Region-specific ontogeny of α-2,6-sialyltransferase during normal and cortisone-induced maturation in mouse intestine

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Dai, Dingwei, N. Nanda Nanthakumar, Tor C. Savidge, David S. Newburg, and W. Allan Walker. Region-specific ontogeny of α-2,6-sialyltransferase during normal and cortisone-induced maturation in mouse intestine. Am J Physiol Gastrointest Liver Physiol 282: G480–G490, 2002.—Regional differences in the ontogeny of mouse intestinal α-2,6-sialyltransferase activities (α-2,6-ST) and the influence of cortisone acetate (CA) on this expression were determined. High ST activity and α-2,6-ST mRNA levels were detected in immature small and large intestine, with activity increasing distally from the duodenum. As the mice matured, ST activity (predominantly α-2,6-ST) in the small intestine decreased rapidly to adult levels by the fourth postnatal week. CA precociously accelerated this region-specific ontogenic decline. A similar decline of ST mRNA levels reflected ST activity in the small, but not the large, intestine. Small intestinal sialyl α-2,6-linked glycoconjugates displayed similar developmental and CA induced-precocious declines when probed using Sambucus nigra agglutinin (SNA) lectin. SNA labeling demonstrated age-dependent diminished sialyl α-2,6 glycoconjugate expression in goblet cells in the small (but not large) intestine, but no such regional specificity was apparent in microvillus membrane. This suggests differential regulation of sialyl α-2,6 glycoconjugates in absorptive vs. goblet cells. These age-dependent and region-specific differences in sialyl α-2,6 glycoconjugates may be mediated in part by altered α-2,6-ST gene expression regulated by trophic factors such as glucocorticoids.

sialyl α-2,6 glycoconjugates; hormonal regulation; ontogeny of the gut; lectin

SIALIC ACIDS ARE A FAMILY of negatively charged sugars whose most common form in mammals is N-acetyl neuraminic acid (NANA). Sialic acid can be attached to penultimate galactose (Gal), N-acetylgalactosamine (GlcNAc), or N-acetylgalactosamine (GalNAc) sugars of glycoconjugates (glycoproteins, mucins, and glycolipids). In addition, sialic acid can form polysialic acid side chains of up to 50 residues in length via the 8-hydroxyl group (39). Sialic acid-containing glycoproteins represent a major class of cell surface receptors important for both physiological transcellular communication (20, 53, 54) and pathogenesis of major pathogens (63). During postnatal development, rodent intestinal membrane glycoconjugates undergo a striking alteration from an initially high sialic acid-to-fucose ratio to a low ratio during maturation (11, 35, 52). This inversion has been attributed to diminished sialylation and increased fucosylation of glycoconjugates during the transition from the suckling to the adult phenotype (50). Sialylation involves transfer of sialic acid from its activated form [cytidine monophosphate (CMP)-NANA] onto the acceptor sugar and is catalyzed by a sialyltransferase (ST) family of enzymes. The glyc an sequences most commonly found in intestine are NANA2,6Gal and NANA2,3Gal in membrane glycoproteins (43), NANA2,6GalNAc, NANA2,6Gal, and NANA2,3Gal in mucins (32), and NANA2,3Gal and NANA2,8NANA in gangliosides (62). Because there is a specific enzyme for each transfer reaction, the existence of up to 19 individual ST enzymes has been proposed (20, 53). Despite the similarity in the reaction catalyzed, all ST cloned to date exhibit very little homology, except for a short consensus sequence (the sialyl motif) to which the activated sugar donor is thought to bind (28, 53). Among these ST, β-galactoside α-2,6-ST (EC 2.4.99.1), which forms the terminal NANA2,6Galβ1-4GlcNAc sequence on the carbohydrate side chain of glycoproteins, was the first to be purified from rat liver (60). The corresponding cDNA has since been cloned (61), allowing molecular analysis of tissue-specific expression of ST mRNA (34, 36) and its regulation by glucocorticoids and cytokines (57). In the colon of the rat, mucus glycoprotein contains carbohydrate chains that terminate in sialic acid attached by α-2,3 and α-2,6 linkages (12, 48). However,
the adult rat colon contains less α-2,6-ST activity than α-2,3-ST activity, and the α-2,6-ST activity is more predominant in the small intestine. Furthermore, the α-2,6-transferase activity in the small intestine decreases from birth to weaning in the developing rat (12). The amount of mRNA and protein of this α-2,6-ST likewise decrease in the small intestine during rat development, with the most pronounced decrease occurring first in the distal small intestine (55). Administration of hydrocortisone in vivo results in a reduction of ST mRNA in immature rat small intestine (18) but causes upregulation in vitro in organ culture (19), indicating that regulation of ST can be mediated by corticosteroids (18). This decrease in ST is accompanied by a reciprocal increase in fucosyltransferase in rats, indicating an inverse relationship in the activities of the two principal enzymes involved in glycosylating the nonreducing termini of the glycans of rat intestinal glycoprotein (9, 52). This inversion of enzyme activities corresponds to a shift from sialylation to fucosylation of intestinal membrane glycoconjugates (50, 52). These changes in glycosylation may explain the differential expression of isoforms of membrane glycoproteins during postnatal gut development. For example, alkaline phosphatase (56) and γ-glutamyltransferase (25) both have sialic acid-rich immature isoforms and sialic acid-poor adult isoforms. Similarly, lactase has a sialic-rich neonatal isoform and a fucose-rich adult isoform (6). These observations support the hypothesis that intestinal maturation involves developmental control of membrane glycoconjugates. Their glycosylation is regulated primarily by the altered expression of specific glycosyltransferases, possibly mediated by modified enzyme gene transcription, activity, and stability. The shift in glycosylation from terminal sialic acid to terminal fucose may also relate to differential susceptibility to enteric pathogens in immature mammals.

The expression of sialic acid in cell surface glycoconjugates of mouse intestine is somewhat different from that of the rat, and its developmental expression and control of expression have not been well defined. Genetic deletion of specific glycosyltransferases in mice has been reported (14, 27, 29). Mice homozygous for the β1,4-galactosyltransferase deletion die during the third postnatal week. In wild-type mice, this period coincides with dramatic increases of the enzyme in the small intestine and changes in the brush-border membrane glycoproteins (9, 11); these changes coincide with a shift in microflora. The inability of the galactosyltransferase knockout mouse to effect these changes in gut physiology could contribute toward lethality in β1,4-galactosyltransferase mutant mice. Inactivation of fucosyltransferase (Fuc-TVII) is not lethal in mice, but the mice do respond inappropriately to experimental colitis of the gut (3, 14).

Thus intestinal glycosyltransferases may be central to the normal development of the gut and can participate in enteric pathophysiology. In addition, the documentation of normal development of ST provides an important basis for understanding the microbial-epithelial interaction in the developing mouse intestine.

To better understand the regulation of regional specificity of intestinal sialylation during intestinal development, we quantitatively studied the expression of α-2,6-ST enzyme activity, its mRNA accumulation, and microvillus membrane (MVM) sialylation in the mouse intestine from duodenum to colon during postnatal development. The effect of glucocorticoids, known trophic factors that promote precocious gut maturation, on α2,6-ST expression in the suckling mouse was also investigated using cortisone acetate (CA).

**MATERIALS AND METHODS**

**Reagents.** Fetuin, submaxillary mucin, α1-acid glycoprotein, neuraminidase (Clostridium perfringens), BSA, 2-mercaptoethanol, OCT, phenylmethylsulfonyl fluoride (PMSF), and ultra-pure sucrose were purchased from Sigma (St. Louis, MO). CMP-N-acetyl-[4,5,6,7,8,9-3H]neuraminic acid (CMP-[3H]NeuAc; 0.1 μCi, sp act, 1.8 mCi/mmol) was purchased from New England Nuclear Life Sciences (Boston, MA). TaqMan reverse transcription reagents and TaqMan Gold RT-PCR kits were purchased from Perkin-Elmer (San Ramone, CA). Streptavidin-horseradish peroxidase conjugate was purchased from Amersham Life Sciences (Piscataway, NJ). Biotinylated *Sambucus nigra* agglutinin (SNA), fluorescein-conjugated SNA, and *Maackia amurensis* II were purchased from Vector Laboratories (Burlington, CA). Control glycoprotein transferrin was purchased from Roche Diagnostics (Indianapolis, IN). CA was from Merck, Sharp and Dohme (West Point, PA). All other reagents were of analytic or molecular biology grade from Fisher Biotech (Pittsburgh, PA) or Sigma.

**Animals.** Timed-pregnant dams of Black Swiss mice were purchased from Taconic Farms (Germantown, NY) at 16–18 days of gestation. All dams were housed individually in opaque polystyrene cages. The dams gave birth in our animal facility with a 12:12-h light-dark cycle and were fed mouse chow and water ad libitum. On the due date, cages were checked every 4 h for the presence of pups. The date of birth of the pups was designated as day 0. The following day (day 1), litters were reduced to 9 pups per dam. To avoid circadian influences, all pups were killed between 1200 and 1400.

**Cortisone treatment.** Each litter of suckling mice was divided into two groups. At 10 days of age, one group was injected subcutaneously with a single dose (5 mg/100 g body wt) of CA in a saline suspension. The other control group was injected with the same volume of normal saline (0.9% NaCl). The animals were maintained with their dams until they were killed at 14 days of age.

**Preparation of the microsomal fraction.** Animals were killed by cervical dislocation, and the entire small intestine and whole colon were removed and thoroughly flushed with ice-cold 0.9% NaCl. The small intestine was divided into the duodenum, jejunum, and ileum. The small intestine from the stomach to the ligament of Treitz was defined as the duodenum. The proximal and distal halves of the remaining small intestine were defined as the jejunum and ileum, respectively. Intestine samples were placed on a glass plate maintained at 4°C and cut open; mucosa was harvested by scraping with a microscope glass slide. All subsequent procedures were performed at 4°C. A 10% mucosal homogenate in 0.1 M Tris-HCl buffer (pH 7.4) was centrifuged at 1,000 g for 15 min to remove nuclei and cellular debris. The supernatant was then centrifuged at 105,000 g for 1 h in a Beckman L6–65 ultracentrifuge, producing a microsomal pellet (9, 52). Enzyme activities were determined from this fraction (recon-
stained in the same buffer), which was either stored at 
-80°C or used immediately for the enzyme assay.

Protein determination. Protein was determined using a 
biocinchonic acid protein assay (Pierce, Rockford, IL) modi-
fied for use in 96-well microtiter plates as specified by the 
supplier. Absorbance was measured at 560 nm (BT 2000 
microkinetics reader spectrophotometer, Fisher). The protein 
centration of each sample was calculated using a BSA 
standard curve.

ST assay. Asialofetuin, asialo bovine submaxillary mucin, 
and asialo α₁-acid glycoprotein were prepared from their 
sialylated parent compounds by incubation with neuramin-
dase (1 U/10 mg glycoprotein) at 37°C, pH 5.0, in 100 mM 
acetate buffer in 150 mM NaCl for 8 h followed by boiling for 
15 min. Combined α-2,3-ST and α-2,6-ST activity on both N- 
and O-linked glycans was assayed using asialofetuin as an 
exogenous acceptor. Because of the ability of this acceptor to 
measure all of these activities, this acceptor was used to 
monitor changes in ST activity in specific regions of intestine 
as a function of time. To determine the relative contribution 
of each ST activity to the change in general activity, 
individual ST activities were measured in selected tissues 
through the use of specific substrates. Asialo submaxillary 
mucin was the receptor used to measure the addition of sialic 
acid by α-2,3 and α-2,6 linkages to O-linked glycans (mucin-
type). Asialo α₁-acid glycoprotein was used to measure the 
addition of sialic acid by α-2,3 and α-2,6 linkages to N-linked 
glycans, with a preference for α-2,6 linkages (37, 61). Lacto-
N-tetraose was used to measure the activity that catalyzes 
the addition of sialic acid by α-2,3 linkages to N-linked 
glycans, whereas N-acetyllactosamine was used to measure 
the activity in which addition of sialic acid by α-2,6 linkages 
to N-linked glycans occurs (37, 61).

The reaction mixture (total vol, 0.1 ml) contained 2.5 mM 
substrates, 12.5 μmol sodium phosphate buffer (pH 6.8), 
0.5% Triton X-100, 55 nmol CMP[14C]NeuAc, and 50–100 μg 
of microsomal fraction. Incubation was carried out at 37°C 
for 1 h and terminated by the addition of 0.5 ml 15% TCA 
containing 5% phosphotungstic acid. Assays were 
processed by rapid vacuum filtration for measurement of radioactive 
products as previously described (9, 52). Mixtures containing 
oligosaccharides were applied to a column of A1 X8 (100 
× 20 mm; Tetko, Elmsford, NY). RNA was 
dereproteinized by extraction with phenol, chloroform, and 
isoamyl alcohol and stored at −20°C (8). Purity of the RNA 
was determined by the ratio of absorbances at 260 to 280 nm, 
and the concentration was determined from optical density at 
260 nm.

Quantitative real-time RT-PCR assays. PCR primers and 
TaQMan probes for α-2,6-ST were designed using applica-
tion-based primer design software (Primer, Perkin-Elmer) 
according to the published mouse α-2,6-ST cDNA sequence 
(GenBank accession no. D16106) (17). Primer and probe 
sequences were as follows: forward, 5′-CGGGACCAGAGT-
CAGGTT-3′; reverse, 5′-ACATTCAGGTGCTCAGGAAGG-3′; 
and probe, 5′-ACCGTACAGGCTGCTGCTGCC-3′. The Taq-
Man probe was labeled with a reporter fluorescent dye, 6-car-
boxyfluorescein, at the 5′ end and a fluorescent dye quencher, 
6-carboxytetramethyl-rhodamine, at the 3′ end. PCR primers 
and a TaqMan probe for glyceraldehyde-3-phosphate dehydro-
genase (GAPDH) were obtained from Perkin-Elmer.

RT reactions used 1 μg total RNA in a total volume of 20 μl 
containing 1× PCR buffer II, 5 mM MgCl2, 1 mM of each 
dNTP, 2.5 μM random hexamers, 1 U/μl RNase inhibitor, 
and 2.5 U/μl Moloney murine leukemia virus RT. Reactions 
were performed in a PTC-200 Peltier thermal cycler (MJ 
Research, Watertown, MA). RT was carried out at 42°C for 15 
min, 99°C for 5 min, and 5°C for 5 min followed by incubation 
at 4°C. The relative abundance of α-2,6-ST and GAPDH 
mRNAs was assessed using TaqMan real-time PCR (15). The 
latter was used as the endogenous control. PCR reactions 
were carried out in duplicates in 25-μl volume consisting of 
TaqMan 1× PCR buffer A, 5.5 mM MgCl2, 0.2 μM 2′-de-
oxynucleoside 5′-triphosphate, 200 nM primers (forward and 
reverse), 100 nM TaqMan probe, 0.01 U/μl AmpErase, 0.025 
U/μl AmpliTaq Gold DNA polymerase, and 1 μl RT products. 
PCR was performed at 50°C for 2 min (for AmpErase UNG 
incubation to remove any uracil incorporated into the cDNA) 
and 95°C for 10 min (for AmpliTaq Gold activation) and then 
run for 40 cycles at 95°C for 15 s and at 60°C for 1 min (ABI 
 Prism 7700 sequence detection system, Perkin-Elmer). PCR 
reactions lacking RT product were run as controls.

The number of threshold cycles (Ct) was measured for 
each sample during PCR. Ct indicates the fractional cycle 
number at which the amount of amplified target reaches a 
fixed threshold. Ct is an important parameter for quantita-
tion (15). A duodenal sample from a 6-wk-old mouse was used 
as the calibrator and was run in every experiment. The 
relative amount of α-2,6-ST mRNA for each sample, normal-
ized to GAPDH and relative to the calibrator, was 
Req = 2 - Ct. Here, Ct value is determined by subtracting the average 
GAPDH Ct value from the average α-2,6-ST Ct value. 
The calculation of Ct involves subtraction by the Ct 
calibrator value.

Preparation of MVM. Mucosal scrapings were homoge-
nized in 500 mM mannitol, 10 mM HEPES, 5 mM EGTA, 
and 1 mM PMSF (pH 7.4), for 5 min, diluted with ice-cold distilled 
water (1:5), and filtered under suction through fine nylon 
mesh (mesh size, 40 μm; Tetko, Elmsford, NY). MgCl2 (1 M) 
was added to the crude homogenate (with stirring) to a 
final concentration of 10 mM, and the suspension was allowed to 
sediment for an additional 15 min. The homogenate was then 
frozen centrifuged at 5,000 g for 15 min. The pellet was discarded, 
and the supernatant was centrifuged at 28,000 g for 30 min. 
The resulting pellet was suspended and homogenized in 100 
mM mannitol, 10 mM HEPES, 5 mM EGTA, and 1 mM 
PMSF (pH 7.4) and centrifuged again at 28,000 g for 30 min. 
This purified MVM pellet was suspended and homogenized in 
10 mM Tris·HCl and 1 mM PMSF (pH 7.4) and stored at 
−80°C (6, 9, 31). The above procedures were all performed 
at 4°C.

Slot-blot analysis with SNA lectin. Mucosal protein (25 μg) 
was loaded onto a 0.45-μm nitrocellulose membrane with the 
use of the Bio-Dot SF microfiltration apparatus (Bio-Rad, 
Heracles, CA). Sialylglycoconjugates were detected on the 
blot using biotin-conjugated SNA lectin (Vector Laboratories, 
Burlingame, CA). SNA recognizes both sialic acid α-2,6-ga-
loactomoieties and sialic acid α-2,6-galactosamine moieties 
(46). The slot blot was blocked with solution containing 1% 
BSA in TTBS (0.05 mM Tris·HCl, 0.15 M NaCl, 1 mM MgCl2, 
and 1 mM CaCl2, pH 7.5) at room temperature for 1 h and 
washed twice for 10 min in TTBS. The membrane was sub-

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sequently probed with biotin-SNA (0.1 μg/ml) for 1 h, washed three times for 10 min each with TTBS, then incubated with streptavidin-horseradish peroxidase conjugate in the blocking solution (1:5,000 dilution) for 1 h. After washing the blot three times for 10 min each in TTBS, the blot was processed with SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL) and exposed to enhanced chemiluminescence Hyperfilm (Amersham, Piscataway, NJ). Densitometric analysis of the sample bands was performed with National Institutes of Health image analysis software. The amount of sialyl α-2,6 glycoconjugate in each sample was expressed in densitometry units. Transferrin was loaded and used as a positive control.

Lectin-fluorescent staining with SNA. Analysis of α-2,6 sialyl glycoconjugates was performed on frozen tissue sections using FITC-conjugated SNA (Vector Laboratories). Staining for α-2,3-sialylglycoconjugates was performed using Maackia amurensis lectin II (MAA II; Vector Laboratories). The middle 1 cm of tissues from each region of the gut was fixed for 2 h in 4% paraformaldehyde at 4°C, washed in ice-cold PBS containing 30% sucrose overnight at 4°C, and embedded in OCT. Frozen sections (6–7 μm thick) were blocked with PBS containing 2% BSA and then stained with labeled lectin for 1 h (10 μg/ml). Sections were then washed three times in cold PBS, mounted using Anti-Fade (Vector Laboratories), and analyzed by confocal microscopy.

Statistical analysis. Results are means ± SE. Effects of age and treatment were analyzed by two-way ANOVA. After overall significance was confirmed, post hoc tests for individual variables were performed by a two-tailed unpaired t-test. Differences with $P < 0.05$ were considered significant.

RESULTS

Age-dependent and region-specific changes in intestinal ST activity. To determine if there was a regional specificity in the developmental expression of ST, enzyme activity was measured in the duodenum, jejunum, ileum, and colon of mice from postnatal days 1 to 42 using asialofetuin as the acceptor, as it has broad specificity. The results (Fig. 1) showed that the highest ST activity was detected on day 1 in all regions, with a proximal-to-distal increasing gradient with the lowest activity in the duodenum ($P < 0.01$). During the first 3 wk (days 1 to 21), enzyme activities decreased to adult levels, with significant 1.4-, 3.4-, and 9.7-fold reductions recorded in the duodenum ($P < 0.01$), jejunum ($P < 0.001$), and ileum ($P < 0.001$), respectively. No developmental change was observed in the colon. Loss of activity was gradual in all tissues of the small intestine from days 1 to 21 and was not restricted to abrupt changes during the weaning period.

Relative distribution of α-2,3-ST and α-2,6-ST in mouse intestine. Enzymatic sialylation of asialofetuin was the measure of α2,3/6-ST, the combined activity of α-2,3- and α-2,6-ST activities for both N- and O-linked glycans. To investigate the relative contribution of the specific ST activities to the above changes in general ST activity, we used specific acceptors: asialo submaxillary mucin (O-linked ST activity), asialo α1-acid glycoprotein (N-linked ST activity, preference for α-2,6 glycoconjugates).
linked), N-acetyllactosamine (N-linked α-2,6-ST activity), and lacto-N-tetraose (N-linked α-2,3-ST activity) to measure the specific ST activities in immature (2 wk old) and mature (4 wk old) ileum and colon from the same mice as described above. The results are shown in Fig. 2. In the ileum, 80% of the ST activity was N-linked (Fig. 2B) rather than O-linked (Fig. 2C). Most of the N-linked activity was accounted for by α-2,6-ST activity (Fig. 2D) with minimal contributions by α-2,3-linked ST (Fig. 2E). In the colon, there was an appreciable contribution by N-linked ST activity (Fig. 2B), but there was a much higher relative contribution by O-linked ST activity (Fig. 2C). Note that the contribution by O-linked ST increases during colonic development whereas the contribution by N-linked ST decreases in the colon during development, similar to N-linked ST in the small intestine. As a result of these opposing trends, the combined N- and O-linked activity for colon (Fig. 2A) shows no significant change with development. Furthermore, in the colon, unlike the small intestine, there is a significant contribution to the N-linked activity by the α-2,3-ST (Fig. 2E), although the change in activity during development is again due largely to the α-2,6-ST activity. These data are consistent with mRNA of all α-2,3-ST genes responsible for α-2,3-ST activities that are not altered in the developing colon (24). These data strongly support the conclusion that the loss of α-2,6-ST activity during maturation of the small intestine in the mouse is primarily due to a loss of α-2,6-ST activity specific for N-linked glycans. There also seems to be a loss of this enzyme activity during maturation of the colon that may be somewhat offset by an increase in ST activity specific for O-linked glycans strongly expressed in goblet cells.

Age-dependent and region-specific changes in intestinal α-2,6-ST mRNA levels. To investigate possible control mechanisms for the above changes in α-2,6-ST activity, we examined developmental patterns of α-2,6-ST mRNA accumulation. Of the three known genes whose products have α-2,6-ST activity specific for N-linked glycans, two are known to be expressed in the small intestine. Quantitative RT-PCR was applied to total RNA prepared from the duodenum, jejunum, ileum, and colon from 2-, 4-, and 6-wk-old mice. The α-2,6-ST mRNA levels of one of these two intestinal genes (24, 51) were expressed in a pattern similar to that for enzyme activity, with a proximal-to-distal increase in mRNA accumulation (Fig. 3). A developmental decline in mRNA accumulation was observed in the duodenum, jejunum, and ileum, but not in the colon. In the colon, loss of specific N-linked α-2,6-ST activity may be partly related to undetermined posttranslational modification or differential promoter utilization. Despite this decline in mRNA levels, a similar regional gradient was maintained in all ages examined in the mouse small intestine.

Effect of exogenous glucocorticoid on α-2,6-ST mRNA accumulation and enzyme activity in suckling mouse intestine. Injection of CA on day 10 significantly accelerated the developmental decline of α-2,6-ST activity by day 14 in duodenum (2.5-fold), jejunum (2.3-fold), and ileum (13.5-fold) (Fig. 4A; P < 0.01). CA did not induce significant changes in the colon. The levels of α-2,6-ST mRNA accumulation after CA treatment paralleled α-2,6-ST activity (Fig. 4B). To confirm the general maturational effect of CA in the developing small intestine, we measured sucrase activity, which is a sensitive enzyme marker for small intestinal development (31), in the same tissue extracts. CA treatment significantly induced sucrase activity in the jejunum (5.64 ± 0.2 vs. 0.12 ± 0.05 μmol h⁻¹·mg protein⁻¹; P < 0.001).

SNA lectin staining to identify developmental and region-specific intestinal sites of sialylated glycoconjugate expression. To determine qualitative changes in the location of α2,6-linked sialylglycosylation in tissue

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Fig. 2. Developmental expression of specific ST activities in the mouse ileum and colon. Ileal and colonic tissues from 4 independent preparations were used for these assays. The overall N- and O-linked 2,3/6-ST activity was measured using asialofetuin as the substrate (A); N-linked 2,3/6-ST activity with preference to 2,6 linkage was measured with asialo α1-acid glycoprotein as the substrate (B); O-linked specific 2,3/6-ST activity was measured with asialo submaxillary mucin as the substrate (C); N-linked specific 2,6-ST activity was measured with N-acetyllactosamine as the substrate (D); and N-linked specific 2,3-ST activity was measured with lacto-N-tetraose as the substrate (E). All substrates were used at a final concentration of 2.5 mM in duplicates. Results are means ± SE; 16 2- and 4-wk-old animals were used.
sections, SNA lectin fluorophore labeling with visualization by Nomarski imaging was applied to intestinal tissues (Fig. 5). Representative sections of immature (day 1) ileum (Fig. 5, A, B, E, and F) and colon (Fig. 5, C, D, G, and H) compared with 4-wk-old ileum (Fig. 5, I and J) and colon (Fig. 5, K and L) are depicted. These images show that in immature 1-day-old gut, H9251 2,6-linked sialglycosylation is abundant and principally located in mucus-producing goblet cells in the small and large bowel (Fig. 5, F and H). Weak labeling was also observed in the mesenchyme and brush-border membrane. A marked loss of staining was observed in the small intestine during development, the most pronounced feature being an absence of sialoglycosylation in the goblet cell population. This change was not apparent in the colon where goblet cells were still labeled strongly with FITC-conjugated SNA after 4 wk (Fig. 5). CA treatment appeared to precociously diminish the level of lectin labeling in the small intestine, but not in the large intestine where goblet cells still expressed sialyl H9251 2,6 glycoconjugates (data not shown). Staining for H9251 2,3-sialylglycoconjugates was performed using MAA II in 1-day-, 2-wk-, and 4-wk-old ileum and colon (data not shown). In contrast with SNA staining, MAA II staining is not developmentally altered and exhibits uniform labeling of crypt, villus, and colonic cuff epithelium in all tissue. The goblet cell mucus was not labeled with MAA II lectin staining. This data is consistent with the N-linked specific H9251 2,3-ST activity (Fig. 2E). These data suggest that although the developmental decline in H9251 2,6-linked sia-

lyglycosylation was found in a number of different cell types and extracellular matrix components throughout the intestinal mucosa, the most prominent loss of expression in the small intestine was in the goblet cell population.

Sialyl H9251α 2,6-linked glycoconjugate expression in MVM during development and after cortisone treatment of suckling mice. Measurements of H9251α 2,6-ST enzyme activity and mRNA accumulation in mucosal scrapings reflect the combined changes in goblet cell and mesenchymal expression, as well as expression in MVM. Because H9251α 2,6-linked sialylglycoproteins in the MVM of intestinal absorptive epithelial cells constitute potential bacterial binding sites for components of the gut microflora, we examined MVM from all the tissues to measure any developmental and region-specific alterations in sialylglycoconjugate content. A proximal-to-distal increase in brush-border sialylglycoconjugate content was observed in all age groups examined (Fig. 6), consistent with data on whole mucosal scrapings. Likewise, a developmental decrease in sialylglycoprotein content was observed in the small intestine and, in

**Fig. 3.** Real-time RT-PCR assay of H9251α 2,6-ST mRNA in the developing gastrointestinal tract of the mouse. Total cellular RNA was prepared from the duodenum, jejunum, ileum, and colon of mice at different ages. RT-PCR was carried out using the TaqMan approach. The number of threshold cycles (Ct) was an important parameter for quantitation. A 6-wk-old duodenum sample served as a calibrator. The relative amount of H9251α 2,6-ST mRNA for each sample, normalized to glyceraldehyde-3-phosphate dehydrogenase and relative to the calibrator, was Req = 2 − Ct (see MATERIALS AND METHODS for detailed calculation). Each bar represents a relative amount of H9251α 2,6-ST mRNA for a single sample.

**Fig. 4.** Effect of cortisone acetate (CA) on H9251α 2,6-ST activity (A) and mRNA levels (B) in the mouse gastrointestinal tract. Four litters of pups were used; on postnatal day 10, half of the pups in each litter were treated with cortisone and the other half were treated with saline only. All pups were killed on day 14. The tissues were collected for the enzyme assay and total cellular RNA extraction. Values are means ± SE of 3 samples for H9251α 2,6-ST activity (A) or relative amount of mRNA for 1 sample (B). Each sample was pooled from 6 mice. *P < 0.05 vs. control group.
contrast to the finding on whole scrapings, the colon. Similarly, these developmental declines of α,2,6-linked sialylglycoprotein in the MVM fraction were accelerated by CA treatment in all tissues examined (P < 0.001). Even though α,2,6-ST mRNA levels and activity were not altered in the colon during development or by CA (Figs. 1–3), α,2,6-linked sialylglycoprotein was significantly reduced from 2 to 4 wk of age (P < 0.05). These age and regional differences in α,2,6-linked sialylated glycoconjugate patterns may be due to changes in α,2,6-ST enzyme activity as well as the availability and the turnover rate of MVM glycoproteins that function as substrates for α,2,6-ST activity.

**DISCUSSION**

This study provides the first evidence in the mouse of distinct developmental and region-specific control of β-galactoside α,2,6-ST (N-linked α,2,6-ST) expression in the small and large intestine. In all tissues examined, regulation of combined ST (N- and O-linked α,2,6-ST) activity reflected an anterior-to-posterior gradient, with the highest enzyme activity recorded in the colon. In the small intestine, most of the ST activity could be attributed to N-linked α,2,6-ST activity that diminished during development. This decrease in N-linked α,2,6-ST activity was most likely regulated at the transcriptional level. In contrast, developmental diminution of colonic N-linked α,2,6-ST activity was not associated with a similar alteration in mRNA accumulation, indicating possible posttranscriptional modification (34), differential expression other ST genes (24, 36, 51, 53, 59), and/or alternate promoter utilization (36, 58). However, a clearer understanding of region-specific regulatory events of all α,2,3/6-ST genes is beyond the scope of this work. Importantly, although a loss of N-linked α,2,6 ST activity is evident during development of the large bowel, nonspecific ST activity is not altered. This is principally due to a developmental and tissue-specific increase of O-linked α,2,3/6-ST activities most likely being expressed in colonic goblet cell populations. However, further investigation is required to examine whether measurement of cell lineage-specific α,2,6-ST expression accurately reflects developmental changes that are apparent in specific cell lineages such as colonocytes. Exogenous glucocorticoid treatment of these mice precociously induced gut maturation, as evidenced by a 47-fold increase in intestinal sucrase activity, a classical indicator of development (31). Treatment with CA in the small intestine precociously accelerated the postnatal decline of α,2,6-ST expression at the mRNA level, suggesting that regulation of α,2,6-ST represents an im-

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**Fig. 5. Sambucus nigra agglutinin (SNA) lectin staining of α,2,6-linked sialylglycoprotein in developing colonic and ileal tissue.** Representative sections of 1-day-old ileum (A, B, E, and F) and colon (C, D, G, and H) as well as 4-wk-old ileum (I and J) and colon (K and L) are depicted. Nomarski and SNA lectin immunofluorescence staining (SNA-FITC) show the developmental decline of ileal expression and unchanged colonic expression of α,2,6-linked sialylglycoproteins.
important component of gut maturation. These changes partly coincide with weaning, suggesting that luminal factors present in maternal milk and the establishment of microflora in the gut may also contribute to this developmental decline of α-2,6-ST expression.

Antibodies specific to α-2,6-ST have demonstrated that this enzyme is expressed predominantly in the intestinal epithelium in the rat (55). In the mouse, we found that the glycosylation product of α-2,6-ST is also abundantly expressed in the epithelium, especially in goblet cells. This pattern of sialylation demonstrated a clear regional specificity between the small and large bowel, the latter site maintaining high levels of α-2,6-ST expression. In contrast, MVM preparations isolated from colonocytes showed sialoglycoconjugates decreasing with maturation, indicating a possible differential regulation of α-2,6-ST activity in absorptive and goblet cells. The overall developmental decline of α-2,6-ST expression, which seems to be primarily α-2,6-ST in the small intestine, may likely be due to a combination of regulation in goblet cells and in enterocytes.

In contrast to the developmental decline of the IgG-Fc receptor expression, which shows a high proximal-to-low distal gradient in suckling rats (40, 47) or ileal bile acid binding and absorbing proteins (42, 21) that are only expressed in the distal small intestine during the third postnatal week, α-2,6-ST expression developed an increasing gradient along the cephalo-caudal (anterior-posterior) axis of the gut. The developmental decline of α-2,6-ST activity in the small intestine is most likely controlled at the transcriptional level, similar to the postweaning rise of sucrase and trehalase activity (31, 33). The developmental decline of IgG-Fc receptor expression in rat small intestine also relates to its mRNA level (47) and in a similar manner to α-2,6-ST expression precedes the weaning period. This differs markedly from the postweaning decline of lactase activity where both transcriptional and posttranscriptional processes are apparent (6, 30). These new findings support previous suggestions (21, 31, 42) that different, region-specific factors are involved in establishing cephalo-caudal gradients of gene expression in the developing small intestine.

In vitro studies on the mechanism of α-2,6-ST control support a role for transcriptional regulation of rat α-2,6-ST activity (49, 59). With the use of a hepatic cell line (H35 cells) and primary hepatocytes, glucocorticoid treatment induced increased expression of α-2,6-ST via transcriptional control and not by altering mRNA stability. Unlike hepatocytes, in the small intestine, steroids promote a decline of α-2,6-ST expression. A number of other enzymes involved in gluconeogenesis (e.g., phosphoenolpyruvate carboxykinase) are induced by glucocorticoids in the liver but are repressed by the same treatment in the small intestine (2, 16, 64). In contrast to our in vivo data, Kolinska et al. (23) observed an upregulation of α-2,6-ST mRNA levels and enzyme activity after dexamethasone treatment of 7-day-old rat jejunum in organ culture (18, 22). However, limitations in organ culture techniques prevent elucidation of the precise control mechanism of α-2,6-ST regulation. Also, distinct promoter utilization has been demonstrated for α-2,6-ST expression in the liver and kidney (58), but it is not known whether intestinal expression uses a comparable hepatic promoter or a unique promoter specific for the α-2,6-ST gene. However, availability of the genomic DNA clones for mouse and rat α-2,6-ST (17, 59) should help to delineate the mechanisms of developmental and glucocorticoid-mediated regulation of α-2,6-ST expression in the small intestine, which is beyond the scope of the current study.

The α-2,6-ST is a membrane-bound protein, located almost exclusively in the trans-Golgi network, whose catalytic domain faces into the lumen of the Golgi cisternae. It is responsible for the addition of terminal sialic acid residues on diverse membrane glycoproteins and glycolipids and on secreted mucins. Sialylated N- and O-glycans are important glycosylation products that regulate tissue development and disease processes. For example, appropriate cell-to-cell interactions during the development of the central nervous system require that the neural cell adhesion molecule be highly sialylated during embryogenesis (41). Aberrant sialylation may also adversely modify cellular-matrix interactions. Both cell-to-cell and epithelial-
matrix interactions have been implicated in the development of the intestine (30), but developmental changes in ST and its role in intestinal ontogenesis have not been systematically evaluated. In extreme cases, aberrant sialylation may promote metastasis of colorectal carcinomas (4). Enhanced sialylation is also associated with c-Ha-ras oncogene transformation, mediated by an increased gene expression and enzyme activity of α-2,6-ST (28). Evidence is also mounting that the product of α-2,6-ST activity is a necessary component of a recognition epitope required for homotypic B cell interactions (38). Moreover, sialylglycoproteins can also contain a critical component of an epitope for cellular recognition such as the sialyl-Lewis structures recognized by selectins (7, 27).

The presence of a highly sialylated intestinal surface at the time of birth may directly influence the binding of gut microflora on the epithelial surface of the small and large intestine. Attachment sites for several organisms may be masked by sialic acids, whereas other pathogenic and nonpathogenic bacterial strains secrete sialidase enzymes that enable them to overcome host defensive mechanisms and create novel binding sites for colonization (10). In contrast, many pathogenic bacteria and viruses recognize specific sialylated glycan structures as their adhesion receptors (11, 54, 63). For example, enterotoxigenic Escherichia coli K99 causes diarrhea in newborn piglets, calves, and lambs by expressing K99 fimbrial adhesins that bind to sialylated glycoproteins and glycolipids (44). N-glycolylneuraminyl-lactosyl-ceramide (NeuGc-GM3) has been identified as a major receptor for K99. The membrane content of NeuGc-GM3 is highest in newborn pigs and gradually decreases during development, as does the susceptibility to E. coli K99 enteritis (64). The regional and temporal variation in the expression of sialylated cell surface glycoconjugate receptors may explain the regional and temporal differences in susceptibility to pathogens that exist within a species, just as differential expression among species accounts for the species specificity of pathogens. Furthermore, developmental regulation of sialylation may contribute toward specificity of intestinal colonization.

Environmental factors may also regulate ST activity. Bacterial colonization and its fermentation products such as short-chain fatty acids (SCFA) may provide a direct luminal signal to switch off α-2,6-ST activity and mRNA. A major constituent of SCFA, n-butyrate, is also a major fermentation product of intestinal microflora and reduces up to 90% of α-2,6-ST mRNA in the human hepatoma cell line Hep G2 (45). A dose- and time-dependent effect of n-butyrate on glycosyltransferase mRNA in T84 (human colonic adenocarcinoma) cells was demonstrated (26) in which n-butyrate (3–5 mM) caused approximately an 80% inhibition of α-2,6-ST mRNA accumulation. The effect on α-2,6-ST was near maximum by 6 h (26). However, SCFA are mostly concentrated in the colon and substantially lower levels are found in the proximal small intestine (5), although it is feasible that these levels could elevate significantly as a result of bacterial overgrowth in the small bowel during various pathophysiological conditions. While it remains possible that α-2,6-ST repression in adult enterocytes is mediated in part by the n-butyrate pathway, the relative contribution of this mechanism to the regulation of glycosyltransferase expression in vivo remains to be investigated. Therefore, SCFA, fermentation byproducts of microflora in the animals, might be one contributing luminal factor in the developmental decline of α-2,6-ST in the mouse gut. Their role in secondary regulation of ST activity may be examined further under germ-free conditions.

Our findings in the developing mouse intestine agree with prior observations on developing rat intestine with regard to the drop in α-2,3/6-ST in small intestine from birth to weaning, the premature drop in activity with corticosteroid treatment, the persistence of the activity in colon, and the proximal-to-distal differences in activity in the gut. In the mouse, the α-2,6-ST activity predominates over the α-2,3-ST activity in the small intestine; however, in the mouse, the α-2,6-ST activity also comprises a significant portion of the activity of the colon (12). These studies suggest that such phenomena are general to mammals and that similar studies in developing human intestine may produce information that will be helpful in understanding normal functions in the gut, such as colonization, as well as pathological enteric processes.

In the present study, we demonstrate that intestinal α-2,6-ST mRNA accumulation, enzyme activity, and the abundance of α-2,6-linked sialic acid residues on MVM glycoproteins are under developmental and region-specific regulation and can be modified by cortisone treatment. These results support the hypothesis that changes in the sialic acid content in intestinal membrane glycoproteins are primarily due to altered α-2,6-ST gene expression and that transcription may be regulated by region-specific factors modulated by glucocorticoids.

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REFERENCES


