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The Presence of CA19-9 in Serum and Saliva from Lewis Blood-group Negative Cancer Patients

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Eighteen cancer patients showed high levels of CA19-9 in sera, even though the blood-group phenotypes of their red blood cells were Le(a−b−). Seven of these patients (group I) were determined as Le(a−b−) from both red blood cells and saliva consistently, whereas eleven other patients (group II) secreted either Le^a or Le^b antigen in saliva and showed the expression of incompatible Lewis blood-group antigens. GDP-fucose: *N*-acetyl-glucosaminide $\alpha(1\rightarrow4)$-L-fucosyltransferase was demonstrated to be present in salivas from both group I and group II. These results suggest that a cancer-associated alteration of Lewis blood-group antigen expression occurs in cancer patients.

Key words: CA19-9 — Lewis blood groups — Salivary $\alpha(1\rightarrow4)$-L-fucosyltransferase — Incompatible expression of blood-groups

The antigenic determinant of CA19-9, one of the cancer-associated antigens, has been characterized as sialylated Le^a (NANA[*5] $\alpha2\rightarrow3$Gal$\beta1\rightarrow3$[Fuc$\alpha1\rightarrow4$]GlcNAc).[1] This antigen has been found to be increased in colorectal, biliary tract, gastric and pancreatic cancers.[1-6] Although it is also found in normal salivas from Lewis-positive individuals [Le(a+b−) and Le(a−b+)], CA19-9 is absent in Lewis-negative ones [Le(a−b−)] because they lack the *Le* gene which specifies the fucosyltransferase responsible for formation of the Fuc$\alpha1\rightarrow4$GlcNAc linkage.[7] As a result, it has been suggested that serum CA19-9 level could not be useful as a tumor-marker for diagnosis and therapy of cancer in patients belonging to the Le(a−b−) blood-group.[1,7] Recently, with the aid of our chemically synthesized substrate, 2-acetamido-2-deoxy-4-O-(2-O-methyl-β-D-galactopyranosyl)-D-glucopyranose 2′-O-methyllactosamine, we were able to detect elevated activities of α-(1→3)-L-fucosyltransferase related to the synthesis of the Lewis X antigen (Gal$\beta1\rightarrow4$[Fuc$\alpha1\rightarrow3$]GlcNAc) in patients suffering from various cancers.[8-10] Based upon a similar strategy, we used another synthetic substrate, benzyl 2-acetamido-2-deoxy-3-O-(2-O-methyl-β-D-galactopyranosyl)-β-D-glucopyranoside [2′-O-methyllacto-*N*-biose I βBz], to detect α-(1→4)-L-fucosyltransferase related to the synthesis of Lewis and CA19-9 antigens (unpublished results). In the present paper, we report that eighteen cancer patients were found to show high levels of CA19-9 in sera, though the Lewis phenotypes of their red blood cells were Le(a−b−). Eleven of these patients showed the expression of incompatible Lewis blood-group antigens and secreted either Le^a or L^b antigen in saliva. It was also demonstrated that GDP-fucose: *N*-acetyl-glucosaminide α-(1→4)-L-fucosyltransferase was present in saliva from Le(a−b−) cancer patients.

MATERIALS AND METHODS

GDP-[^{14}C]fucose (262 mCi/mmol) was purchased from New England Nuclear. GDP-Fucose was obtained from Seitetsu Kagaku, Hyogo. CA19-

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[*5] Abbreviations used in this paper: NANA, *N*-acetylneuraminic acid; Gal, D-galactose; Fuc, L-fucose; GlcNAc, *N*-acetyl-D-glucosamine; RIA, radiometric immunoassay; Bz, benzyl; BSA, bovine serum albumin; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

9 immunoradiometric assay kit (Lot No. 126C) was from Compagnie Oris Industrie S.A., France. Anti-A and anti-B were from Ortho Diagnostic Inc. Anti-Le^a (Lot No. AU2-070) and anti-Le^b (Lot No. LAR-1018) monoclonal antibodies were obtained from Chembiomed, Canada. 2′-O-Methyllacto-*N*-biose IβBz was synthesized as described elsewhere. Blood and saliva samples were collected from patients with various cancers admitted to Gunma Cancer Center and Gunma University Hospital, Gunma. All patients had clinically measurable tumor parameters and were undergoing treatment at the time of drawing blood. Control sera were collected from randomly selected volunteers of both sexes. Microsomes from human stomach mucosa were prepared by a method described previously.[11] Blood specimens were allowed to clot in a sterile plastic tube and sera were separated and stored at $-80°$ until use. Saliva samples were collected without any artificial stimulus or preservative and prepared as described previously.[10, 12]

Washed erythrocytes were treated with an equal volume of 1% papain in saline for 30 min at 37° prior to use. These papain-treated erythocytes were examined for their Lewis phenotypes by using anti-Le^a and anti-Le^b monoclonal antibodies. Moreover, saliva samples were heated for 30 min at 100° and then used in a hemagglutination inhibition test to determine their Lewis phenotypes.

CA19-9 levels of serum and saliva were measured by RIA according to the manufacturer's instructions. The cut-off level for CA19-9 in serum was set at 37 U/ml. The standard reaction mixture for α-(1→4)-L-fucosyltransferase assay contained the following components in a final volume of 80 μl: 1.05 nmol of GDP-[^{14}C]fucose; 4 μmol of HEPES-NaOH buffer (pH 7.0); 1 μmol of NaN_3; 0.2 μmol of ATP; 20 μl of serum or saliva and 0.5 μmol of 2′-O-methyllacto-*N*-biose I βBz. This substrate is a specific acceptor for α-(1→4)-L-fucosyltransferase (unpublished results). After incubation at 37° for 16 hr, an equal volume of ethanol was added and the mixture was centrifuged. Supernatant was spotted on Whatman 3MM paper. The paper was chromatographed in *n*-butanol-ethanol-water=4:1:5 (v/v/v). The area of the paper containing radioactivity was counted with an Aloka LSC-700 scintillation spectrometer.

The fucosylation of erythrocytes was performed with cold GDP-fucose, papain-treated erythrocytes and fucosyltransferases from human stomach mucosa in the presence of the following components: 60 nmol of GDP-fucose, 4 μmol of sodium cacodylate buffer (pH 7.0), 0.5 μmol of $MnCl_2$, 10 μmol of NaCl, 100 mg of BSA, 25 μl of packed erythrocytes and 50 μl of enzyme preparation from human stomach of Le(a−b+) solubilized with Emulgen 109P.[13] The reaction mixture was incubated at 37° for 2 hr.

Results

Lewis Blood-group Phenotypes of Erythrocytes from Cancer Patients Lewis blood-group phenotypes of erythrocytes from cancer patients (n=360) and healthy controls (n=454) are compared in Table I. The frequency of the Le(a−b−) blood-group in cancer patients was found to be almost twice as high as that in healthy controls. The frequency of the Le(a+b−) blood-group, on the contrary, was nearly the same in both patients and healthy controls. There was no correlation between the frequencies of the ABO and the Lewis phenotypes of erythrocytes in patients (data not shown).

CA19-9 Levels in Cancer Patients CA19-9 levels of serum and saliva were examined in 360 cancer patients. Eighteen patients suffering from various cancers showed high levels of CA19-9 in their sera, even though the Lewis phenotypes of their red blood cells were Le(a−b−) (Table II). Most of the patients (14/18) were in the late stage of diseases. Seven of the patients (group I) were also determined as Le(a−b−) from their salivas. However, the rest (group II) secreted either Le^a or Le^b antigen in saliva and the salivas were capable of inhibiting hemagglutination of Le(a+b−) and Le(a−b+) erythrocytes caused by anti-Le^a and anti-Le^b antibodies, respectively. Thus, patients from group II showed the expression of incompatible Lewis blood-group antigens in their erythrocytes and salivas. The anti-Lewis antibodies were also adsorbed with the patients' salivas from group II, but not with their erythrocytes (data not shown).

Enzymatic Fucosylation of Lewis Blood-group Precursor on Erythrocytes Table III

Table I. Frequencies of Lewis Blood-group Phenotypes in Cancer Patients and Healthy Controls

Lewis blood-group	Frequency (No.)	
	Patients	Healthy controls
Le(a−b−)	26.9% (97)	12.1% (55)
Le(a+b−)	20.6 (74)	21.2 (96)
Le(a−b+)	52.5 (189)	66.7 (303)

Table II. Elevated CA19-9 Levels in Lewis-negative Cancer Patients

Donor	ABO & Lewis phenotype		CA19-9 (U/ml)		Carcinoma	Stage
	RBC	Saliva	Serum	Saliva		
Group I						
K.M.	ALe(a−b−)	Le(a−b−)	690	6.7	Stomach	I
C.I.	ABLe(a−b−)	Le(a−b−)	710	7.4	Thyroid	IV
W.N.	ALe(a−b−)	Le(a−b−)	1,300	87.2	Stomach	IV
S.F.	ALe(a−b−)	Le(a−b−)	2,500	110.0	Breast	IV
S.A.	ALe(a−b−)	Le(a−b−)	820	520.0	Stomach	IV
C.M.	BLe(a−b−)	Le(a−b−)	2,200	261.0	Stomach	III
E.W.	OLe(a−b−)	Le(a−b−)	650	171.0	Lingua	IV
Group II						
N.S.	ALe(a−b−)	Le(a−b+)	690	92.0	Uterus	I
K.Y.	BLe(a−b−)	Le(a−b+)	720	180.0	Stomach	IV
M.K.	ALe(a−b−)	Le(a−b+)	14,760	379.0	Liver	IV
S.I.	ABLe(a−b−)	Le(a+b−)	9,940	129.0	Pancreas	IV
M.U.	ALe(a−b−)	Le(a−b+)	17,120	82.0	Pancreas	IV
Y.O.	OLe(a−b−)	Le(a−b+)	680	74.0	Stomach	I
T.K.	ALe(a−b−)	Le(a+b−)	810	837.0	Lung	IV
T.S.	ALe(a−b−)	Le(a+b−)	22,640	111.0	Colon	IV
T.H.	ALe(a−b−)	Le(a−b+)	770	2,500.0	Stomach	IV
K.H.	ABLe(a−b−)	Le(a−b+)	1,180	629.0	Esophagus	IV
K.S.	ALe(a−b−)	Le(a−b+)	1,225	1,200.0	Esophagus	IV

Table III. Treatment of Erythrocytes from Lewis-negative Cancer Patients with α-Fucosyltransferases from Human Stomach Mucosa and GDP-Fucose

RBC donor	ABO & Lewis	Enzyme	GDP-Fuc	Hemagglutination titer	
				anti-Le^a	anti-Le^b
M.U.	ALe(a−b−)	+	+	1:2	1:512
		+	−	< 1:2	1:16
		−	−	< 1:2	< 1:2
W.N.	ALe(a−b−)	+	+	1.2	1:128
		+	−	< 1:2	1:2
		−	−	< 1:2	< 1:2
Control	ALe(a−b−)	+	+	1:128	1:512
		+	−	1:2	1:16
		−	−	< 1:2	< 1:2

shows the treatment of erythrocytes from Le(a−b−) cancer patients whose salivas were Le(a−b+) (M.U.) and Le(a−b−) (W.N.) and from a healthy control, with an α-fucosyltransferase preparation from human stomach mucosa. In this preparation, not only α-(1→2)- and α-(1→3)- but also α-(1→4)-L-fucosyltransferase were detected (data not shown). Lewis-negative erythrocytes from the two cancer patients as well as erythrocytes from the healthy control acquired Le^b activity, whereas, without GDP-fucose in the incubation mixture, only slight Le^b activity could be detected in all erythrocytes mostly because of the adsorption of Le^b active fucolipids onto acceptor erythrocytes coexisting in the enzyme preparation used. Conversion of all these Lewis-negative erythrocytes to Le^b active ones was also observed after incubation with a preparation of purified Le^b active glycolipids from human plasma (data not shown).

Table IV. α-(1→4)-L-Fucosyltransferase Activities in Cancer Patients

Donor	Lewis phenotype		α-(1→4)-L-Fucosyltransferase[a]
	RBC	Saliva	
T.K.	Le(a−b−)	Le(a+b−)	1,969
K.H.	Le(a−b−)	Le(a−b+)	4,750
S.F.	Le(a−b−)	Le(a−b−)	4,989
S.A.	Le(a−b−)	Le(a−b−)	1,875
C.M.	Le(a−b−)	Le(a−b−)	3,016
J.O.	Le(a−b−)	Le(a−b−)	139
M.M.	Le(a−b−)	Le(a−b−)	98
Control			
	Le(a+b−)	Le(a+b−)	3,046
	Le(a−b+)	Le(a−b+)	2,572
	Le(a−b−)	Le(a−b−)	129

a) [^{14}C]Fucose (DPM) incorporated into 2′-O-methyllacto-*N*-biose I βBz per 20 μl of saliva.

α-(1→4)-L-Fucosyltransferase Activity in Saliva α-(1→4)-L-Fucosyltransferase activities in salivas from patients whose erythrocytes were in the Le(a−b−) blood-group and from healthy controls were determined by using 2′-O-methyllacto-*N*-biose I βBz as an acceptor (Table IV). Saliva samples from five patients who showed high levels of CA19-9 in sera (Table II) and from Lewis-positive controls contained the enzyme activity. On the other hand, this enzyme activity could not be detected either in Lewis-negative healthy controls or in Lewis-negative patients (J.O. and M.M.) whose serum CA19-9 levels were within the normal range (<37 U/ml).

Discussion

The frequency of Le(a−b−) erythrocytes was found to increase in cancer patients. However, some patients showed the expression of incompatible Lewis blood-group antigens in their erythrocytes and salivas, so that the frequencies in cancer patients may change if the Lewis blood-group phenotypes are determined with their salivas. Indeed, eleven of the 18 patients whose CA19-9 levels were extremely high and who were determined as Le(a−b−) from their erythrocytes, secreted either Le^a or Le^b antigen in saliva.

Lewis blood-group specificities of erythrocytes are characterized by fucolipids in plasma as well as in the erythrocyte membranes.[14,15] It is also well known that the Lewis active fucolipids do not originate from erythrocytes, but are adsorbed by cells from plasma.[16] On the other hand, Le(a−b−) erythrocytes carry blood-group Lewis precursor glycolipids on the surface of their cell membranes. They could be converted to Le^a and Le^b active cells by enzymatic fucosylation with GDP-fucose and α-(1→4)- and α-(1→2)-L-fucosyltransferase.[17] The results from the enzymatic fucosylation of erythrocytes and from the adsorption of Lewis blood-group substances on erythrocytes in this study demonstrated that there was no abnormality in the erythrocyte membranes of Le(a−b−) cancer patients for acquiring Lewis blood-group substances.

Previous reports suggested that the Lewis-negative individuals lack the fucosyltransferase that catalyzes the synthesis of the sugar sequence, Fucα1→4GlcNAc, so that cancer patients belonging to the Le(a−b−) blood-group cannot synthesize CA19-9.[1,2,5,7] However, the results presented here demonstrated that at least seven cancer patients whose erythrocytes and salivas were in the Le(a−b−) blood-group (group I) did show elevated CA19-9 levels not only in serum but also in most of the salivas examined. Recently,[12] we found that α-(1→4)-L-fucosyltransferase related to the synthesis of Lewis antigenic determinant was present in salivas from Le(a+b−) and Le(a−b+) individuals, but not from Le(a−b−) ones. More recently, we succeeded in synthesizing chemically a specific acceptor, 2′-O-methyllacto-*N*-biose IβBz, for detecting this enzyme (unpublished results). It is of particular interest that three patients who showed high levels of CA19-9 have α-(1→4)-L-fucosyltransferase in their salivas, even though they were determined consistently as Le(a−b−) from both red blood cells and saliva. This enzyme activity, on the contrary, could not be detected either in Lewis-negative healthy controls or in Lewis-negative patients (J.O. and M.M.) whose CA19-9 levels in serum were within the normal range (<37 U/ml). In addition, we have successfully synthesized CA19-9 from the serum preparation using GDP-fucose and α-(1→4)-L-fucosyltransferase from an Le(a−b−) patient (results will be published elsewhere). These results, therefore, suggest that

the presence of α-(1→4)-L-fucosyltransferase in Le(a−b−) cancer patients is associated with the elevated CA19-9 levels in their sera and salivas.

The expression of ABH and Lewis antigens in cancer has been studied and the incompatible expression of the antigens has been observed in various types of cancers.[18-22] The Lewis phenotypes of erythrocytes were also found to change during pregnancy, i.e., from Lewis-positive to Lewis-negative, though the phenotypes of salivas were constant.[23] The mechanism for the incompatible blood-group expression in cancer is unclear, but it has been suggested to occur as the result of activation of cancer-altered genes, change in specificity of glycosyltransferases, or competition for substrate between enzymes.[10, 18, 21] The findings described here on the expression of incompatible Lewis blood-group phenotypes indicate, though not conclusively, that the alteration of the expression of these antigens is a cancer-associated phenomenon. It remains to be seen whether there might be at least two distinct α-(1→4)-L-fucosyltransferases, one of which is responsible for the biosynthesis of Lewis blood-group substance, and the other for the CA19-9 antigen.

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