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Immunohistochemical Expression of Blood Group Substances and Related Carbohydrate Antigens in Breast Carcinoma

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In forty-one carcinomas and sixteen benign lesions (fibroadenoma and mastopathy) of the human breast, immunohistochemical expression of sialylated and non-sialylated forms of both Le^a and Le^x, and the A, B, and H type 2 blood group substances were studied by using an indirect immunoperoxidase staining. In normal ductal epithelium and benign lesion of breast, Lewis-related antigens were mostly expressed. Breast carcinomas showed these antigens with the following frequencies: Le^a, 31.7% (13/41); sialyl Le^a, 56.1% (23/41); Le^x, 46.3% (19/41); sialyl Le^x, 68.3% (28/41); A/B/H type 2, 38.1% (16/41). Sialylated forms of Le^a and Le^x were observed more frequently than their respective non-sialylated forms in breast carcinomas. In both one normal epithelium and four carcinomas of breast with $Le^{(a-b-)}$ phenotype, the expressions of type 2 antigens were observed, while type 1 antigens were not consistently expressed. Although compatible expression was observed in all specimens of both normal epithelium and benign lesion of breast, twenty-four cases with the deletion of A and/or B antigens, six cases with H type 2 accumulation and one case with incompatible expression were demonstrated in breast carcinoma. Thirty-one breast carcinomas which showed the deletion of A/B/H type 2 expressed the Lewis-related antigens more frequently than nine cases which showed compatible expression. These results suggested that the activation of terminal fucosyltransferase and sialyltransferase as well as inactivation of some glycosyltransferases had occurred in cancer cell membrane, and sialyl Le^x, defined by a new monoclonal antibody CSLEX1, may be useful as a tumor-associated antigen independent of Lewis blood group type in breast cancer.

Key words: Carbohydrate antigen — Blood group substance — Breast carcinoma

Recently, many MoAbs[5] have been produced using the procedure described by Koehler and Milstein.[1] It has been elucidated that most of these MoAbs recognize the terminal carbohydrate chains of glycolipid or glycoprotein in cancer cell membrane. CA19-9 defined by MoAb N-19-9[2] is well known as a tumor marker for carcinomas of pancreas and other digestive organs. Its epitope was determined to be sialylated lacto-N-fucopentaose II,[3] which is a sialyl derivative of human Le^a blood group antigen. We have previously reported a new MoAb CSLEX1,[4] directed to the sialyl Le^x antigen, which is a chemical structure isomeric to the sialyl Le^a antigen defined by MoAb N-19-9 and is also a tumor-associated antigen.[4]

The recently evolved MoAb approach, which defines tumor-associated antigens, has elucidated the aberrant glycosylation of glycolipid and glycoprotein in tumor cell membranes.[5] Expression of certain carbohydrate antigens on the cell surface seems to be closely related to oncogenesis. Although we had already reported a significant increase in the incidence of sialyl Le^x antigen in various human carcinomas and in sera of patients with breast cancer using the MoAb CSLEX1, and proposed that sialyl Le^x antigen might be a useful marker of cancer,[4,6] no detailed work has been reported on the immunohistochemical expression of sialyl Le^x antigen detected by CSLEX1 antibody in normal ductal epithelium, benign lesion and breast carcinoma. In the present work, we examined the expression of sialyl Le^x antigen in various breast tissues classified by blood group status and histopathological types. At the same time, the expression of blood group substances and related carbohydrate antigens such as Le^a, sialyl Le^a, Le^x, A, B and H type 2 was investigated in the same cases.

MATERIALS AND METHODS

Tissue specimens The tissues of primary breast carcinomas were obtained from forty-one patients undergoing surgical resection at Nagasaki University Hospital. Six were papillo-tubular, twenty-one were solid-tubular, six were scirrhous, seven were mucinous and one was non-invasive carcinoma. Benign breast lesions such as fibroadenoma and mastopathy were obtained from six-

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[5] Abbreviations: MoAb(s), monoclonal antibody(antibodies); Le^a, $Lewis^a$; Le^x, $Lewis^x$; TBS, Tris-buffered saline (50 m*M* Tris-HCl 200 m*M* NaCl, pH 7.6).

Table I. Carbohydrate Structure of Antigenic Determinants in Blood Group Substances and Related Antigens

Antigen	Carbohydrate structure	Monoclonal antibody
Le^a	Galβ1→3GlcNAcβ1→ 4 ↑ Fucα1	CLEA1
Sialyl Le^a	NeuAcα2→3Galβ1→3GlcNAcβ1→ 4 ↑ Fucα1	CSLEA1
Le^x	Galβ1→4GlcNAcβ1→ 3 ↑ Fucα1	CLEX1
Sialyl Le^x	NeuAcα2→3Galβ1→4GlcNAcβ1→ 3 ↑ Fucα1	CSLEX1
A	GalNAcα1→3Galβ1→$^3_4$GlcNAcβ1→ 2 ↑ Fucα1	anti-A
B	Galα1→3Galβ1→$^3_4$GlcNAcβ1→ 2 ↑ Fucα1	anti-B
H type 2	Galβ1→4GlcNAcβ1→ 2 ↑ Fucα1	anti-H type 2

Fuc, fucose; GlcNAc, N-acetylglucosamine; GalNAc, N-acetylgalactosamine; NeuAc, N-acetylneuraminic acid.

teen patients. All tissue samples were fixed in formalin, embedded in paraffin, and deparaffinized for immunoperoxidase staining.

Antibodies MoAbs and their antigenic determinants employed in this study are listed in Table I. MoAbs CLEA1, CSLEA1, CLEX1 and CSLEX1 recognize Le^a, sialyl Le^a, Le^x and sialyl Le^x, respectively, and were kindly provided by Professor P. I. Terasaki (UCLA Tissue Typing Laboratory, Los Angeles, CA). The anti-A and anti-B MoAbs recognize both type 1 and type 2 of their respective A and B antigens. The anti-H type 2 MoAb recognizes only the type 2 H antigen. MoAbs anti-A, anti-B and anti-H type 2 were purchased from DAKO Co., Ltd. (Copenhagen). Peroxidase-conjugated goat $F(ab')_2$ anti-mouse IgG and IgM were purchased from Cappel Lab. (Pennsylvania).

Immunoperoxidase staining Indirect immunoperoxidase staining was accomplished as follows: the MoAbs were appropriately diluted in 0.05 *M* Tris, 2.5% bovine serum albumin, mouse liver acetone powder (100 μg/liter; Sigma Chemical Co.) and 0.05% sodium azide. After treatment with 3% hydrogen peroxide in 0.05 *M* TBS for 5 min, the MoAb was incubated with tissue sections for one hour at room temperature in a moist chamber, followed after 3 washes in TBS by incubation with goat anti-mouse peroxidase-conjugated IgG or IgM at 1:100 in TBS for 45 min. The slides were washed 3 times in TBS, and were flooded for 10 min with 0.05 mg% of 3,3′-diaminobenzidine and 0.03% hydrogen peroxide in TBS. Sections were then counter-stained with hematoxylin and mounted in glycerol-PBS. Slides were evaluated under a light microscope with results expressed as a score based on the percentage of the total field staining positively with the various MoAbs. Scores were based on the following scale: +++, over 50% of the field showing positive staining; ++, 30% to 50%; +, 1% to 30%; −, negative staining. Positive and negative control tissues were used in all cases, giving the expected results.

ABO(H) and Lewis blood grouping The ABO(H) and Lewis blood group typings of erythrocytes were determined by means of a hemagglutination test using anti-A, B, O(H), Le^a and Le^b anti-serum (Ortho Diag. Sys. Inc.).

Statistical analysis Statistical significance was assayed using the chi-square test or Fisher's exact probability test. A *P* value below 0.05 was considered significant.

RESULTS

Normal ductal epithelium and benign lesion of breast In seven cases of normal ductal epithelium adjacent to breast carcinoma and sixteen tissues from benign lesions such as mastopathy and fibroadenoma, Lewis-related antigens were mostly expressed (Table II), and antigen expression corresponding to ABO(H) blood groups were demonstrated. Incompatible expression was not observed (Table III).

Breast carcinoma In forty-one breast carcinomas, the percent expression of the sialylated and non-sialylated forms of both Le^a and Le^x, and the A/B/H type 2 antigens are shown in Fig. 1. The type 2 antigens (Le^x and sialyl Le^x) were expressed more often than the type 1 antigens (Le^a and sialyl Le^a), and the sialylated forms (sialyl Le^a and sialyl Le^x) showed higher expression rate than the non-sialylated forms (Le^a and Le^x). Furthermore, sialyl Le^x gave the highest positive rate (68.3%) among these antigens.

The relationship between the degree of differentiation of the breast carcinomas and the expression of the carbohydrate antigens studied was not statistically significant,

Table II. Expression of Blood Group Substances and Related Antigens in Normal Ductal Epithelium and Benign Lesion of Breast

	No. (%) with antigen expression			
	Le^a	sialyl Le^a	Le^x	sialyl Le^x
Normal epithelium (n=17)	15 (88.2)	16 (94.1)	16 (94.1)	16 (94.1)
Benign lesion				
Fibroadenoma (n=5)	5 (100)	5 (100)	5 (100)	5 (100)
Mastopathy (n=11)	8 (72.7)	8 (72.7)	11 (100)	11 (100)

Table III. ABO Status and Expression of A/B/H Type 2 Antigens in Normal Ductal Epithelium, Benign Lesions (Fibroadenoma and Mastopathy) and Carcinoma of Breast

ABO status			No. (%) with antigen expression		
			A	B	H type 2
A	Normal epithelium	(n=12)	12 (100)	0 (0)	5 (41.7)
	Benign lesion	(n=1)	1 (100)	0 (0)	1 (100)
	Breast carcinoma	(n=21)	7 (33.3)	1 (4.8)[a)]	5 (23.8)
B	Normal epithelium	(n=3)	0 (0)	3 (100)	2 (66.7)
	Benign lesion	(n=4)	0 (0)	4 (100)	1 (25.0)
	Breast carcinoma	(n=9)	0 (0)	1 (11.1)	3 (33.3)
AB	Normal epithelium	(n=3)	3 (100)	3 (100)	2 (66.7)
	Benign lesion	(n=5)	5 (100)	5 (100)	4 (80.0)
	Breast carcinoma	(n=3)	0 (0)	0 (0)	2 (66.7)
O	Normal epithelium	(n=4)	0 (0)	0 (0)	3 (75.0)
	Benign lesion	(n=6)	0 (0)	0 (0)	3 (50.0)
	Breast carcinoma	(n=8)	0 (0)	0 (0)	1 (12.5)

a) Incompatible expression.

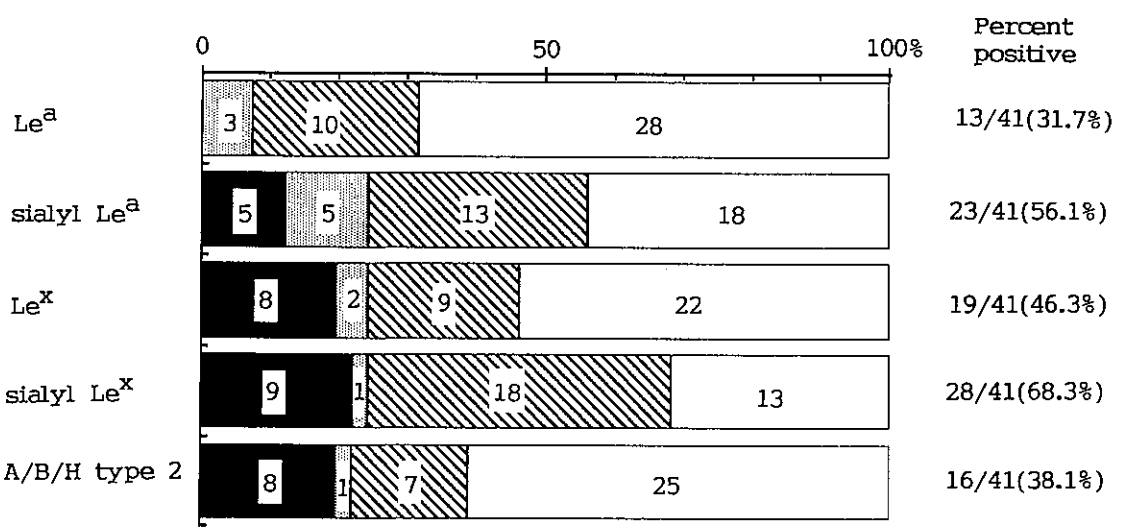

Fig. 1. The expression of blood group substances and related antigens in breast carcinomas. Results are shown as percentage of positive cases in each group. Staining results were scored from "−" to "+++" according to the percentage of the field showing positive staining, as follows: ■ +++, over 50%; ▨ ++, 30% to 50%; ▧ +, 1% to 30%; □ −, negative.

Table IV. Expression of Blood Group Substances and Related Antigens in Breast Carcinoma According to Histopathological Classification

		No. (%) with antigen expression				
		Le^a	sialyl Le^a	Le^x	sialyl Le^x	A/B/H type 2
Non-invasive	(n=1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
Papillo-tubular	(n=6)	2 (33.3)	4 (66.7)	4 (66.7)	6 (100)	1 (16.7)
Solid-tubular	(n=21)	7 (33.3)	12 (57.1)	7 (33.3)	12 (57.1)	8 (38.1)
Scirrhous	(n=6)	1 (16.7)	3 (50.0)	4 (66.7)	5 (83.3)	3 (50.0)
Mucinous	(n=7)	3 (42.9)	4 (57.1)	4 (57.1)	5 (71.4)	3 (42.9)

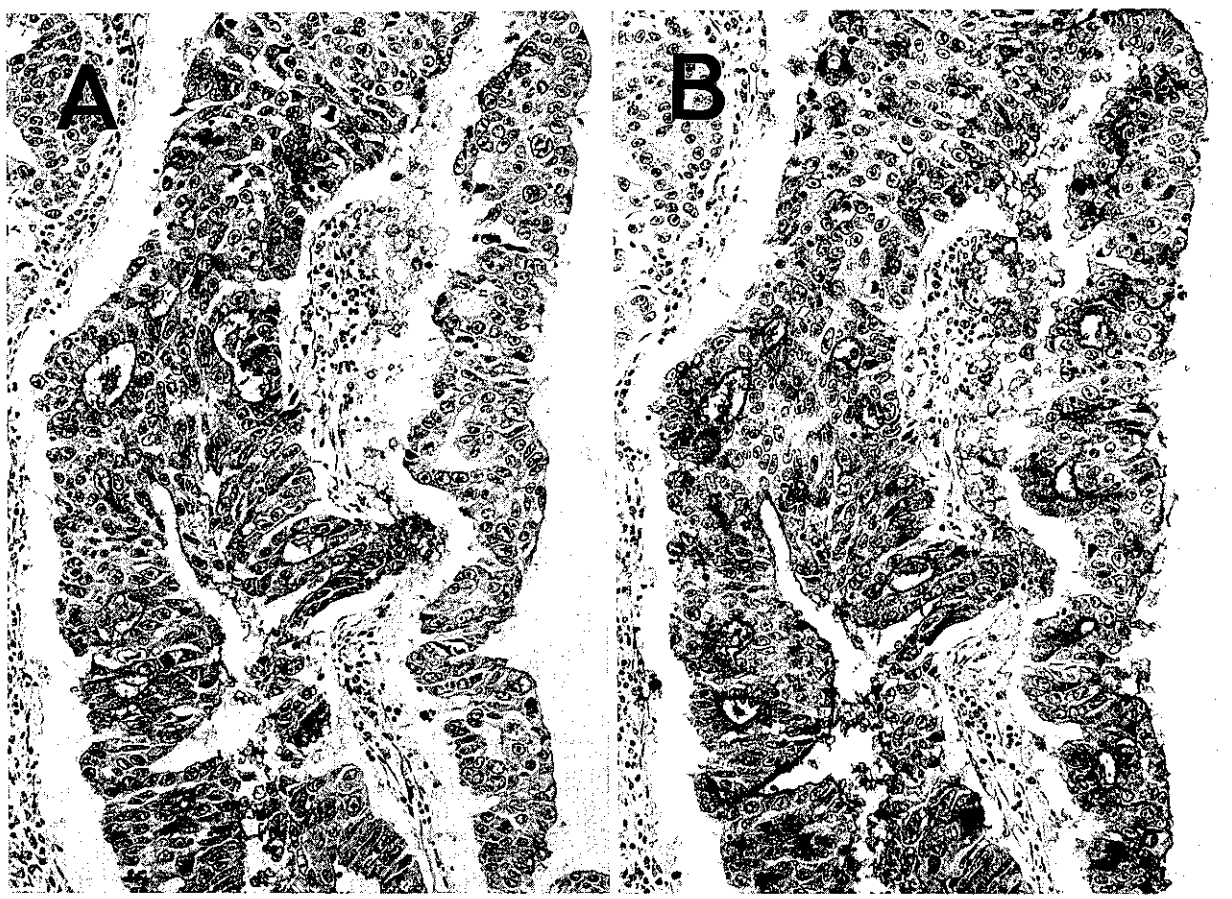

Fig. 2. The expression of sialyl Le^a (A) and sialyl Le^x (B) in solid-tubular carcinoma of breast. The apical cytoplasma of cancer tubules, the luminal contents and the cytoplasma of the cancer cells are positively stained with MoAbs CSLEA1 (A) and CSLEX1 (B) (×50). Staining procedure is described in "Materials and Methods."

but sialyl Le^a, Le^x and sialyl Le^x antigens, the carbohydrate antigens other than blood group substances, tended to be strongly expressed in well-differentiated papillo-tubular carcinoma (Table IV).

Localization of the carbohydrate antigens was observed in the apical cytoplasma of cancer tubules, the luminal contents and the cytoplasma of the cancer cells (Fig. 2) as well as in mucin lakes.

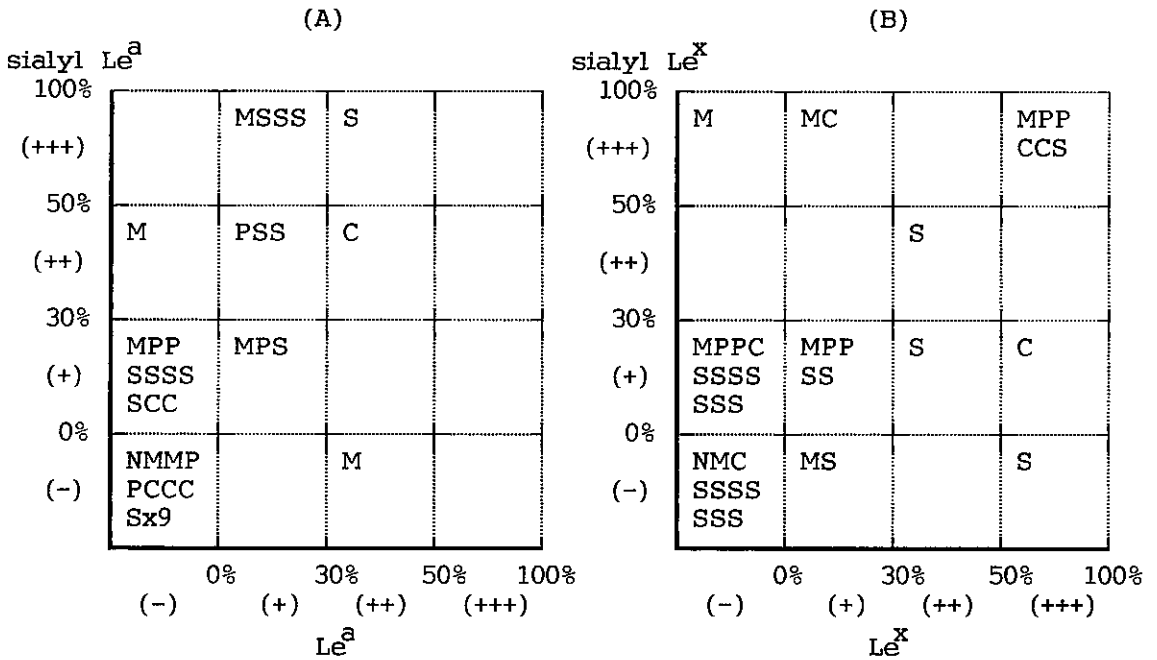

Fig. 3. The correlation between the expression of non-sialylated and sialylated forms of Le^a and Le^x. Staining results were scored from "−" to "+++" according to the percentage of the field showing positive staining (see "Materials and Methods"). Histopathological classification is indicated as follows: N, non-invasive; P, papillo-tubular; S, solid-tubular; C, scirrhous; M, mucinous carcinoma.

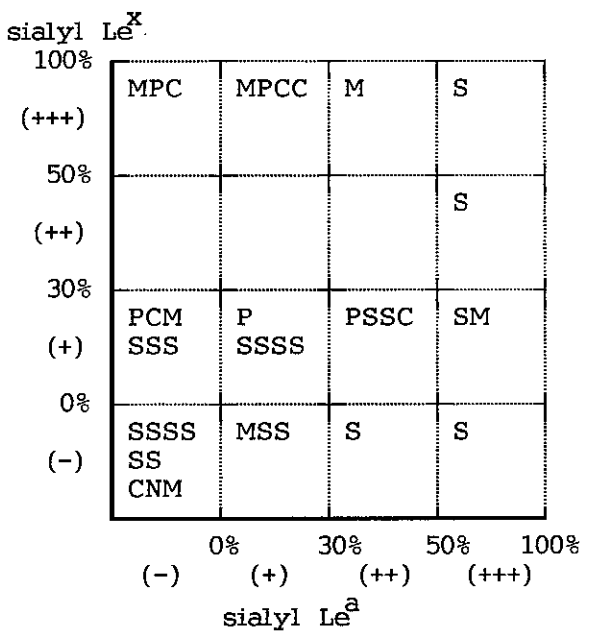

Fig. 4. The correlation between the expression of sialyl Le^a and that of sialyl Le^x. Staining results were scored from "−" to "+++" according to the percentage of the field showing positive staining (see "Materials and Methods"). Histopathological classification is indicated as in Fig. 3.

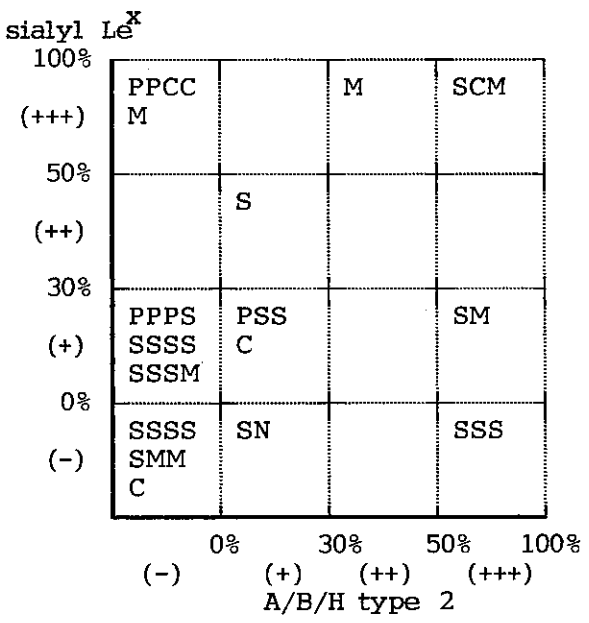

Fig. 5. The correlation between the expression of A/B/H type 2 and that of sialyl Le^x. Staining results were scored from "−" to "++" according to the percentage of the field showing positive staining (see "Materials and Methods"). Histopathological classification is indicated as in Fig. 3.

Sialylated and non-sialylated antigens in breast carcinoma The relationship between expression of the sialylated and non-sialylated forms of Le^a and Le^x is shown in Fig. 3. Twelve of the thirteen cases positive for Le^a were also positive for sialyl Le^a (92.3%), while 12 of the 23 cases positive for sialyl Le^a were also positive for Le^a (52.2%) (Fig. 3-A). Similarly for type 2 antigens, 16 of the 19 cases positive for Le^x were also positive for sialyl Le^x (84.2%), while 16 of the 28 cases positive for sialyl Le^x were also positive for Le^x (57.1%) (Fig. 3-B). Sialylated forms of Le^a and Le^x were observed more frequently than their respective non-sialylated forms ($P<0.01$ and $P<0.05$, respectively).

Sialyl Le^x and sialyl Le^a in breast carcinoma As shown in Fig. 4, eighteen of the twenty-three cases positive for sialyl Le^a were also positive for sialyl Le^x (78.3%), while eighteen of the twenty-seven cases positive for sialyl Le^x were also positive for sialyl Le^a (66.7%). Sialyl Le^x

Table V. Relation between Expression of A/B/H Type 2 Antigens and That of Lewis Blood Group-related Antigens

Expression of A/B/H type 2	No. (%) with antigen expression			
	Le^a	sialyl Le^a	Le^x	sialyl Le^x
Compatible (n=9)	2 (22.2)	5 (55.5)	3 (33.3)	5 (55.6)
Negative (n=31)	10 (32.3)	18 (58.1)	16 (51.6)	22 (71.0)

Table VI. Expression of Blood Group Substances and Related Antigens in Normal Ductal Epithelium and Carcinoma of Breast According to Lewis Blood Group

Lewis blood group			No. (%) with antigen expression			
			Le^a	sialyl Le^a	Le^x	sialyl Le^x
$Le^{(a+b-)}$	Normal epithelium	(n=0)	—	—	—	—
	Breast carcinoma	(n=2)	1 (50.0)	2 (100)	1 (50.0)	2 (100)
$Le^{(a-b+)}$	Normal epithelium	(n=6)	5 (83.3)	6 (100)	6 (100)	5 (83.3)
	Breast carcinoma	(n=13)	4 (30.8)	6 (46.2)	5 (38.5)	5 (38.5)
$Le^{(a-b-)}$	Normal epithelium	(n=1)	0 (0)	0 (0)	1 (100)	1 (100)
	Breast carcinoma	(n=4)	0 (0)	0 (0)	2 (50.0)	3 (75.0)

Table VII. ABO Status and Expression of A/B/H Type 2 Antigens in Breast Carcinoma

ABO status	Antigen expression[a]			No. of cases	No. with antigen expression		
	A	B	H type 2		Negative	Compatible	Incompatible
A (n=21)	(−)	(−)	(−)	11	13	7	1
	(−)	(−)	(+)[b]	2			
	(+)	(+)	(−)	1			
	(+)	(−)	(−)	4			
	(+)	(−)	(+)	3			
B (n=9)	(−)	(−)	(−)	6	8	1	0
	(−)	(−)	(+)[b]	2			
	(−)	(+)	(+)	1			
AB (n=3)	(−)	(−)	(−)	1	3	0	0
	(−)	(−)	(+)[b]	2			
O (n=8)	(−)	(−)	(−)	7	7	1	0
	(−)	(−)	(+)	1			

a) "(−)" and "(+)" denote negative and positive staining, respectively.
b) H type 2 accumulation.

antigen was expressed more frequently than sialyl Le^a, but the difference is not statistically significant. Furthermore, sialyl Le^x MoAb gave a positive reaction with tissue from certain carcinomas that were negative with sialyl Le^a MoAb. Some tissues from carcinomas showed the reverse result.

Lewis-related antigens and A/B/H type 2 antigens in breast carcinoma As shown in Fig. 5, eleven of the twenty-eight cases positive for sialyl Le^x were also positive for A/B/H type 2 (39.3%), while 11 of the 16 cases positive for A/B/H type 2 were positive for sialyl Le^x (68.8%). Furthermore, thirty-one breast carcinomas which showed the deletion of A/B/H type 2 expressed the Lewis-related antigens more frequently than nine cases which showed compatible expression (Table V).

Lewis blood group and Lewis-related antigens Table VI summarizes the relation between the Lewis blood group phenotypes and expression of Lewis-related antigens. In one normal epithelium and four carcinomas of breast with $Le^{(a-b-)}$ phenotype, the expression of type 2 antigens (Le^x and sialyl Le^x) was observed, while type 1 antigens (Le^a and sialyl Le^a) were not expressed consistently.

ABO status and expression of A/B/H type 2 antigens The distribution of the A/B/H type 2 antigens is shown in Tables III and VII grouped according to the ABO blood group status of the patients. Although compatible expression was observed in all specimens of both normal epithelium and benign lesion of breast, deletion, incompatible expression and H type 2 accumulation of A/B/H type 2 antigens were noted in breast carcinoma. Neither A nor B antigens were detected in thirteen cases of type A, eight cases of type B and three cases of type AB, and H type 2 accumulation with the deletion of A and/or B

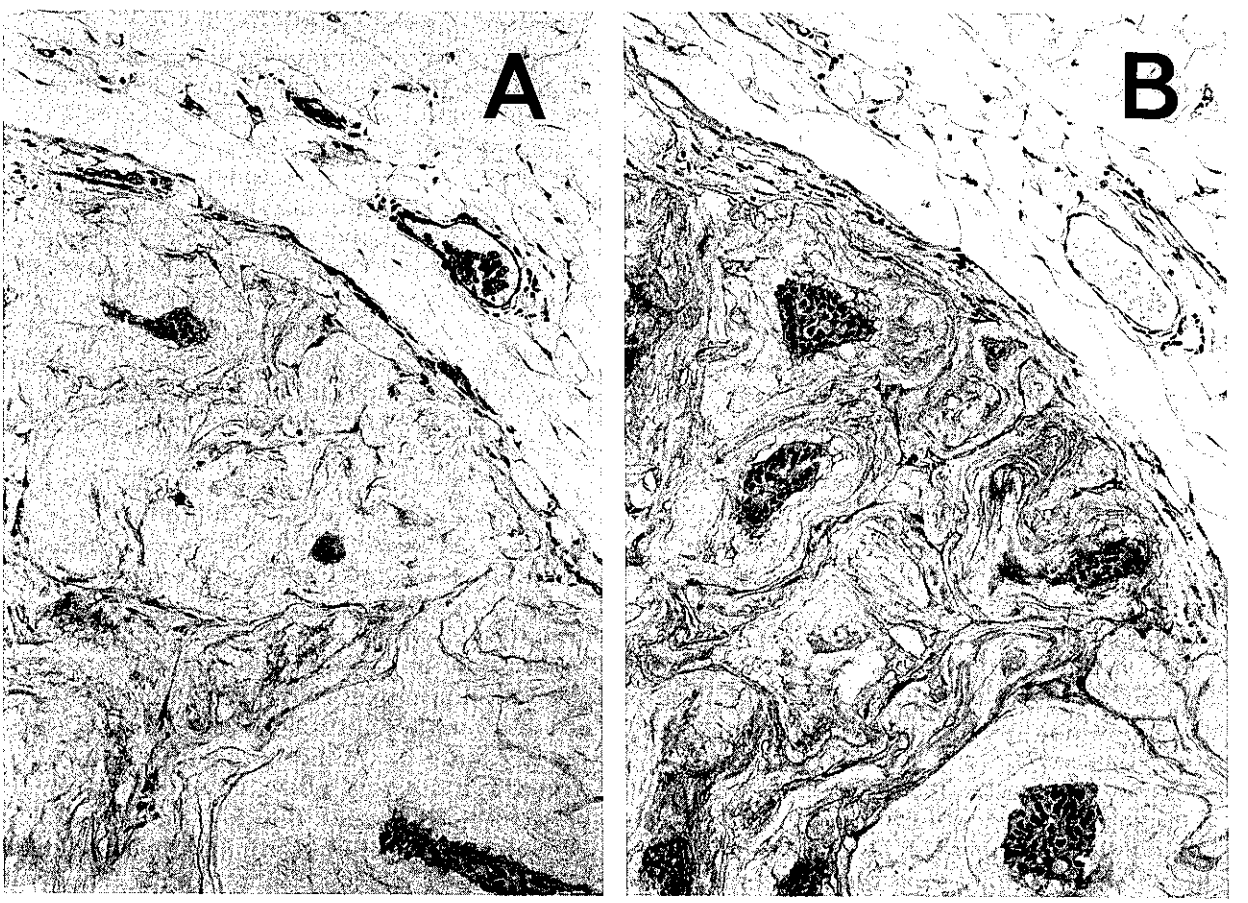

Fig. 6. MoAb anti-A reacted with erythrocytes, endothelium of blood vessels and mucous lake in breast carcinoma with blood group A (A), and furthermore this mucous lake was immunohistochemically stained by MoAb anti-B, while B antigen was not expressed in red blood cells or endothelial cells of blood vessels (B) (×50). Staining procedure is described in "Materials and Methods."

antigens was seen in two cases of type A, two cases of type B and two cases of type AB. In one case with blood group A, mucinous carcinoma incompatibly expressed B antigen (Fig. 6).

DISCUSSION

Recently, extensive biochemical studies have elucidated the alterations in glycolipid expression of the tumor cell surface, and two types of aberrant glycosylation have been observed, as follows. (a) Incomplete synthesis with or without precursor accumulation. (b) Neosynthesis through activation of new addition of a glycosyl residue.[5] The same changes that have been found in glycolipids have also been detected in the peripheral region of glycoprotein carbohydrate.[5,7] Either the precursor accumulation or the appearance of neoglycolipid results in the formation of human tumor-associated carbohydrate markers defined by specific MoAbs.[5]

The antigenic determinant of MoAb N-19-9, reported by Koprowski *et al.*[2] in 1979, has been identified as a ganglioside containing sialylated lacto-N-fucopentaose II, which is a sialyl derivative of human Le^a blood group antigen and is classified as a lacto-series type 1 chain, consisting of Galβ1→3GlcNAc unit.[3,5] This monosialoganglioside antigen is accumulated in many carcinomas originating from colorectum, stomach, pancreas and endometrium.[8] On the other hand, Le^x (X-hapten) and its di- or trimeric structure, classified as lacto-series type 2 chain, consisting of an N-acetyllactosamine unit (Galβ1→4GlcNAc), are characteristic human oncofetal antigens,[4,9,10] and glycolipid with sialyl 2→3 substitution of Le^x structure has also been found to accumulate in human adenocarcinomas.[11] Although sialyl Le^x antigen is recognized by CSLEX1 and FH-6 MoAb, the antigenic determinant defined by MoAb FH-6 is more restrictive than the epitope of CSLEX1. This is consistent with the fact that FH-6 needs two repeating N-acetyllactosamine structures present in the core chain besides the sialyl Le^x structure at the terminus.[4,11,12]

In the current study, the type 2 antigens (Le^x and sialyl Le^x) were expressed more often than the type 1 antigens (Le^a and sialyl Le^a), and sialyl Le^x indicated the highest positive rate among blood group-related carbohydrate antigens, although these Lewis-related antigens were already expressed in normal ductal epithelium of breast. In normal glandular and mucosal epithelia of the digestive organs, the distribution of sialyl Le^x is more restricted than that of sialyl Le^a, while adenocarcinomas, originating from the gastrointestinal tract and pancreato-biliary system, expressed sialyl Le^x more frequently than sialyl Le^a.[4,8] These results suggested that the activity of type 2 sugar chain synthesis is increased in these cancers, and that the tumor specificity of sialyl Le^x is higher than that of sialyl Le^a.

In addition, sialylated forms of both Le^a and Le^x were observed more frequently than their respective non-sialylated forms in breast carcinomas. The significance of sialylation in neoplasma has been the subject of many reports. An increase in sialyltransferase activity in cancer tissues[13] and in the sera of cancer patients has been reported.[14] Our results in this study suggested the possible activation of both 2→3 sialyltransferase and 1→3 fucosyltransferase (coded by the X gene) in breast carcinoma, resulting in the detection of sialyl Le^x in 68.3% of tissue specimens studied.

Chia *et al.*[15] reported that the combined use of two monoclonal antibodies, CSLEX1 and CSLEA1, directed against sialyl Le^x and sialyl Le^a respectively, detects a wider range of sera from cancer patients including breast cancer than the use of a single MoAb alone. This finding is supported by our current results that sialyl Le^x MoAb detected tissue from certain carcinomas that were negative with sialyl Le^a MoAb, and some tissues from breast carcinomas showed the opposite reaction.

In 1982, Koprowski *et al.*[16] pointed out that individuals who are $Le^{(a-b-)}$ can not synthesize the Le^a and Le^b antigens and should also be unable to synthesize sialyl Le^a antigen because they lack the fucosyltransferase specified by the Le gene. Our current study also indicated that in one normal epithelium and four carcinomas of breast with $Le^{(a-b-)}$ phenotype, the expression of type 2 antigens was observed, while type 1 antigens were not completely expressed. As the X gene of type 2 chain is activated in all populations, sialyl Le^x may be independent of Lewis blood type. Thus, type 2 chain antigens such as sialyl Le^x should be more applicable as a universal tumor marker than type 1 chain antigens such as sialyl Le^a.

There are a few reports concerning the immunohistochemical and serological expression of sialyl Le^x detected by MoAb CSLEX1. The distribution of CSLEX1-reactive antigen was very limited in normal tissues. Strong positive staining was observed in the proximal tubules of the kidney and on granulocytes, and weaker staining was observed in some deep crypts of colon, some acinar cells of pancreas, hepatic cells, ureter, and granulocytes.[4] In many adenocarcinomas, the antigen recognized by CSLEX1 could be detected with high frequencies: stomach, 94%; colon, 76%; lung, 63%; breast, 25%; pancreas, 100%.[4] On the other hand, Hirota *et al.*[6] reported that in a cell-binding inhibition assay using MoAb CSLEX1, sera from cancer patients and controls yielded the following percentages of positive inhibition; lung, 43.8%; stomach, 26.0%; colon, 44.4%; gall bladder and bile duct, 47.8%; pancreas, 37.5%; breast, 26.7%; benign diseases, 0.9% and normal healthy

donors, 0.7%. Iguro *et al.*[17] have reported similar results in sera of cancer patients, using the reverse passive hemagglutination test with CSLEX1 MoAb. These reports suggested that sialyl Le^x defined by CSLEX1 may be a useful tumor marker with high tumor-specificity in the monitoring of patients with cancer of digestive organs, lung and breast.

Blood group substances are the major allogenic antigens in humans, and their presence is not limited to blood cells; they are also found in various epithelial cells including the mammary duct.[18, 19] Alterations of the ABH antigens may occur relatively early in tumor progression. ABH deletions are detected not only in areas of carcinoma *in situ* but also in surrounding histologically normal epithelium.[19] Changes in the expression of blood group antigens, such as the deletion of A/B determinant with or without precursor accumulation, are also well-known to occur in invasive carcinoma.[20] A likely mechanism for these alterations is the loss or reduction in activity of glycosyltransferase enzymes required for the synthesis of A/B/H antigens.[20, 21] A blocked synthesis of ABH antigens can be associated with the accumulation of Le^a, Le^x and their sialylated derivatives.[20] These findings are consistent with our current study.

It is of great interest to know whether the alteration of ABH blood group antigens in carcinomas is related to the clinical behavior of tumors. The possibility that the extent of deletion of ABH antigens could parallel malignant transformation, the degree of anaplasia, and subsequent metastasis has been extensively studied in transitional carcinoma of the urinary tract since 1975.[19, 21, 22] Many studies have confirmed that patients whose tumors do not express these antigens are at greater risk for subsequent bladder wall invasion and metastasis.[22, 23] In breast cancer, however, ABH antigens status did not serve as a useful prognostic factor.[21] Further investigation is needed to elucidate this problem and also the biological function of carbohydrate chains, including Lewis-related antigens, in cancer cell membrane.

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