Postnatal development of rat colon epithelial cells is associated with changes in the expression of the β 1,4-*N*-acetylgalactosaminyltransferase involved in the synthesis of Sd^a antigen and of α 2,6-sialyltransferase activity towards *N*-acetyl-lactosamine

Fabio DALL'OLIO, Nadia MALAGOLINI, Giuseppina DI STEFANO, Manuela CIAMBELLA and Franca SERAFINI-CESSI*

Dipartimento di Patologia Sperimentale dell'Università di Bologna, Via S. Giacomo 14, 40126 Bologna, Italy

β 1,4-*N*-Acetylgalactosaminyltransferase (β 1,4GalNAc-transferase) and α 2,3-sialyltransferase are both involved in the biosynthesis of the Sd^a blood group antigen, which is also present in cells of large intestine. The expression of these enzymes and of α 2,6-sialyltransferase activity towards *N*-acetyl-lactosamine was investigated in rat intestinal cells and correlated with both cell differentiation and extent of postnatal maturation. The β 1,4GalNAc-transferase activity was exclusively found in epithelial cells of the large intestine, preferentially in the proximal segments suggesting a proximal–distal gradient of expression. The β 1,4GalNAc-transferase and α 2,3-sialyltransferase activity towards *N*-acetyl-lactosamine were expressed in all cell fractions of the colonic crypt, with a maximum activity in the deeply located cells; therefore Sd^a antigen biosynthesis appears to occur preferentially at a specific stage of cell differentiation. By using *N*-acetyl-lactosamine as an acceptor, the predominant sialyltransferase in the colon cells was that capable of adding sialic acid in the α 2,3- linkage, whereas in the ileum cells the major enzyme was that forming the α 2,6-isomer. There were dramatic changes in the expression of colonic β 1,4GalNAc-transferase and of α 2,6-sialyltransferase activity towards *N*-acetyl-lactosamine during postnatal maturation. The former enzyme, practically absent at birth, increased slowly in the first days of life and then rapidly after weaning; by contrast, the latter enzyme was largely expressed only in newborn animals. As the colonic α 2,3-sialyltransferase activity towards *N*-acetyl-lactosamine did not change during the postnatal period, the ratio between the α 2,6- and α 2,3-sialyltransferase activities was reversed after weaning.

INTRODUCTION

The rat intestinal epithelium represents a convenient and unique model for studying both cellular differentiation and ontogenic maturation. Indeed, epithelial cells replicate at the base of the crypt and differentiate as they migrate towards the apical portion, and undergo biochemical and functional changes during postnatal development (Henning, 1985; Gordon, 1989). One of the major changes associated with the cell differentiation and ontogenesis is the altered structure and biosynthesis of carbohydrates which are bound to proteins and lipids (Feizi, 1985; Fukuda, 1985). Consistent with this fact, several lines of evidence indicate that the biosynthesis and structure of membrane glycoproteins vary in functionally distinct compartments of the crypt–villus unit (Weiser *et al.*, 1986) and during postnatal maturation (Torres-Pinedo & Mahmood, 1984). Most investigations in this field have centred on the small intestine, and significant changes were found in the expression of glycosyltransferases, such as sialyl- and fucosyl-transferases, which are involved in the assembly of the terminal portion of glycoprotein oligosaccharides (Kim *et al.*, 1975; Chu & Walker, 1986; Taatjes & Roth, 1988; Kolinska *et al.*, 1988). Changes were also observed in the carbohydrate structure of both mucus and hydrolase glycoproteins (Herscovics *et al.*, 1981; Shub *et al.*, 1983; Srivastava *et al.*, 1987). The altered glycosylation of these glycoproteins has been correlated with the regulatory role of the

apical membrane in the differentiation and maturation of intestinal cells (Weiser *et al.*, 1986).

We are concerned with the occurrence and tissue distribution of the β 1,4-*N*-acetylgalactosaminyltransferase (β 1,4GalNAc-transferase) responsible for the addition of the immunodominant sugar of the Sd^a phenotype (Serafini-Cessi & Dall'Olio, 1983; Serafini-Cessi *et al.*, 1986, 1988; Malagolini *et al.*, 1989). This enzyme is involved in the elongation of α 2,3-sialylated oligosaccharides of glycoproteins, and thus it is included in the class of terminal glycosyltransferases. Although the β 1,4GalNAc-transferase is expressed in few tissues, all of the mammals studied to date (humans, mice and guinea pigs) have been found to express this enzyme at a high level in intestinal cells (Dall'Olio *et al.*, 1987; Conzelman & Bron, 1987; Malagolini *et al.*, 1989). Since in human colonic carcinomas the activity of this enzyme is drastically reduced and in guinea-pig kidney its expression increases significantly after the postnatal period, we proposed that β 1,4GalNAc-transferase is oncodevelopmentally regulated (Dall'Olio *et al.*, 1987; Malagolini *et al.*, 1989).

The purpose of the present study was to investigate the expression of the β 1,4GalNAc-transferase in the rat intestine and to measure the activity in variously differentiated compartments and during postnatal maturation. Since in human colon carcinomas the activity of α 2,6-sialyltransferase towards *N*-acetyl-lactosamine is significantly increased in comparison with that in normal mucosa (Dall'Olio *et al.*, 1989), the expression of

Abbreviations used: T-H glycoprotein, Tamm–Horsfall glycoprotein; β 1,4GalNAc-transferase, β 1,4-*N*-acetylgalactosaminyltransferase; ALP, alkaline phosphatase; PBS, 0.02 M-sodium phosphate buffer, pH 7.4, containing 0.15 M-NaCl.

* To whom correspondence and reprint requests should be sent.

this enzyme was investigated and correlated with intestinal cell differentiation and ontogenic development.

EXPERIMENTAL

Materials

CMP-[¹⁴C]NeuAc (262 Ci/mol) and [³H]thymidine (1.5 Ci/mmol) were from Amersham. UDP-[6-³H]GalNAc (8.7 Ci/mmol) was from New England Nuclear. Unlabelled UDP-GalNAc, CMP-NeuAc, fetuin, human transferrin and *N*-acetyl-lactosamine were from Sigma. Tamm-Horsfall (T-H) glycopeptide (multiantennary) was prepared by Pronase digestion of T-H glycoprotein as previously described (Serafini-Cessi & Dall'Olio, 1983). Asialofetuin and asialo-T-H glycopeptides were prepared by hydrolysis in 0.05 M-H₂SO₄ at 80 °C for 2 h. 3'-Sialyl-lactose was purified from a commercial mixture of 3'- and 6'-isomers (Sigma) by h.p.l.c. (Bergh *et al.*, 1981). GalNAc β 1,4(NeuAca2,3)Gal β 1,4Glc was prepared as previously described (Serafini-Cessi *et al.*, 1986). DEAE-Sephacel was from Pharmacia and Bio-Gel P-10 (200–400 mesh) was from Bio-Rad. Pronase (*Streptomyces griseus*) and β -hexosaminidase (jack-bean) were from Sigma. The ALP Kine test (*p*-nitrophenyl-phosphate kinetic method) for alkaline phosphatase (ALP) determination was from Sclavo Diagnostic, Milan, Italy. All chemicals were of reagent grade.

Animals

Wistar rats were used in all experiments. Newborn animals, weighing 4–7 g, were allowed to suckle and were killed at the indicated age by decapitation. Animals over 3 months of age were used as adult rats.

Isolation of epithelial cells of large and small intestine

Immediately after decapitation, the large intestine was dissected out from the ileo-caecal valve to the first centimetre of the rectum; the small intestine included only the ileum. The intestine portion was flushed with ice-cold 150 mM-NaCl containing 1 mM-dithiothreitol until the faeces were entirely removed. The intestine was then washed twice with a buffer, prewarmed at 37 °C, containing 96 mM-NaCl, 1.5 mM-KCl, 27 mM-sodium citrate, 5.6 mM-Na₂HPO₄ and 8 mM-KH₂PO₄, pH 7.3 (buffer A). The distal end of the intestine was tied with a firm yarn and filled with a solution containing 137 mM-NaCl, 3 mM-KCl, 1.5 mM-KH₂PO₄, 6.5 mM-Na₂HPO₄, 1.5 mM-EDTA and 0.5 mM-dithiothreitol, pH 7.3, (buffer B), and then the proximal end of the intestine was also tied (Breimer *et al.*, 1981). In experiments with newborn animals the colon was washed *in situ* by injecting buffer A from the ileum until all solid materials were expelled through the anus. The colon, still attached to the abdominal cavity, was filled with buffer B and tied, and was then dissected out. The gut was totally immersed in 0.15 M-NaCl and incubated at 37 °C. In experiments performed in order to fractionate cells according to their location along the crypt-superficies axis, the subsequent incubation times were 6, 10, 10, 15 and 20 min. When the total cells of the crypt and villus were to be collected the incubation time was 1 h. After this time period no more cells were released. At the end of each incubation period the fluid and released cells were removed and the gut was filled again with buffer B. The cells from each incubation were collected by centrifugation at 5000 *g* for 25 min, washed twice with PBS (0.02 M-sodium phosphate/0.15 M-NaCl, pH 7.4) and homogenized in ice-cold distilled water using a Potter homogenizer.

Glycosyltransferase assays

GalNAc-transferase assay. The standard assay mixture had following final concentrations in a total volume of 25 μ l: 80 mM-Tris/HCl buffer, pH 7.5, 10 mM-MnCl₂, 80 μ M-UDP-[³H]GalNAc (264 d.p.m./pmol) and 0.5 % Triton X-100. Usually, 10 μ l of cell homogenate at a concentration of 2.5 mg of protein/ml was added as enzyme source. The concentrations of various acceptors are given in the Figure or Table legends. The reaction was incubated at 37 °C for 1 h. When glycoproteins were used as acceptors, the incubation was stopped by addition of 1 ml of 1 % phosphotungstic acid in 0.5 M-HCl (FTA) and samples were centrifuged at 1500 *g* for 10 min. Pellets were washed three times with 1 ml of FTA and once with 1 ml of methanol, and each washing was centrifuged as described above. Final pellets were solubilized with 0.3 ml of Soluene (Packard) and counted for radioactivity. When T-H glycopeptide or 3'-sialyl-lactose were used as acceptors, incubations were stopped by the addition of 1 ml of 0.05 M-pyridine/acetic acid buffer, pH 5. [³H]GalNAc-labelled T-H glycopeptide and [³H]GalNAc-sialyl-lactose were isolated from the reaction mixture as previously described (Malagolini *et al.*, 1989).

Sialyltransferase assay. The assay mixture contained 80 mM-sodium cacodylate buffer, pH 6.5, 10 mM-MnCl₂, 250 μ g of *N*-acetyl-lactosamine, 80 μ M-CMP-[¹⁴C]NeuAc (37 d.p.m./pmol), 0.5 % Triton X-100 and 25 μ l of cell homogenates in a final volume of 50 μ l. After 2 h at 26 °C the reaction was stopped by addition of 1 ml of 50 mM-pyridine/acetate buffer, pH 5. NeuAca2,3- and NeuAca2,6-*N*-acetyl-lactosamine were isolated from the reaction mixture and fractionated by h.p.l.c. as previously described (Dall'Olio *et al.*, 1989).

Thymidine kinase and alkaline phosphatase assays

Thymidine kinase activity was measured according to Klemperer & Haynes (1968) with some modifications. The assay mixture contained 50 mM-Tris/HCl buffer, pH 8, 2.5 mM-MgCl₂, 8 mM-ATP, 4 μ M-[³H]thymidine and 140 μ l of cell homogenate in a final volume of 200 μ l. Samples were incubated for 15 min at 37 °C, and then 1 ml of water was added. After boiling for 2 min, samples were filtered through a 0.45 μ m membrane. A 200 μ l aliquot was injected in a Waters h.p.l.c. apparatus equipped with a Whatman Partisil 10 Sax column (0.4 cm $\times$ 25 cm) equilibrated and eluted isocratically with 15 mM-KH₂PO₄, pH 3.5, at a speed of 1 ml/min. Fractions of 1 ml were collected and the radioactivity co-eluting with a TMP standard was taken as a measure of thymidine kinase activity.

ALP activity was determined by the ALP Kine test (*p*-nitrophenylphosphate kinetic method) as described by the manufacturer.

Affinity chromatography on Blue-Sepharose

The cell pellet from rat colon was washed twice with PBS, then suspended in 3 vol. of 1 % Triton X-100 and mixed with a stirrer (250 rev./min) for 4 h at 4 °C. The suspension was centrifuged at 15000 *g* and the supernatant was applied to a Blue-Sepharose column (1 cm $\times$ 20 cm). The chromatography was performed as previously described, except that 0.5 % Triton X-100 was present in each eluent (Serafini-Cessi *et al.*, 1988).

Analysis of [³H]GalNAc-labelled oligosaccharides

The [³H]GalNAc(NeuAca2,3)Gal β 1,4Glc tetrasaccharide isolated by DEAE-Sephacel chromatography from the transferase reaction mixture was analysed by t.l.c. on plates of silica gel, and twice developed with ethanol/pyridine/butan-1-ol/acetic acid/water (100:10:10:3:30, by vol.) (Serafini-Cessi

et al., 1986). The radioactive tetrasaccharide was also isolated by h.p.l.c. by a Merck NH₂-Lichrosorb column (12.5 cm × 0.4 cm) as previously described (Malagolini *et al.*, 1989). For both analyses the [³H]GalNAc(NeuAc α 2,3)Gal β 1,4Glc tetrasaccharide standard was isolated as described by Serafini-Cessi *et al.* (1986).

RESULTS

β 1,4GalNAc-transferase in intestine of adult rat

When epithelial cells from the small and large intestines of the rat were totally released by a long incubation with the EDTA-containing buffer and tested for GalNAc-transferase activity with fetuin as an acceptor, a high GalNAc-transferase activity was found only in cells from the large intestine. This enzyme exhibited a low capability for transferring [³H]GalNAc from UDP-[³H]GalNAc to asialofetuin, and this suggested the acceptor specificity of an authentic β 1,4GalNAc-transferase, that strictly requires sialic acid in the acceptor for activity (Serafini-Cessi & Dall'Olio, 1983). No GalNAc-transferase activity was recovered in the residual colon wall, indicating that the enzyme was characteristic of the luminal epithelial cells. The enzyme required Triton X-100 in the assay (optimum 0.5%). As for the β 1,4GalNAc-transferase from other sources, Mn²⁺ was required for activity (maximum at 10 mM), and a broad optimum of pH was found between 7.2 and 8.0. The activity of crude cell homogenates was linear from 0.2 to 4 mg of protein/ml and within a time period of 1 h. Like the soluble form of β 1,4GalNAc-transferase from human urine of Sd(a+) individuals, the rat colon enzyme exhibited a high affinity for Blue-Sepharose (Serafini-Cessi *et al.*, 1988). The enzyme activity of the supernatant from the 1% Triton X-100 extract of the cell homogenate (4 h at 4 °C) was 60% of that detected in the total homogenate, and affinity chromatography on Blue-Sepharose of this supernatant resulted in an enrichment of the enzyme activity of more than 10-fold.

Table 1 shows the substrate specificity of β 1,4GalNAc-transferase from colonic cells. Among the glycoproteins tested as acceptors, fetuin carrying NeuAc α 2,3Gal groups behaved as an efficient acceptor. Since the amount of [³H]GalNAc incorporated into BSA, asialofetuin and human serum transferrin, all of which

are devoid of NeuAc α 2,3Gal units, was slightly higher than that found in endogenous acceptors, one can postulate the presence of a contaminant GalNAc-transferase, probably that involved in the initiation of O-linked chains. This enzyme is largely present in mucin-secreting cells. Consistent with this interpretation, the T-H glycopeptide, isolated after exhaustive Pronase digestion of T-H glycoprotein, behaved as an efficient acceptor, whereas the asialo form was completely devoid of acceptor activity (Table 1). Even α 2,3NeuAc-lactose (but not lactose itself) served as an acceptor, and the [³H]GalNAc-NeuAc α 2,3-lactose tetrasaccharide isolated from the GalNAc-transferase reaction mixture behaved on t.l.c. and h.p.l.c. as an authentic standard of GalNAc β 1,4(NeuAc α 2,3)Gal β 1,4Glc (results not shown).

Finally, the radiolabelled GalNAc transferred to T-H glycopeptide was released by β -hexosaminidase treatment only after the removal of sialic acid by mild acid hydrolysis. When taken together, these results indicate that the enzyme is a β 1,4GalNAc-transferase, and we used as a good index of its activity the amount of [³H]GalNAc transferred into fetuin minus that found in asialofetuin.

When rat β 1,4GalNAc-transferase activity was separately detected in cells released from caecum, proximal and distal colon, the highest activity was detected in the caecum (Table 2). These results are consistent with previous data suggesting a decreasing proximal-distal gradient of the β 1,4GalNAc-transferase in human colon (Malagolini *et al.*, 1989).

Distribution of β 1,4GalNAc-transferase and of α 2,3- and α 2,6-sialyltransferase activity towards N-acetyl-lactosamine along the crypt-surface axis of small and large intestine

Sequential incubations of the gut with an EDTA-containing buffer initially remove the cells located at the surface of the intestinal lumen and subsequently those confined to the crypt base (Weiser *et al.*, 1986). When epithelial cells of rat colon were selectively removed with this procedure, a broad distribution of thymidine kinase activity among the different cell fractions was observed (Fig. 1a). This result was consistent with the observation that in the large intestine the proliferative zone occupies the basal three-quarters of the crypt (Lipkin & Higgins, 1988). On the other hand, the alkaline phosphatase activity occurred at higher levels in cell fractions corresponding to surface crypt regions, and this suggested that along the crypt basal-surface axis there is a gradient of differentiation in spite of rather uniform distribution of thymidine kinase activity. A high β 1,4GalNAc-transferase activity was measured in all cell fractions of the large intestine, with a prevalence in cells more deeply located (Fig. 1b).

Table 1. β 1,4GalNAc-transferase activity in colonic cells towards various sialylated and desialylated acceptors

Epithelial cells were isolated from the rat colon by a 1 h incubation at 37 °C as described in the Experimental section. The amount of NeuAc α 2,3Gal β units of glycoproteins was deduced from their primary structures, whereas that in T-H glycopeptide was calculated on the basis of the structural analysis as performed by Williams *et al.* (1984). GalNAc-transferase activity was measured as described in the text and is expressed as nmol of [³H]GalNAc incorporated per mg of protein in the cell homogenate. Values are means of duplicate experiments.

Acceptor	NeuAc α 2,3Gal β -R in the acceptor (nmol)	GalNAc-transferase activity (nmol/h per mg of protein)
None	—	0.77
Fetuin (250 μ g)	45	7.43
Asialofetuin (250 μ g)	0	1.28
BSA (250 μ g)	0	1.04
Human transferrin (250 μ g)	0	1.15
T-H glycopeptide (250 μ g)	50	6.30
Asialo-T-H glycopeptide (250 μ g)	0	0

Table 2. β 1,4GalNAc-transferase activity in different segments of rat intestine

Intestines from two rats were excised, and cells released from each segment by a 1 h incubation at 37 °C with the EDTA-containing buffer were collected and homogenized in 1% Triton X-100. The transferase reaction was performed as described in the text with 25 μ g of cell homogenate protein and 250 μ g of fetuin or asialofetuin as acceptor. The activity was the amount of [³H]GalNAc transferred into fetuin minus that found in asialofetuin. Values are means of duplicate experiments.

Enzyme source	β 1,4GalNAc-transferase activity (d.p.m./h per μ g of homogenate protein)
Ileum	38
Caecum	7826
Proximal colon	5250
Distal colon	4348

Fig. 1. Crypt-superficies gradient of $\beta 1,4\text{GalNAc}$ -transferase and $\alpha 2,3$ - and $\alpha 2,6$ -sialyltransferase activities in epithelial cells from the large intestine

Epithelial cells were isolated from the large intestine as described in the text after 6, 10, 10, 15 and 20 min of subsequent incubation periods. (a) Thymidine kinase (□) and ALP activity (○), the latter expressed in arbitrary units, were quantified as described in the Experimental section. (b) $\beta 1,4\text{GalNAc}$ -transferase activity (●) was the amount of $[^3\text{H}]\text{GalNAc}$ incorporated into fetuin (250 μg) minus the amount incorporated into asialofetuin (250 μg). $\alpha 2,3$ -Sialyltransferase (■) and $\alpha 2,6$ -sialyltransferase (▲) activities were measured as $[^{14}\text{C}]\text{NeuAc}$ incorporated into $\text{Gal}\beta 1,4\text{GlcNAc}$ and recovered as $\text{NeuAc}\alpha 2,3$ - and $\text{NeuAc}\alpha 2,6$ - $\text{Gal}\beta 1,4\text{GlcNAc}$ respectively. The conditions for the glycosyltransferase assay are given in the text. Values are means of duplicate experiments.

Various sialyltransferases have been described in different tissues (Beyer *et al.*, 1981). Besides the type of linkage with which they add sialic acid to the acceptor, they have been distinguished with respect to the acceptor substrate specificity. In human colon we observed that *N*-acetyl-lactosamine ($\text{Gal}\beta 1,4\text{GlcNAc}$) is an efficient acceptor for both $\alpha 2,3$ - and $\alpha 2,6$ -sialyltransferases, and a procedure was carried out to easily separate $\text{NeuAc}\alpha 2,3\text{Gal}\beta 1,4\text{GlcNAc}$ from the corresponding $\text{NeuAc}\alpha 2,6$ -isomer (Dall'Olio *et al.*, 1989). By using this procedure, the activity of the $\alpha 2,3$ -sialyltransferase towards $\text{Gal}\beta 1,4\text{GlcNAc}$ was found to be from five to seven times higher than that of the corresponding $\alpha 2,6$ -sialyltransferase, and the distribution of the former enzyme among the cell fractions was very similar to that of $\beta 1,4\text{GalNAc}$ -transferase (Fig. 1b).

There is evidence that the proliferative and differentiation gradient along the crypt-villus axis is much more evident in the small intestine than in the colon (Gordon, 1989). Our results on the distribution of thymidine kinase and ALP among cell fractions released by different incubation periods are consistent with this evidence (Fig. 2a). As Fig. 2(b) shows, the distribution in the ileum of the terminal glycosyltransferases that we studied is completely different from that in the colon: practically no $\beta 1,4\text{GalNAc}$ -transferase activity could be detected, and in all cell

Fig. 2. Crypt-villus gradient of $\beta 1,4\text{GalNAc}$ -transferase, $\alpha 2,3$ - and $\alpha 2,6$ -sialyltransferase activities towards *N*-acetyl-lactosamine in epithelial cells of the small intestine

Epithelial cells from the ileum were released by subsequent incubation periods with EDTA-containing buffer of 6, 10, 10, 15, and 20 min. (a) Thymidine kinase (□) and ALP activity (○). (b) $\beta 1,4\text{GalNAc}$ -transferase activity (●), $\alpha 2,3$ -sialyltransferase activity (■) and $\alpha 2,6$ -sialyltransferase activity (▲). Details of the procedure are given in the text and in the legend to Fig. 1.

Fig. 3. Expression of $\beta 1,4\text{GalNAc}$ -transferase, $\alpha 2,3$ - and $\alpha 2,6$ -sialyltransferase activities towards *N*-acetyl-lactosamine in colonic cells during postnatal maturation

Colons of 4-8 animals were used to isolate cells from suckling animals, whereas cells of two colons were mixed in experiments with adult rats. The arrow indicates the day of weaning. Epithelial cells were totally released from the gut walls by an incubation period of 1 h at 37 °C with the EDTA-containing buffer. $\beta 1,4\text{GalNAc}$ -transferase activity (●), $\alpha 2,3$ -sialyltransferase activity (■) and $\alpha 2,6$ -sialyltransferase activity (▲) are shown. Values are means of duplicate experiments.

fractions the $\alpha 2,3$ -sialyltransferase activity towards *N*-acetyl-lactosamine was very low. On the contrary, there was a high expression of the corresponding $\alpha 2,6$ -sialyltransferase, which correlated with the proliferative activity of cell fractions.

Changes in terminal glycosyltransferases in colon epithelial cells during postnatal development

Fig. 3 shows the expression of the three colon glycosyltransferases during postnatal development. No β 1,4GalNAc-transferase activity was found in epithelial cells released from the neonatal rat, or in total colon homogenates of fetal rats at 20 days after conception (results not shown). The activity of this enzyme increased slowly during the suckling period and there was a rapid increase during weaning. An opposite pattern of expression of α 2,6-sialyltransferase towards Gal β 1,4GlcNAc was observed: the activity was very high at birth but hardly detectable a few days after weaning. Since no significant change in the activity of the sialyltransferase able to produce NeuAc α 2,3-Gal β 1,4GlcNAc occurred during the postnatal development, the ratio between the α 2,3- and α 2,6-sialyltransferases was reversed after weaning.

DISCUSSION

Current results indicate that in the rat the β 1,4GalNAc-transferase is confined in the large intestine and is clearly expressed only after weaning. These results strengthen previous observations that few tissues express the enzyme and that the activity is ontogenically regulated (Conzelmann & Bron, 1987; Dall'Olio *et al.*, 1987). Several lines of evidence indicate that the β 1,4GalNAc-transferase is responsible for the addition of the immunodominant sugar of the Sd^a character and that its activity correlates with the expression of Sd(a+) phenotype (Serafini-Cessi & Dall'Olio, 1983; Donald *et al.*, 1987; Serafini-Cessi *et al.*, 1988; Soh *et al.*, 1989).

At the moment there is no indication of the role of the Sd^a antigen or of the event(s) acting as selective effect for the Sd(a+) phenotype. It may be postulated, as it has been suggested for other blood group antigens, that microbial infections operate as selective agents for the high frequency of Sd^a antigen in some tissues. It is worth noting that pathogenic strains of *Escherichia coli* contain adhesins which specifically recognize the NeuAc α 2,3Gal units of cell surface glycoproteins (Korhonen *et al.*, 1984; Sjöberg *et al.*, 1988). This is the same carbohydrate structure on which the β 1,4GalNAc-transferase acts. The structural change due to the addition of GalNAc onto the NeuAc α 2,3Gal unit might hinder the binding of *E. coli* to the cells, with a consequent decrease in susceptibility to infection. Our results indicating a delayed expression of β 1,4GalNAc-transferase at weaning are consistent with this idea. Indeed, it has been reported that in some mammals *E. coli* with K99 fimbriae having high affinity for sialylated chains adhere to intestinal epithelial cells, but only in animals from the day of birth up to a few week of age (Runnels *et al.*, 1980). In this context, it is of interest that even pyelonephritogenic *E. coli* strains possess fimbriae adhesins specific for the NeuAc α 2,3Gal group (Moch *et al.*, 1987; Parkkinen *et al.*, 1988). Since the Sd^a antigen is largely present in distal kidney and urine (Morton *et al.*, 1970), mainly associated with T-H glycoprotein (Soh *et al.*, 1980), a correlation between the resistance to *E. coli* infection and the expression of the Sd^a determinant may be suggested. This point requires further investigations, such as whether the Sd(a-) individuals are more sensitive to these types of infection. Moreover, other possibilities, such as *O*-acetylation of sialic acid must be considered as putative mechanisms which interfere with the binding of *E. coli* fimbriae to the intestinal epithelium. Lindahl *et al.* (1987) reported that K99 fimbriae from enterotoxigenic *E. coli* have a higher affinity for 4-*O*-acetyl-NeuAc and a lower affinity for *N*-acetylneuraminic acid with an *O*-substituent at C-7-C-9 as compared with *N*-acetylneuraminic acid.

The distribution of α 2,3- and α 2,6-sialyltransferase activities towards *N*-acetyl-lactosamine in cell fractions released from the colon crypt indicates the predominance of the former enzyme. The distribution of this enzyme along the crypt axis parallels that of β 1,4GalNAc-transferase, with a maximum activity in a relatively deep region. Although there is no evidence that the colon α 2,3-sialyltransferase that we studied is the only enzyme involved in the sialylation of the Sd^a determinant, the distribution of β 1,4GalNAc-transferase suggests that the biosynthesis of Sd^a antigen occurs preferentially in deep regions of the crypt at a defined stage of colonic cell differentiation.

Current results indicate that when *N*-acetyl-lactosamine is used as an acceptor, the major sialyltransferase detectable in colon cells of the adult rat is that able to add sialic acid in a α 2,3-linkage. This result is consistent with that of Taatjes *et al.* (1988), even though they used different acceptors to discriminate between the α 2,3- and the α 2,6-sialyltransferases. It is worth noting that *O*-glycosidically linked oligosaccharides of rat colonic mucin carry the sialic acid α 2,6-linked to the innermost *N*-acetylglactosamine residues (Slomiany *et al.*, 1980). The α 2,6-sialyltransferase involved in the assembly of these oligosaccharides is different from that which we and Taatjes *et al.* (1988) have studied (Beyer *et al.*, 1981). We suggest that the high α 2,3-sialyltransferase activity towards *N*-acetyl-lactosamine detected in the rat colon cells might be that involved in the termination of *N*-linked chains of glycoproteins which terminate with *N*-acetyl-lactosamine units.

The expression of the colonic α 2,3-sialyltransferase activity towards *N*-acetyl-lactosamine does not appear to change significantly during postnatal development, whereas a rapid decline in the corresponding α 2,6-sialyltransferase occurs. The fall in the latter sialyltransferase after weaning may contribute to the decrease in total sialyltransferase activity previously reported as being characteristic of the postnatal development of the rat intestine (Torres-Pinedo & Mahmood, 1984; Chu & Walker, 1986).

The sialylated terminal portions of glycoproteins and glycolipids have been identified as specific ligands for viruses, bacteria, toxins and lectins (Gottschalk, 1959; Adair & Kornfeld, 1974; Eidels *et al.*, 1983; Karlsson, 1989; Paul *et al.*, 1989), and increasing evidence indicates that the binding affinity varies according to the linkage of the sialic acid (Rogers & Paulson, 1982; Rogers *et al.*, 1985; Suzuki *et al.*, 1986; Shibuya *et al.*, 1987). The expression of one or another sialyltransferase should modify the terminal structure of oligosaccharides (Paulson *et al.*, 1989), and because of this modification even the susceptibility to pathogenic agents might vary.

Another important point is the location of glycoproteins in basolateral or apical plasma membranes of intestinal cells and the putative role of sialic acid for their routing and sorting (Taatjes & Roth, 1988). The addition of *N*-acetylglactosamine to oligosaccharides terminating with a α 2,3-linked sialic acid, or the replacement of the latter residue with the α 2,6 isomer, might result in a change in the anion charge, and in the immunological and conformational properties of the terminal carbohydrate structure of the glycoproteins. Therefore one may suggest that both cell polarization and cellular adhesion are affected by the alteration of β 1,4GalNAc-transferase expression and by the inversion of the ratio between the α 2,3- and α 2,6-sialyltransferase activities towards *N*-acetyl-lactosamine, both of which occur during ontogenesis.

The major conclusion of our study is that in the rat intestine both β 1,4GalNAc-transferase and α 2,6-sialyltransferase activity towards *N*-acetyl-lactosamine are ontogenetically regulated; the former is poorly expressed at the early stage of ontogenesis and in colon carcinoma cells (Malagolini *et al.*, 1989); the latter is

high in activity in cells which are undifferentiated because of incomplete maturation or neoplastic transformation (Dall'Olio *et al.*, 1989).

This research was supported by grants from A.I.R.C. and C.N.R. Progetto Finalizzato Oncologia.

REFERENCES

- Adair, W. L. & Kornfeld, S. (1974) *J. Biol. Chem.* **249**, 4696–4704
- Bergh, M. L. E., Koppen, P. & van den Eijnden, D. H. (1981) *Carbohydr. Res.* **94**, 225–229
- Beyer, T. A., Sadler, J. E., Rearick, J. I., Paulson, J. C. & Hill, R. L. (1981) *Adv. Enzymol.* **52**, 23–175
- Breimer, M. E., Hansson, G. C., Karlsson, K.-A. & Leffler, H. (1981) *Exp. Cell Res.* **135**, 1–13
- Chu, S.-H. W. & Walker, W. A. (1986) *Biochim. Biophys. Acta* **883**, 496–500
- Conzelmann, A. & Bron, C. (1987) *Biochem. J.* **242**, 817–824
- Dall'Olio, F., Malagolini, N. & Serafini-Cessi, F. (1987) *Biosci. Rep.* **7**, 925–932
- Dall'Olio, F., Malagolini, N., Di Stefano, G., Minni, F., Marrano, D. & Serafini-Cessi, F. (1989) *Int. J. Cancer* **44**, 434–439
- Donald, A. S. R., Soh, C. P. C., Feeney, J. & Watkins, W. M. (1987) in *Glycoconjugates, Proceeding of the XIth International Symposium*, Lille, France (abstract F66)
- Eidels, L., Proia, R. L. & Har, D. A. (1983) *Microbiol. Rev.* **10**, 229–260
- Feizi, T. (1985) *Nature (London)* **314**, 53–57
- Fukuda, M. (1985) *Biochim. Biophys. Acta* **780**, 119–150
- Gordon, J. I. (1989) *J. Cell Biol.* **108**, 1187–1194
- Gottschalk, A. (1959) in *the Viruses* (Burnet, F. V. & Stanley, W. V., eds.), vol. 3, pp. 51–61, Academic Press, New York
- Henning, S. J. (1985) *Annu. Rev. Physiol.* **47**, 231–245
- Herscovics, A., Bugge, B., Quaroni, A. & Kirsch, K. (1981) *Biochem. J.* **192**, 145–153
- Karlsson, K.-A. (1989) *Annu. Rev. Biochem.* **58**, 309–350
- Kim, Y. S., Perdomo, J., Ochoa, P. & Isaacs, R. A. (1975) *Biochim. Biophys. Acta* **391**, 39–50
- Klemperer, H. G. & Haynes, G. R. (1968) *Biochem. J.* **108**, 541–546
- Kolinska, J., Ivanov, S. & Chelibonova-Lorer, H. (1988) *FEBS Lett.* **242**, 57–60
- Korhonen, T. K., Vaisanen-Rhen, V., Rhen, M., Pere, A., Parkkinen, J. & Finne, J. (1984) *J. Bacteriol.* **159**, 762–766
- Lindahl, M., Brossme, R. & Wadstrom, T. (1987) *Glycoconjugate J.* **4**, 51–58
- Lipkin, M. & Higgins, P. (1988) *Adv. Cancer Res.* **50**, 7–24
- Malagolini, N., Dall'Olio, F., Di Stefano, G., Minni, F., Marrano, D. & Serafini-Cessi, F. (1989) *Cancer Res.* **49**, 6466–6470
- Moch, T., Hoschutsky, H., Hacker, J., Kroncke, K.-D. & Jann, K. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3462–3466
- Morton, J. A., Pickles, M. M. & Terry, A. M. (1970) *Vox Sang.* **19**, 58–65
- Parkkinen, J., Virkola, R. & Lorhonen, T. K. (1988) *Infect. Immun.* **56**, 2623–2630
- Paul, R. W., Choi, A. H. C. & Lee, P. W. K. (1989) *Virology* **172**, 382–385
- Paulson, J. C., Weinstein, J. & Schauer, A. (1989) *J. Biol. Chem.* **264**, 10931–10934
- Rogers, G. N. & Paulson, J. C. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42**, 2181
- Rogers, G. N., Daniels, R. S., Skehel, J. J., Wiley, D. C., Wang, X., Higa, H. H. & Paulson, J. C. (1985) *J. Biol. Chem.* **260**, 7362–7367
- Runnels, P. L., Moon, H. W. & Schneider, R. A. (1980) *Infect. Immun.* **28**, 298–300
- Serafini-Cessi, F. & Dall'Olio, F. (1983) *Biochem. J.* **215**, 483–489
- Serafini-Cessi, F., Dall'Olio, F. & Malagolini, N. (1986) *Carbohydr. Res.* **151**, 65–76
- Serafini-Cessi, F., Malagolini, N. & Dall'Olio, F. (1988) *Arch. Biochem. Biophys.* **266**, 573–582
- Shibuya, N., Goldstein, I. J., Broekaert, W. F., Nsimba-Lubaki, M., Peeters, B. & Peumans, W. J. (1987) *J. Biol. Chem.* **262**, 1596–1601
- Shub, M. D., Pang, K. Y., Swann, D. A. & Walker, W. A. (1983) *Biochem. J.* **215**, 405–411
- Sjoberg, P.-O., Lindahl, M., Porath, J. & Wadstrom, T. (1988) *Biochem. J.* **255**, 105–111
- Slomiany, B. L., Murty, V. L. N. & Slomiany, A. (1980) *J. Biol. Chem.* **255**, 9719–9723
- Soh, C. P. C., Morgan, W. T. J., Watkins, W. M. & Donald, A. S. R. (1980) *Biochem. Biophys. Res. Commun.* **93**, 1132–1139
- Soh, C. P. C., Donald, A. S. R., Feeney, J., Morgan, W. T. J. & Watkins, W. M. (1989) *Glycoconjugate J.* **6**, 319–332
- Srivastava, O. P., Steele, M. I. & Torres-Pinedo, R. (1987) *Biochim. Biophys. Acta* **914**, 143–151
- Suzuki, Y., Nagao, Y., Kato, H., Matsumoto, M., Nerome, K., Nakajima, K. & Nobusawa, E. (1986) *J. Biol. Chem.* **261**, 17057–17061
- Taatjes, D. J. & Roth, J. (1988) *Eur. J. Cell Biol.* **46**, 289–298
- Taatjes, D. J., Roth, J., Weinstein, J. & Paulson, J. C. (1988) *J. Biol. Chem.* **263**, 6302–6309
- Torres-Pinedo, R. & Mahmood, A. (1984) *Biochem. Biophys. Res. Commun.* **125**, 546–553
- Weinstein, J., de Souza-e-Silva, U. & Paulson, J. C. (1982) *J. Biol. Chem.* **257**, 13845–13854
- Weiser, M. M., Walters, J. R. F. & Wilson, J. R. (1986) *Int. Rev. Cytol.* **101**, 1–57
- Williams, J., Marshall, R. D., van Halbeek, H. & Vliegthart, J. F. G. (1984) *Carbohydr. Res.* **134**, 141–155

Received 2 January 1990/14 May 1990; accepted 17 May 1990