Tissue-specific loss of fucosylated glycolipids in mice with targeted deletion of α(1,2)fucosyltransferase genes

Masao IWAMORI*[1] and Steven E. DOMINO†

*Department of Biochemistry, Faculty of Science and Technology, Kinki University, 3-4-1 Kowakae, Higashiosaka, Osaka 577-8502, Japan, and †Department of Obstetrics and Gynecology, The University of Michigan Medical School, Ann Arbor, Michigan 48109-0617, U.S.A.

Glycolipids in epithelial tissues of the gastrointestinal tract act as receptors for enteric bacteria and are implicated in the activation of the intestinal immune system. To clarify the genes involved in the fucosylation of the major glycolipids, substrate glycolipids and fucosylated products were measured in tissues of wild-type and mutant mice lacking α(1,2)fucosyltransferase genes *FUT1* or *FUT2*. Quantitative determination was performed by TLC-immunostaining for GA1 (Gg_4Cer), FGA1 (fucosyl GA1), GM1 ($II^3NeuAc\text{-}Gg_4Cer$), FGM1 (fucosyl GM1), and Forssman glycolipids. Both FGM1 and FGA1 completely disappeared from the antrum, cecum, and colon of FUT2-null mice, but not those of FUT1-null and wild-type mice. Precursor glycolipids, GM1 and GA1, accumulated in tissues of FUT2-null mice, indicating that the *FUT2*-encoded enzyme preferentially participates in the fucosylation of GA1 and GM1 in these tissues. Female reproductive organs were similarly found to utilize FUT2 for the fucosylation of glycolipids FGA1 (uterus and cervix), and FGM1 (ovary), due to their absence in FUT2-null mice. In FUT1-null mice FGA1 was lost from the pancreas, but was present in wild-type and FUT2-null mice, indicating that FUT1 is essential for fucosylation of GA1 in the pancreas. *Ulex europaeus* agglutinin-I lectin histochemistry for α(1,2)fucose residues confirmed the absence of α(1,2)fucose residues from the apical surface of pancreatic acinar glands of FUT1-null mice. Ileum, epididymis, and testis retained specific fucosylated glycolipids, irrespective of targeted deletion of either gene, indicating either compensation for or redundancy of the α(1,2)fucosyltransferase genes in these tissues.

Key words: fucosyltransferase (FUT1 and FUT2), gangliosides, gastrointestinal system, germ-free mice, glycolipids, knockout mice.

INTRODUCTION

α(1,2)Fucosyltransferase (GDP-L-fucose:β-D-galactosyl-R 2-α-L-fucosyltransferase, EC 2.4.1.69) catalyses the transfer of α-L-fucose to the terminal β-D-galactose residue of glycoconjugates through an α(1,2) linkage. The resultant Fucα(1,2)Galβ structures exhibit H-antigenicity for blood-group carbohydrates and provide ligands for microbe adhesion, morphogenesis, and metastasis of cancer cells [1,2]. Two distinct structural genes, *FUT1* (*H* gene) and *FUT*2 (secretor or *Se* gene) encode α(1,2)fucosyltransferases responsible for the syntheses of H, A and B antigens in the erythroid lineage and vascular endothelial cells, and of Le^b and Le^Y (Lewis) antigens in endoderm-derived epithelial tissues, including the digestive tract and salivary glands respectively [3]. The *SEC1* pseudogene is similar in overall sequence to the *FUT2* gene, but encodes an inactive enzyme due to a frameshift mutation in humans [4].

Counterparts of the human α(1,2)fucosyltransferase genes have been found in mouse, specifically *FUT1* (*MFUT-I*), *FUT2* (*MFUT-II*), and *SEC1* (*MFUT-III*) genes, and their enzymic properties have been examined using homogenates of COS-7 cells after transfection of the open reading frames in mammalian expression vectors [5–8]. Homogenates of *FUT1*- and *FUT2*-transfected cells, but not of *Sec1*-transfected cells, exhibit α(1,2)fucosyltransferase activity with *p*-nitrophenyl β-D-galactoside. Substrate specificity and specific activity toward various oligosaccharides differ between FUT1 and FUT2 enzymes, as well as between human and mouse enzymes.

Mouse FUT1 and FUT2 enzymes exhibit the ability to fucosylate the non-reducing terminal galactose of GA1 (Gg_4Cer) to give the type 4-H structure as the major glycosphingolipid in murine non-neuronal tissues [7]. FGA1 (fucosyl GA1) was found in the small intestine of conventionally bred mice after the weaning period, but not in that of germ-free mice, in which its expression was induced by the administration of microbes under epigenetic control. The gene responsible for the expression of FGA1 in the small intestine was demonstrated to be *FUT2* [6,7]. In knockout mice, no distinct effect on development or histology of the reproductive tract was observed in mice with either the *FUT1* or *FUT2* gene deleted [9]. In order to determine the tissue-specific modifications induced in the carbohydrate structure of glycolipids by the targeted deletion of the *FUT1* and *FUT2* genes, we made quantitative determinations of glycolipids exhibiting type 4-H antigenicity of FUT1- and FUT2-null mice by TLC-immunostaining with monoclonal antibodies.

MATERIALS AND METHODS

Animals

FUT1- and FUT2-null mice were generated as reported previously [9], and tissues of F2- to F4-generation null mice, together

Abbreviations used: *FUT1*, α(1,2)fucosyltransferase *H* gene; *FUT2*, α(1,2)fucosyltransferase *Se* gene; GA1, Gg_4Cer [Gal(β1,3)GalNAc(β1,4)Gal-(β1,4)Glc-Cer]; FGA1, fucosyl GA1 ($IV^2Fuc\text{-}Gg_4Cer$); GM1, $II^3NeuNAc\text{-}Gg_4Cer$ {Gal(β1,3)GalNAc(β1,4)[Sia(α2,3)]Gal(β1,4)Glc-Cer}; FGM1, fucosyl GM1 ($IV^2Fuc,II^3NeuNAc\text{-}Gg_4Cer$); Lc_4Cer, lactotetraosylceramide; nLc_4Cer, paragloboside (neolactotetraosylceramide); Le, Lewis (antigen); $IV^3NeuNAc\text{-}nLc_4Cer$, sialylparagloboside; *Sec1*, third α(1,2)fucosyltransferase gene; UEA-I, *Ulex europaeus* agglutinin-I. Nomenclature of glycolipids follows the recommendations by IUPAC-IUB Commission on Biochemical Nomenclature (1977) Lipids **12**, 455–463.

[1] To whom correspondence should be addressed (e-mail Iwamori@chem.kindai.ac.jp).

with those from wild-type controls (C57BL/6J × 129 × 1/SvJ F1 hybrid background), were used for analysis.

Glycolipids

GM1, FGM1, IV^{3}NeuAc-nLc$_4$Cer (sialylparagloboside), Lc$_4$Cer (lactotetraosylceramide or paragloboside) and Forssman glycolipids were purified from bovine brain, porcine thyroid, human erythrocytes, human meconium and equine kidney respectively, in our laboratory, and nLc$_4$Cer (neolactotetraosylceramide), GA1 and FGA1 were prepared from IV^{3}NeuAc-nLc$_4$Cer, GM1 and FGM1 respectively by treatment with *Arthorobactor ureafaciens* sialidase [10,11].

Antibodies

Polyclonal antibodies to GA1, GM1 and Forssman glycolipids, and monoclonal antibodies to FGA1 (LFA-1, IgG2) and FGM1 (LFM-1, IgG3) were generated in our laboratory by immunizing rabbits or mice with purified glycolipids together with *Mycobacterium tuberculosis* or *Salmonella minnesota* as the adjuvant, and were proven to exhibit monospecific reactivity toward the carbohydrate moieties of the respective antigen glycolipids [6].

Quantitative TLC-immunostaining

The extraction of lipids from the freeze-dried tissues of mice was performed by incubating them with chloroform/methanol/water (20:10:1, 10:20:1, and 1:1; by vol.), the volume of the combined extracts being adjusted with chloroform/methanol (1:1, v/v). The lipid extracts, each corresponding to 0.1 mg of dry tissue weight, were chromatographed on plastic-coated TLC plates (Macherey-Nagel, Düren, Germany), successively with n-hexane/diethyl ether (4:1, v/v) and chloroform/methanol/0.5 % $CaCl_2$ in water (55:45:10, v/v), and then the spots were visualized by immunostaining with the above anti-glycolipid antibodies, followed by immunostaining with peroxidase-conjugated anti-rabbit or anti-mouse IgG and IgM antibodies (Jackson Immunoresearch Laboratories, PA, U.S.A.), and with biotin-conjugated *Ulex europaeus* agglutinin-I (UEA-I) (EY Laboratories Inc., San Mateo, CA, U.S.A.), followed by peroxidase-conjugated avidin (Vector Laboratories, Burlingame, CA, U.S.A.), according to the procedure reported previously [12]. Known amounts of the respective glycolipids were stained on the same plates, and the densities of the spots were determined at 500 nm by TLC-densitometry (CS-9000; Shimadzu, Kyoto, Japan) to obtain standard curves, which were linear between 5 to 50 ng of glycolipids.

COS-7-cell expression and fucosyltransferase activity

The open reading frames of the *FUT1* and *FUT2* genes were ligated into mammalian expression vector pcDNA3.1 (termed pcDNA3.1-Fut1 and pcDNA3.1-Fut2), followed by transfection into COS-7 cells using a Cellphect transfection kit [7]. Cells were homogenized in 0.25 M sucrose and the protein content of the homogenate was then determined by Bradford's method with BSA as the standard [13]. α(1,2)Fucosyltransferase activity was determined with GA1, GM1, nLc$_4$Cer and Lc$_4$Cer as substrates, with homogenates of COS-7 cells transfected with pcDNA3.1, pcDNA3.1-Fut1 and pcDNA3.1-Fut2 as enzyme sources. The standard assay mixture comprised 38 nmol glycolipid, 20 mM $MnCl_2$, 1 % Triton X-100, 50 mM cacodylate/HCl buffer (pH 5.8), 37 pmol GDP-[^{14}C]fucose (270 mCi/mmol), 4 nmol GDP-fucose, and 0.4 mg enzyme protein, in a final volume of 100 μl. After incubation at 37 °C for 1 h, the reaction was terminated with 200 μl of ethanol and the products were separated by TLC with chloroform/methanol/0.5 % $CaCl_2$ in water (55:45:10, v/v). The radioactivity incorporated into glycolipids was determined with an image analyzer (BAS2000; Fuji Film, Tokyo, Japan), and with a liquid scintillation counter (Tri-Carb1500; Packard). The apparent K_m values for the fucosylation of several glycolipids by the FUT1 and FUT2 enzymes were determined, using glycolipids in the range of 0.38–114 nmol, by the Enzyme-Kinetics program (Trinity Software, Campton, NH, U.S.A.).

Germ-free mice and Northern hybridization

Germ-free mice (10-week-old C57BL) received oral administration of faeces (10 mg/100 μl in PBS) from conventionally bred mice, and 48 h later were used to determine the expression of FUT1 and FUT2 mRNA in the digestive tract. Total RNA (25 μg) extracted from tissues by the acid guanidine thyocyanate phenol/chloroform method was separated on a formaldehyde-agarose (1.2 %) gel and then transferred to a nylon membrane. Membranes were prehybridized in 6 × SSC (where SSC is 0.15 M NaCl/0.015 M sodium citrate), 5 × Denhart's solution, 0.1 % SDS, and 100 μm/ml denatured salmon sperm DNA at 65 °C for 4 h. Gene-specific ^{32}P-labelled probes, at positions 953–1040 for *FUT1* and 903-980 for *FUT2*, were hybridized with the membranes at 65 °C for 15 h. After washing the membranes twice with 1 × SSC, 0.1 % SDS at 22 °C, and three times with 0.2 × SSC, 0.1 % SDS at 65 °C, radioactive bands were visualized by autoradiography with X-ray film (RX-U; Fuji, Tokyo, Japan).

Lectin histochemistry

The α(1,2)fucose-specific lectin UEA-I was conjugated to biotin (EY Laboratories). Adult wild-type, FUT1-null, and FUT2-null mice were terminally perfused with PBS, and pancreas tissue was fixed in 10 % buffered formalin at 4 °C overnight. Frozen sections were processed for immunohistochemistry as described previously [9].

RESULTS

Fucosyltransferase activity toward glycolipid substrates *in vitro*

The fucosyltransferases encoded by the *FUT1* and *FUT2* genes in mammalian expression vectors were transfected into COS-7 cells and their enzymic properties were determined with glycolipid substrates. Both enzymes exhibited significantly higher affinity and specific activity toward GA1 than toward GM1, nLc$_4$Cer, or Lc$_4$Cer, indicating that sialic acid at the internal galactose residue of GA1 decreases the fucosyltransferase activity toward the terminal galactose (Table 1). In comparison with the substrate specificity to lacto-series glycolipids, the activity of the FUT1 enzyme toward nLc$_4$Cer was found to be higher than for Lc$_4$Cer, whereas the specific activity of FUT2 enzyme toward Lc$_4$Cer was higher than for nLc$_4$Cer.

Similarly, the apparent K_m for nLc$_4$Cer and Lc$_4$Cer differed between FUT1 and FUT2 enzymes, showing higher affinity (lower K_m) of FUT1 toward nLc$_4$Cer, and higher affinity of FUT2 toward Lc$_4$Cer (Table 1). Thus, FUT1 exhibited higher activity toward the lacto-series type 2 chains, lactose and phenylβ-D-galactoside than FUT2; FUT2 enzyme showed higher activity with the lacto-series type 1 chains and ganglio-series carbohydrates. Since ganglio-series carbohydrates are the major glycolipids in mouse non-neural organs, the substrate activities of the FUT1 and FUT2 enzymes were both found to be in ranges suitable to direct fucosylation of glycolipids in mouse tissues.

Table 1 Specific activity of fucosyltransferases towards glycolipids in pcDNA3.1-Fut1- and pcDNA3.1-Fut2-transfected COS-7 cells

Homogenates of COS-7 cells transfected with pcDNA3.1-Fut1 or pcDNA3.1-Fut2 were used for determination of α(1,2)fucosyltransferase activity as described in the Materials and methods. The specific activity represents the value obtained with 38 nmol of glycolipid substrates. The apparent K_m for each glycolipid is the mean ± S.D. of three separate determinations, using the EnzymeKinetics programs.

	pcDNA-Fut1		pcDNA-Fut2	
Glycolipid	Specific activity (pmol/mg protein/h)	K_m (μM)	Specific activity (pmol/mg protein/h)	K_m (μM)
GA1	22.5 ± 3.8	55.6 ± 4.7	195.9 ± 10.5	50.0 ± 2.1
GM1	4.3 ± 0.1	909.1 ± 10.3	12.1 ± 1.6	500.0 ± 12.8
nLc$_4$Cer	10.8 ± 0.2	80.1 ± 2.3	43.6 ± 3.4	166.7 ± 4.0
Lc$_4$Cer	6.2 ± 0.1	117.6 ± 8.0	60.5 ± 4.8	95.2 ± 6.2

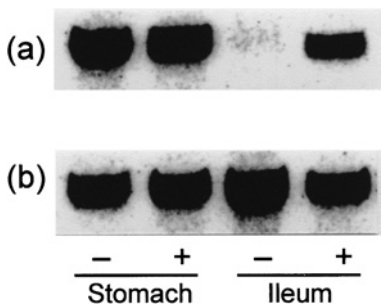

Figure 1 Northern-blot analysis of the FUT2 transcript in germ-free mice

Total RNA (25 μg) extracted from the stomach and ileum of germ-free mice before (−), and 48 h after administration of faeces from conventionally bred mice (+), was electrophoresed and then transferred to a nylon membrane, which was probed with a radiolabelled cDNA for FUT2 (**a**), followed by stripping and reprobing with a radiolabelled cDNA for glyceraldehyde-3-phosphate dehydrogenase (**b**).

Induction of FUT2 transcript in ileum, but not stomach, of germ-free mice

Similar to our previous study involving an ICR strain of mice, a 3.5 kb FUT2 transcript was clearly demonstrated in the stomach, but not in the ileum of germ-free mice (C57BL strain). As shown in Figure 1, the steady-state level of FUT2 transcript in the stomach of germ-free mice was not altered after the administration of faeces. FUT2 transcripts in ileum were induced in germ-free mice 48 h after administration of faeces from conventionally bred mice. FUT2 transcripts became detectable in ileum 6 h after the administration of faeces, and increased dramatically in relative concentration to a maximum level at 24 h (results not shown). In association with the increased expression of the FUT2 transcript, FGA1 was detectable after 8 h and was the major glycolipid in the ileum of mice 24 h after the administration of faeces. The induction of the FUT2 transcript and FGA1 after the administration of faeces was similarly observed in the duodenum, jejunum and ileum, but not in the stomach, cecum or colon (results not shown). On the other hand, the FUT1 transcript was not detected in the stomach or small intestine in germ-free mice, and only in a trace amount in the colon (results not shown).

Glycolipids in the digestive tract of FUT1-null and FUT2-null mice

As shown in Table 2 and Figure 2, the concentrations of Forssman glycolipids belonging to the globo-series glycolipids were quantified in various regions of the digestive tract in wild-type, FUT1-null, and FUT2-null mice. No dramatic differences were found, indicating that the targeted deletion of either fucosyltransferase gene does not have a significant effect upon the metabolism of globo-series glycolipids. However, both FGM1 and FGA1 in the antrum, cecum, proximal colon, and distal colon completely disappeared from FUT2-null, but not FUT1-null, mice. Precursor glycolipids GM1 and GA1, accumulated in these tissues in FUT2-null mice, indicating that the fucosylation of GA1 and GM1 in the antrum, cecum and colon is performed only by FUT2.

The molar percentage of FGA1 in total GA1 plus FGA1, and that of FGM1 in total GM1 plus FGM1, were compared to estimate the proportion of *in vivo* fucosylation (Table 2). GA1 in the antrum, cecum and colon of wild-type mice was fucosylated in 71–82% of samples, and that of FUT1-null mice in 62–82% of samples. Although the proportions of FGM1 in the cecum of wild-type and FUT1-null mice were 95% and 70% respectively, GM1 in the antrum and proximal colon was found to be completely converted to FGM1, only being detected in the antrum and proximal colon of FUT2-null mice. Also, GA1 accumulated in the antrum, cecum and colon of FUT2-null mice amounted to 1.6–6.3-fold the levels in wild-type and FUT1-null mice.

On the other hand, FGA1 in the ileum was unexpectedly detected in wild-type, FUT1-null and FUT2-null mice (Table 2 and Figure 2). The molar percentages of FGA1, representing the proportions of fucosylation of GA1, in the ileum of wild-type, FUT1-null and FUT2-null mice were 17.6%, 81.7% and 9.5% respectively, indicating highly enhanced synthesis of FGA1 in the ileum of FUT1-null mice in comparison with wild-type and FUT2-null mice. As a result, a compensatory reduction of GA1 was observed in the ileum of FUT1-null mice, the level being one-fourth the molar concentrations of those in wild-type and FUT2-null mice.

Glycolipids in male and female reproductive organs and pancreas of FUT1- and FUT2-null mice

FGA1 in the uterus and cervix, and FGM1 in the ovary of wild-type mice were absent in the FUT2-null mice, but not in the FUT1-null mice, indicating the essential involvement of the *FUT2*-encoded enzyme in the fucosylation of glycolipids in the female reproductive organs (Table 2 and Figure 3). Although the accumulation of precursor glycolipids in these organs of FUT2-null mice was not as evident as that in the digestive organs, the proportions of fucosylation of GA1 and GM1 were similar between wild-type and FUT1-null mice, being 30–34% of FGA1 levels in the uterus, 42–47% of FGA1 levels in the cervix and 70–73% of FGM1 levels in the ovary respectively.

In contrast, FGA1 in the epididymis and testis, and FGM1 in the testis of male mice were present in FUT1-null, FUT2-null and wild-type mice, indicating that the enzymes responsible for the

Table 2 Concentrations of glycolipids in wild-type (W), Fut1-null (I) and Fut2-null (II) mice (pmol/mg dry weight)

Values are means ± S.D., values in parentheses are the molar percentages of FGA1 in GA1 plus FGA1, and those of FGM1 in GM1 plus FGM1. P. colon, proximal colon; D. colon, distal colon.

	Forsman antigen			GA1			FGA1			GM1			FGM1		
Tissue	W	I	II	W	I	II	W	I	II	W	I	II	W	I	II
Antrum	205 ± 13	172 ± 6	162 ± 5	44 ± 6	47 ± 6	73 ± 7	185 ± 10 (80.8)	148 ± 12 (75.9)	0	0	0	89 ± 4	389 ± 18 (100)	352 ± 12 (100)	0
Ileum	170 ± 6	183 ± 8	182 ± 2	220 ± 2	55 ± 7	219 ± 10	47 ± 7 (17.6)	245 ± 16 (81.7)	23 ± 1 (9.5)	0	0	0	0	0	0
Cecum	221 ± 7	224 ± 4	259 ± 3	78 ± 5	104 ± 6	251 ± 11	192 ± 8 (71.1)	179 ± 6 (63.3)	0	21 ± 2	144 ± 8	205 ± 12	415 ± 23 (95.2)	343 ± 16 (70.4)	0
P. colon	223 ± 4	221 ± 4	209 ± 2	41 ± 5	49 ± 6	108 ± 9	187 ± 7 (82.0)	194 ± 4 (79.8)	0	0	0	21 ± 1	30 ± 7 (100)	42 ± 5 (100)	0
D. colon	211 ± 3	206 ± 3	207 ± 3	10 ± 5	17 ± 1	63 ± 3	25 ± 2 (71.0)	58 ± 8 (77.3)	0	0	0	0	0	0	0
Epididymis	118 ± 8	106 ± 6	89 ± 10	14 ± 1	80 ± 7	28 ± 1	109 ± 8 (88.6)	52 ± 3 (39.4)	101 ± 8 (83.4)	0	0	0	0	0	0
Testis	194 ± 9	198 ± 7	197 ± 3	92 ± 6	82 ± 7	65 ± 3	218 ± 12 (70.3)	213 ± 11 (72.2)	198 ± 10 (75.3)	12 ± 1	16 ± 2	18 ± 2	26 ± 2 (52.0)	19 ± 1 (54.3)	17 ± 1 (48.6)
Uterus	292 ± 4	351 ± 6	297 ± 5	122 ± 10	106 ± 4	98 ± 8	64 ± 2 (34.4)	43 ± 2 (28.9)	0	6 ± 1	0	3 ± 1	0	0	0
Cervix	184 ± 8	167 ± 5	109 ± 5	30 ± 2	9 ± 1	17 ± 2	22 ± 8 (42.3)	8 ± 1 (47.1)	0	0	0	0	0	0	0
Ovary	210 ± 8	129 ± 6	117 ± 6	6 ± 1	2 ± 1	2 ± 1	0	0	0	21 ± 1	8 ± 1	tr	59 ± 2 (73.8)	19 ± 1 (70.4)	0
Pancreas	144 ± 8	90 ± 4	113 ± 7	6 ± 1	5 ± 2	9 ± 1	43 ± 2 (87.8)	0	7 ± 1 (43.8)	0	0	0	0	0	0

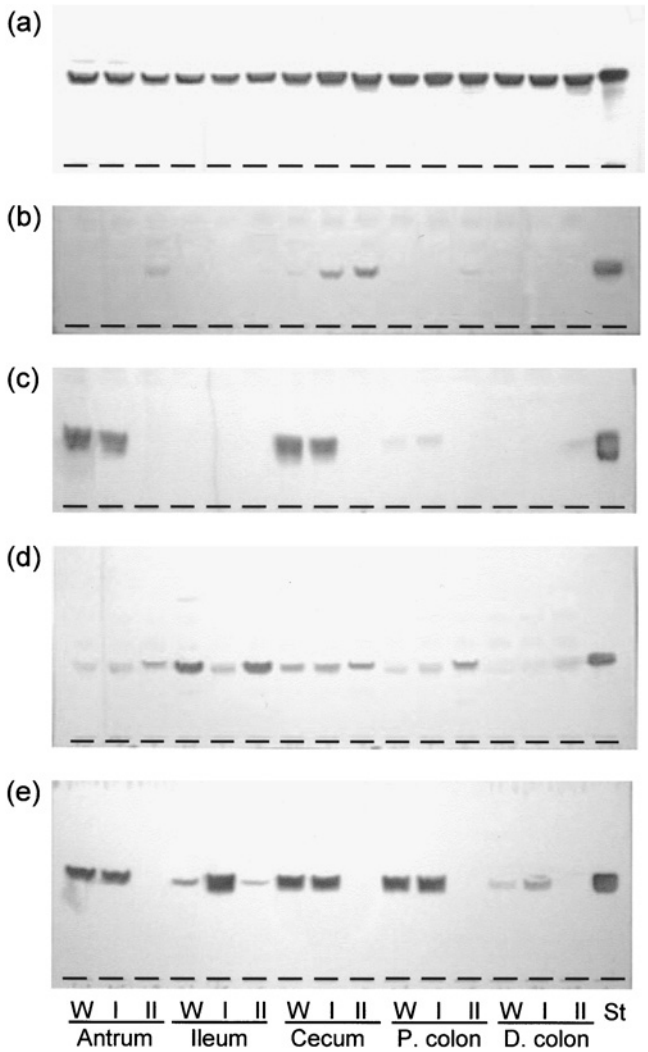

Figure 2 TLC-immunostaining of glycolipids from the digestive tract

Lipid extracts, corresponding to 0.1 mg dry weight, were chromatographed on plastic TLC plates. The spots were visualized by immunostaining with the following antibodies: anti-Forssman (**a**), anti-GM1 (**b**), anti-FGM1 (**c**), anti-GA1 (**d**), and anti-FGA1 (**e**). W, wild-type mice; I, FUT1-null mice; II; FUT2-null mice; P. colon, proximal colon; D. colon, distal colon; St, glycolipid standards of Forssman (**a**), GM1 (**b**), FGM1 (**c**), GA1 (**d**), and FGA1 (**e**).

fucosylation of glycolipids can be driven from either the FUT1 or FUT2 gene in male reproductive organs. In the epididymis, the proportions of FGA1 in GA1 plus FGA1 in wild-type, FUT1-null and FUT2-null mice were 88.6%, 39.4% and 83.4% respectively. GA1 in FUT1-null mice showed compensatory accumulation to a concentration of more than three to five times the levels in wild-type and FUT2-null mice. Fucosylation of GA1 and GM1 in the testis occurred in the proportions of 70–75% and 48–54% respectively, irrespective of the targeted deletion of either gene.

In the pancreas, FGA1 was present in wild-type and FUT2-null mice, but not FUT1-null mice, indicating that synthesis of FGA1 in the pancreas is preferentially performed by the FUT1 enzyme (Table 2 and Figure 4). To detect the presence or absence of α(1,2)fucose glycoconjugate residues, lectin histochemistry was performed on pancreas of wild-type, FUT1-null, and FUT2-null mice with the α(1,2)fucose-specific lectin UEA-I. UEA-I lectin staining confirmed the absence of α(1,2)fucose residues from the apical surface within acinar glands of the pancreas of FUT1-nulls

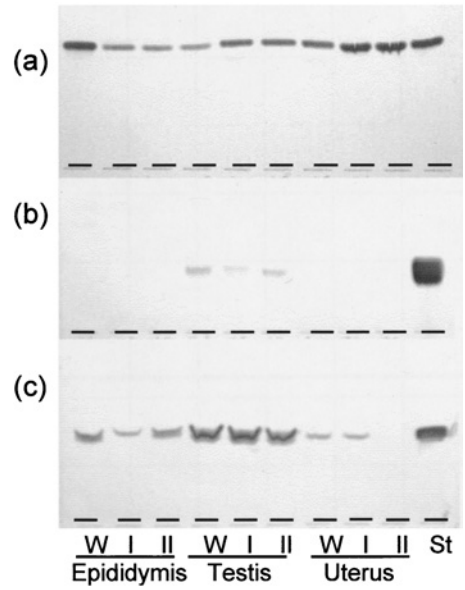

Figure 3 TLC immunostaining of glycolipids from reproductive organs

Lipid extracts, corresponding to 0.1 mg dry weight, were chromatographed on plastic-coated TLC plates. The spots were visualized by immunostaining with the following antibodies: anti-Forssman (**a**), anti-FGM1 (**b**), and anti-FGA1 (**c**). The standard glycolipids (St) were Forssman glycolipids (**a**), FGM1 (**b**), and FGA1 (**c**).

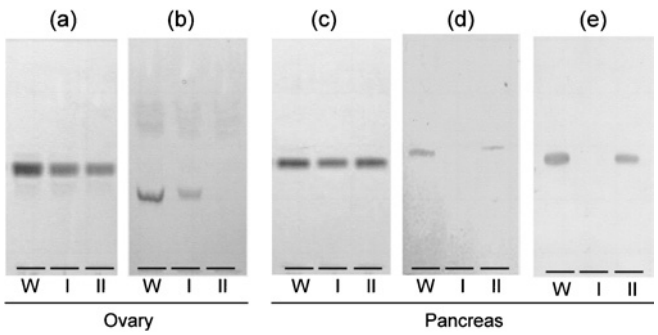

Figure 4 TLC immunostaining of glycolipids from ovary and pancreas

Lipid extracts, corresponding to 0.1 mg dry weight, were chromatographed on plastic-coated TLC plates. The spots were visualized by immunostaining with the following antibodies: anti-Forssman (**a**, **c**), anti-FGM1 (**b**), anti-FGA1 (**d**), and UEA-I lectin (**e**). The bands detected by UEA-I lectin were more intense than bands detected by anti-FGA1.

(Figure 5). For other tissues studied (salivary glands, thymus, spleen, kidney, brain, lung and liver), FGA1 and FGM1 were not detected under the conditions used in these experiments (results not shown).

DISCUSSION

The nucleotide-sequence identity between *FUT1* and *FUT2* is 75.8 %, yet the cognate α(1,2)fucosyltransferases encoded by these genes exhibit similar substrate specificities with the highest activity toward GA1, the major glycolipid detected in mouse tissues. Both enzymes had the ability to fucosylate glycolipids in mouse tissues, however, each gene was found to be expressed in a tissue-specific manner on analysis of FGA1 and FGM1. The expression of the *FUT1* (H) and *FUT2* (Se) genes in human tissues is closely associated with the syntheses of A, B and O antigens by the *FUT1* gene, and of Lewis antigens Le^b and Le^Y by the *FUT2* gene [4,14–17]. Expression of mouse genes *FUT1* and *FUT2*, in contrast, occurred in a tissue-characteristic manner that was unexpectedly overlapping for carbohydrate structure. Only in the pancreas was the *FUT1*-encoded enzyme deduced to be essential for production of FGA1 in FUT1-null mice. *FUT2*-encoded enzyme was essential in antrum, cecum, colon, uterus, cervix and ovary due to the absence of FGA1 or FGM1. In each of these tissues, the fucosylated derivative of GA1 or GM1 was completely lacking due to the targeted deletion of either the *FUT1* or *FUT2* gene. Since FUT2-null mice exhibited fully normal fertility, the fucosylated glycan on the uterine epithelial cell surface was not essential for blastocyst adhesion, which was demonstrated by experiments involving carbohydrate-specific antibodies [18–20]. Absence of fucosylation in the antrum, cecum, colon and pancreas did not cause any visible abnormality in digestive function. On the other hand, the fucosylation potential to form glycolipids with type 4-H antigenicity was maintained in the ileum, epididymis and testis of FUT1-null and FUT2-null mice, indicating that the α(1,2)fucosyltransferases in these tissues are derived alternatively from either the *FUT1* or *FUT2* genes, or potentially a third α(1,2) fucosyltransferase gene (*Sec1*/*MFUT-III*).

It was notable that the *FUT1*-derived enzyme was utilized for the synthesis of FGA1 only in the ileum, i.e. not in the antrum or colon, of FUT2-null mice, in spite of the preferential expression of the FUT2 transcript in the ileum after administration of microbes. As reported previously [6], the expression of glycolipids in the small intestine (duodenum, jejunum and ileum) clearly differed from those in the stomach, cecum and colon. Firstly, the small intestine contained GA1 as the sole precursor glycolipid for α(1,2)fucosyltransferase to form FGA1, while other gastrointestinal tissues contained both GA1 and GM1 for the formation of FGA1 and FGM1 respectively, probably due to the occurrence of GM3 synthase at the key step for the synthesis of GM1 [21]. Secondly, the α(1,2)fucosyltransferase expressed in colon after weaning under developmental regulation, while its expression in the small intestine was epigenetically regulated in response to several stimuli, including microbial invasion of the digestive tract and administration of cycloheximide in the abdominal cavity [6]. Although GA1 in the small intestine of germ-free mice was the predominant glycolipid, GA1 in conventionally bred mice was partly converted to FGA1, which was transcriptionally induced by utilizing either the *FUT1* or *FUT2* gene. Thus, the third characteristic difference between the small intestine and large intestine was the alternative expression of either the *FUT1* or *FUT2* gene in the former, and the preferential expression of the *FUT2* gene in the latter.

In epididymis and testis, α(1,2)fucosyltransferase activity could be derived from either gene. The proportions of fucosylation of GA1 and GM1 in the ileum, epididymis and testis were different among FUT1-null, FUT2-null and wild-type mice. However, no change in the molar proportion of fucosylation of glycolipids in the testis was observed among wild-type, FUT1-null and FUT2-null mice (70.3–75.3 % of GA1 and 48.6–54.3 % of GM1). In contrast, in epididymis and ileum, where the *FUT1*- and *FUT2*-encoded enzymes were mainly involved in the fucosylation of GA1 [6–8], precursor GA1 accumulated. Concentration of GA1 in the epididymis of FUT1-null mice was higher than that in FUT2-null mice, while GA1 in the ileum of FUT2-null mice was higher than that in FUT1-null mice, indicating that the fucosylation activities in the epididymis of FUT1-null mice and the ileum of FUT2-null mice are relatively low. One can suggest that the reduced fucosylation potential in the epididymis of FUT1-null mice causes more severe reduction of H-antigenic glycoproteins

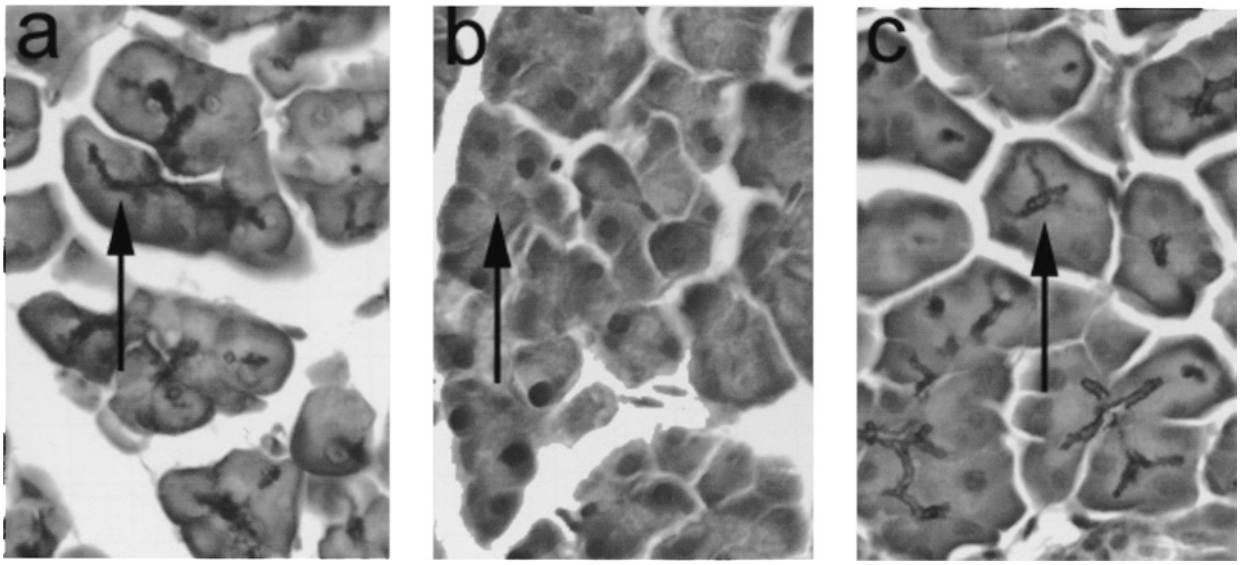

Figure 5 Lectin histochemistry of pancreas from wild-type, FUT1-null and FUT2-null mice

Pancreases from wild-type (**a**), FUT1-null (**b**) and FUT2-null (**c**) mice were processed as described in the Materials and methods section. Note the loss of specific staining from the apical surface within acinar glands of FUT1-nulls (**b**, arrowed).

than that of type 4-H glycolipids in the glycocalyx, resulting in the negative staining of the epididymal epithelium of FUT1-null mice with UEA-1 lectin and anti-(type 1-H) antibodies [8]. In contrast, the higher and lower amounts of FGA1 in the ileum of FUT1-null and FUT2-null mice respectively, than in the ileum of wild-type mice, were thought to be brought about under conventional breeding conditions. Analysis of the factors responsible for the epigenetic transcription of the genes is required to clarify the mechanism underlying their alternative expression. In connection with this finding, the mouse *FUT1* and *FUT2* loci have been shown to be closely located on chromosome 7, in a similar manner to the human *FUT1* and *FUT2* loci on chromosome 19 [4,8].

The overall profile of carbohydrate chains in the digestive tract is determined by a set of glycosyltransferases, among which those at the key step of metabolism play a leading part in the tissue-characteristic expression of glycoconjugates, that is, α-galactosyl, *N*-acetylglucosaminyl, *N*-acetylgalactosaminyl and *N*-acetylneuraminyl transferases for glycolipids, and *N*- and *O*-glycosidic glycoproteins [22–25]. Consequently, the amounts of Forssman glycolipids and GA1 in the stromal and epithelial tissue of the digestive tract are determined by the synthetic activities of Gb_3Cer and Gg_3Cer, respectively, being maintained as the major glycolipids even in the tissues of FUT1-null and FUT2-null mice. The resultant GA1 in the epithelial cells has been proven to be responsible for the receptor activity for bacteria belonging to *Lactobacillus* species, such as *L. casei, L. reuteri and L. johnsonii*, which form the bacterial flora in every part of the digestive tract and are implicated in the activation of intestinal immune systems [26]. Although there is no evidence that FGA1 is involved in the receptor for bacteria, fucosylation of GA1 should prevent the binding of *Lactobacillus* bacteria to the intestinal epithelium, indicating a possible role in the regulation of microbial symbiosis. Also, FGM1 has been noted to act as a target antigen for autoimmune diseases [27], suggesting that fucosylation of glycoconjugates generates a new epitope important for immune recognition. To obtain data as to the functional significance of terminal fucosylation of glycolipids, comparison of cell biological properties in tissues among wild-type, FUT1-null, and FUT2-null, as well as knockouts of more α(1,2)fucosyltransferase genes, including Sec1, is necessary.

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