cells/ml was subjected to each well and incubated for one hr at room temperature. After washing 5 times with 0.05% Tween 20 (Wako) in PBS (T-PBS), 50 µl/well of biotinylated AM-1 or AM-2 (20 µg/ml) was dispensed into the plates. After incubation for 30 min, the wells were washed 5 times with T-PBS. Fifty micro liters of 1:200 diluted ABC was dispensed into each well and the plates were incubated for 30 min. The wells were washed 5 times prior to addition of tetramethyl-benzidine (0.1 mg/ml) and 0.01% H_2O_2 (100 µl/well) in 0.1 M citric-acetate buffer, pH 6.0. Color development in the wells was stopped after 5 to 10 min by addition of 0.5M H_2SO_4, and the optical density of the solution at 450 nm was measured with an IntreMed NJ-2000 automatic ELISA reader (SLT, Labinstruments, Austria).

Binding inhibition assays with MAbs recognizing known TAAAs

We performed binding inhibition assays to compare the antigen defined by AM-1 or AM-2 with other antigens including MUC1 and TAG-72. HMA-1 cells were incubated with biotinylated AM-1 or AM-2 (1 µg/ml) in the presence of MAbs HMFG-1, HMFG-2, SM-3, MUSE11, DF3, B72.3, AM-1 or AM-2 at 20 µg/ml (37°C, 60 min). After washing 3 times with PBS, samples were labeled with FITC-avidin (5 µg/ml) and analyzed by FACScan.

Inhibition assays with synthetic core peptide of MUC1

Synthetic peptide (PDTRPAPGSTAPAHGVTSAAPDTR) utilized as the protein core of MUC1 mucin was supplied from Toray Research Institute, Kama-kura, Japan. After an incubation with excessive synthetic peptide (100 µg/ml) for 1 hr at 37°C, HMA-1 cells were treated with MAbs HMFG-1, HMFG-2, SM-3, MUSE11, AM-1 and AM-2 at the concentration of 1 µg/ml for 30 min at 4°C. The cells were then labeled by FITC anti-mouse IgG and the reactivity was analyzed by FACScan.

RESULTS

Reactivity of AM-1 and AM-2 with human cells in flow cytometry

Thirty five human cell lines including malignant cells, adult fibroblasts, fetal fibroblasts and normal lymphocytes were studied. Immunoreactivity of the samples were analyzed by flow cytometry, and calculated as mean fluorescence
Fig. 1. Immunoreactivity of AM-1 (a) and AM-2 (b) with human cells in flow cytometry. Ten breast carcinoma cell lines, 23 other malignant cell lines and 3 normal cells were analyzed.

intensity (Fig. 1). MAb AM-1 reacted strongly with 4 breast cancer cell lines, HMA-1, YMB-1-E, YMB-1 and MDA-MB-231 cells, moderately with ZR-75-1, MCF-7, MCF-7 R27 and T-47D cells, but only weakly with MDA-MB-453 and SK-Br-3 cells. AM-1 was also reactive with ovarian cancer cell line, OVCAR-3 and uterine cancer cell line, ME180. MAb AM-2 demonstrated a similar but a little broad reaction pattern as compared to AM-1. The reactivity of both MAbs with adult or fetal fibroblast and normal lymphocytes were negligible.

Immunoeroxidase staining

Human normal tissues were stained with MABs AM-1 and AM-2 to examine the distribution of antigen defined by the MABs. Vast majority of normal tissues were not reactive with the MABs. However, AM-2 weakly reacted to the kidney, alveolar wall and ductal cells of the lung, apical surface of the submandibular gland, chief cells of the stomach, apical surface of the thyroid follicle, epithelial cells of the prostate and histiocytes of the spleen.

Benign and malignant breast tissues were tested with the MABs. Whereas MABs AM-1 and AM-2 showed an occasional apical reactivity to normal and benign epithelial cells, they did not show cytoplasmic reactivity to the vast majority of normal and benign epithelial cells. In contrast, the MABs demonstrated strong cytoplasmic reactivity to breast carcinoma cells (Fig. 2). Apical
Fig. 2. Immunoreactivity of AM-1 with breast tissues. (a) Invasive ductal carcinoma. Note the strong apical and cytoplasmic reactivity of carcinoma cells. (Scale bar = 50 μm) (b) In situ carcinoma. AM-1 is reactive with cytoplasm of in situ carcinoma cells (short arrow), but not with normal epithelial cells (long arrow). (Scale bar = 38 μm) (c) Severe atypical ductal hyperplasia. Note the reactivity at marginal cytoplasm of atypical hyperplasia (long arrow). No reactivity with epithelial cells with apocrine metaplasia (short arrow). (Scale bar = 25 μm)
and cytoplasmic reactivity was found in specimens with invasive carcinomas as well as in specimens with in situ carcinomas (Fig. 2a and 2b). Immunoreactivity at the marginal cytoplasm was occasionally observed in epithelial cells with atypical hyperplasia, but not in cells with apocrine metaplasia (Fig. 2c).

Enzyme and chemical treatments of antigens

The reactivity of MAb AM-1 and AM-2 with HMA-1 cells significantly decreased after treatment by heat, pronase or NaIO₄ (Table 1). These results suggest that antigenic epitope defined by AM-1 or AM-2 may be glycoproteins.

Immunoprecipitation with enzyme treatments

Fig. 3 shows a reducing SDS-PAGE of immunoprecipitation with enzymatic digestion. The cell lysate of HMA-1 was immunoprecipitated by successive treatments of AM-1 or AM-2. Without enzyme treatments, several bands with molecular weights ranging from 160 kDa to 210 kDa and the band over 370 kDa were immunoprecipitated by each Mab (Fig. 3, lanes b and g). In addition, a component of molecular weight 63 kDa was immunoprecipitated by AM-1 and that of 88 kDa was immunoprecipitated by AM-2. The enzyme digestion reduced the molecular weight approximately from 450 kDa to 370 kDa by neuraminidase (lane c), and from 370 kDa to 310 kDa by N-glycanase (lanes e and f) in SDS-PAGE using AM-1. Digestion with O-glycanase after prior digestion with neuraminidase reduced the molecular weight approximately 10 kDa (lane d). Reduction of molecular weights with enzyme digestion by AM-2 showed a similar result by AM-1. However, the molecular size of AM-2 digested by O-glycanase did not change from the size digested by neuraminidase alone (lanes h and i). These results suggest that the sialic acid residues, N-linked sugars and a paucity of O-linked carbohydrate may locate on the antigen molecule defined by AM-1 or AM-2.

Sandwich ELISA

Using HMA-1 cell extract as an antigen source a sandwich ELISA was performed with combination of either two of MAb DF3, B72.3, 4C8 (anti-CEA),
Fig. 3. Effects of Neuraminidase (Neu), O-glycanase (O) and N-glycanase (N) on AM-1 or AM-2 binding to $^{125}$I-labeled HMA-1 antigens in immunoprecipitation. The precipitates were assayed by SDS-PAGE. Lane a, normal mouse serum gel without enzyme treatment; Lanes b~f, AM-1 gel; Lanes g~k, AM-2 gel; Lanes b and g, no enzyme treatment; Lanes c and i, Neu; Lanes d and h, NEu + O; Lanes e and j, Neu + O + N; Lanes f and k, N treatment.

AM-1 and AM-2 (Fig. 4). The antigens bound to solid phase AM-1 or AM-2 were efficiently reactive with AM-1. The antigens bound to solid phase 4C8, DF3 or B72.3 were not detectable by AM-1. Furthermore, the antigens bound to solid phase B72.3 were reactive with AM-2, but not with 4C8 or DF3, suggesting that AM-2 may recognize sialyl Tn antigen.

Inhibition assays using existing MAb's

Fig. 5 shows binding curves of biotinylated AM-1 and AM-2 with HMA-1, which indicate that an amount of 1 $\mu$g/ml of both MAb's is the proper concentra-
Fig. 4. Sandwich ELISA using biotinylated AM-1 (open bars), biotinylated AM-2 (dotted bars) and solid phase monoclonal antibodies. The optimal density of the solution was measured at 450 nm.

Fig. 5. Binding of biotinylated AM-1 (●) and AM-2 (○) to HMA-1. The closed circles indicate biotinylated AM-1 and the open circles indicate biotinylated AM-2. Both curves became plateau at the concentration of 1 μg/ml.

formation for the binding inhibition experiments. Reactivity of biotinylated AM-1 with HMA-1 cells was significantly inhibited by AM-2 or DF3, whereas reactivity of biotinylated AM-2 was markedly inhibited by AM-1, but only weakly by HMFG-1 and MUSE11. On the other hand, reactivity of either biotinylated AM-1, or biotinylated AM-2 was not inhibited by B72.3 (Fig. 6).

Inhibition assays using synthetic peptide

After an incubation of HMA-1 cells with the synthetic peptide (PDTRPAPGSTAP-PAHGVTAPDTR) coding MUC1 core protein, the reactivity of MAbS HMFG-1, HMFG-2, SM-3 and MUSE11 to the HMA-1 cells were significantly inhibited, whereas the reactivity of AM-1 and AM-2 were not inhibited (Fig. 7), indicating that the epitopes defined by MAbS AM-1 and AM-2
Fig. 6. Antibody competition assays. HMA-1 cells were treated by (a) biotinylated AM-1 and (b) biotinylated AM-2 after incubation with cold MAbs. Bold lines indicate cell numbers treated by cold MAbs, while thin lines indicate cell numbers without treatment.

Fig. 7. Inhibition of MAbs by synthetic peptides (MUC1 core peptide) using HMA-1 cells. Bold lines indicate cell numbers treated by synthetic peptide, whereas thin lines indicate cell numbers without treatment.

do not reside in MUC1 core protein.

**Discussion**

Breast cancer-associated antigens including MUC1 and TAG-72 have been
characterized and MAbs recognizing such distinct epitopes have been widely utilized in detection of the cell surface antigens expressed in breast cancer. In view of the degree of antigenic heterogeneity which has been observed in most carcinomas, the use of mixtures of MAbs reactive with different TAAs, or reactive with distinct epitopes on the same antigen molecule, is necessary to detect carcinoma, and the generation of new MAbs which might recognize novel antigen would be one of great tasks for management of breast cancer. In this study, we generated MAbs AM-1 and AM-2 using TAA-rich breast cancer cell line, HMA-1, as an immunogen.

Flow cytometry assay demonstrated preferential reactivity of AM-1 and AM-2 to breast carcinoma cell lines vs. to other malignant or normal cell lines, suggesting that the MAbs recognize a breast cancer-associated antigen. In immunohistochemical assay using formalin-fixed tissues the MAbs showed strong reactivity to carcinoma cells. Tissue distribution of the antigen in various human tissues was similar to that of MUC1 (Croghan et al. 1983; Hilkens et al. 1984; Girling et al. 1989; Price et al. 1990), but different from that of TAG-72 (Johnson et al. 1986), MUC2 or MUC3 (Ho et al. 1993).

MUC1, the human gene which encodes for the MUC1 mucin, was developmentally regulated and expressed by secretory epithelial tissues including mammary glands (Zotter et al. 1988). Progesteron and estrogen appeared to up-regulate the expression of MUC1 and specific glyciforms of MUC1 mucin were shown to be present in the endometrium during pregnancy, when the immune system was exposed to MUC1 mucin protein core epitopes, resulting in the priming of T cells which can be detected in vitro by their proliferative response to MUC1 synthetic epitopes (Agrawal et al. 1995).

Immunoprecipitation and flow cytometry demonstrated that antigenic epitopes defined by AM-1 and AM-2 were high molecular weight glycoproteins containing sialic acid residues, N-linked sugars and a paucity of O-linked carbohydrate. The data generated by enzyme treatments suggest that the epitopes may contain mucin-like glycoproteins. Bramwell et al. (1983) and Shimizu and Yamauchi (1982) described that up to 50%-90% of the O-linkages resided on MUC1 mucin. The antigen defined by AM-1 or AM-2, however, appeared to have a few O-linked carbohydrate structure based on the reduced SDS-PAGE. Reduction pattern of molecular weights digested by neuraminidase or endoglycosidases was similar to that of 5T4, but the molecular components of AM-1 or AM-2 were clearly different from the size of 5T4. The molecular size of 5T4 was a 63 kDa component identified by the unglycosilated core protein of mucin in immunoprecipitation (Burchell et al. 1987). Taking this report into consideration, the 63 kDa component of AM-1 and the 88 kDa component of AM-2 might be identified as unglycosilated core proteins.

In inhibition assays using MAbs such as DF3 or B72.3, the binding of AM-1 to the HMA-1 cells was markedly inhibited by AM-2 and partially inhibited by
DF3. The binding of AM-2 was markedly inhibited by AM-1 and partially by HMFG-1 and MUSE11. On the contrary, the binding of both MAbs to the antigen was not inhibited by the synthetic MUC1 core peptide. In sandwich ELISA, the antigens bound to solid phase AM-1 or AM-2 were detected by AM-1, while the antigens bound to solid phase B72.3 were detected by AM-2. In consideration of these results, we raise a hypothesis of possible location of the epitopes defined by AM-1 and AM-2 in relation to the locations of MUC1 and TAG-72 antigens. Kuroki et al. (1990) described the location of TAG-72 epitope by means of serological mapping of the antigen using 19 distinct MAbs. AM-1 and AM-2 may detect a common antigen (AM antigen) but different epitopes each other. AM antigen may be different from MUC1 and TAG-72 antigens, and the 3 antigens may construct a trimeric form in HMA-1 cells. The epitope defined by AM-1 may locate nearby the DF3 defined epitopes, and the epitope defined by AM-2 may be present near the HMFG-1 or MUSE11 defined epitopes. The AM-1 epitope may locate nearby TAG-72, whereas the AM-2 epitope may differ from TAG-72. This hypothesis of trimer form of 3 antigens could be a possible explanation of the results obtained in sandwich ELISA and binding inhibition assays. The result obtained by ELISA was in accordance with a sandwich radioimmunoassay (RIA) using solid phase B72.3 and 125I labeled AM-2. In the sandwich RIA using solid phase AM-1 and 125I labeled AM-2, we detected increased levels of the antigen in serum or urine from breast cancer patients as compared to those from patients without breast cancer (unpublished observation).

In conclusion, AM-1 and AM-2 may recognize a novel breast cancer-associated antigen and they may be clinically useful in the diagnosis of breast cancer. Purification of AM antigen and molecular cloning of cDNA coding for the antigen are now in progress. We are also investigating the anti-tumor effect of drug conjugated AM-1 for treatment of breast cancer.

Acknowledgments

We thank J. Taylor-Papadimitriou, Imperial Cancer Research Fund, U.K. for providing MAbs HMFG-1, HMFG-2 and SM-3, Dr. N. Ida, Toray Research Institute, Kamakura, Japan for providing the MUC1 synthetic peptide, and Mr. H. Ueno, Department of Hygienic Chemistry, Pharmaceutical Institute, Tohoku University, Sendai, Japan for kind technical advice.

References

4) Burchell, J., Gentler, S., Taylor-Papadimitriou, J., Girling, A., Lewis, A., Millis, R. &


