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A soluble form of Sd^a-β1,4-*N*-acetylgalactosaminyl-transferase is released by differentiated human colon carcinoma CaCo-2 cells

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We have previously shown that human colon carcinoma CaCo-2 cells express the Sd^a-β1,4-*N*-acetylgalactosaminyltransferase (Sd^a-βGalNAc-transferase) and that the enzyme activity correlates with the degree of enterocytic differentiation. Here we report that a large amount of this glycosyltransferase is released in soluble form, particularly when CaCo-2 cells are maintained in culture for more than 3 weeks in order to ensure an higher degree of enterocyte differentiation. The soluble enzyme was concentrated and partially purified by Blue-Sepharose and fetuin-Sepharose chromatography. The substrate specificity of the partially purified enzyme was similar to that of Sd^a-enzyme from epithelial cells of colon mucosa, and for its activity strictly required the presence in acceptors of NeuAc in α2,3-linkage to subterminal galactose. Among the low molecular glycans tested, NeuAcα2,3Galβ1,4GlcNAc appeared to be the best acceptor, whereas sialyl-Lewis^x and sialyl-Lewis^a did not serve as acceptors, indicating that the fucosylation of sub-terminal GlcNAc hindered the transferase activity. Contrary to this, the activity towards a disialylated acceptor such as di-sialyl-lacto-*N*-tetraose was reduced but not abolished. When CaCo-2 cells were cultured on porous membranes and the transferase activity assayed in medium collected from chambers corresponding to either the apical or basolateral face of highly differentiated CaCo-2 cells, a preferential release from the basolateral surface was found. Considering that Sd^a-βGalNAc-transferase is mainly located in the large intestine, current results support the notion that colonic cells largely contribute to the presence of the enzyme in human plasma.

Keywords: β1,4-*N*-acetylgalactosaminyltransferase, Sd^a blood-group antigen, CaCo-2 cells, enterocyte differentiation, polarized release

Introduction

The Sd^a β1,4-*N*-acetylgalactosaminyltransferase (Sd^a-βGalNAc-transferase) is the glycosyltransferase that catalyses the transfer of GalNAc from UDP-GalNAc to the Gal residue of the NeuAcα2,3Galβ-R terminal unit of oligosaccharides unbound and bound to glycoproteins [1, 2]. Its occurrence correlates with the Sd(a+) phenotype of the human population [3, 4]. The structure of the Sd^a antigen, originally described as a blood group determinant, was first elucidated by Donald *et al.* [5] as the GalNAcβ1,4(NeuAcα2,3)Galβ1,4GlcNAcGal pentasaccharide carried by *N*-linked glycans of Tamm-Horsfall glycoprotein isolated from urine of Sd(a+) individuals. The human tissue localization of Sd^a-βGalNAc-transferase as well as that of the Sd^a antigen appears restricted to intestine and kidney [6–8]. In all mammals studied to date (human, guinea-pig, mice, rat and pig) a very high enzyme activity has been found in epithelial cells of large intestine with an expression gradient which decreases from the proximal to the distal part of the gut [7, 9–12]. The Sd^a-βGalNAc-transferase activity is onco-ontogenetically regulated: in rat colon it is practically absent at birth but rapidly increases at weaning time, and in humans the activity is dramatically decreased in colorectal carcinomas [7, 11]. Such an enzyme, although at a low level, is released in a soluble form in urine of Sd(a+) individuals, and very likely it is the product of the proteolytic cleavage of the kidney enzyme [4]. The Sd^a enzyme also occurs in human plasma [13]. The screening of several human colon carcinoma cell lines in culture proved that only in the line referred to as CaCo-2 is the

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enzyme detectable and the activity was found to increase with the degree of enterocyte differentiation [14]. This cell line exhibits the property to differentiate spontaneously along the enterocytic lineage when maintained in culture for an extended period of time after confluence [15, 16]. A β1,4GalNAc-transferase with a substrate specificity very similar to that of Sd^a-βGalNAc-transferase had been shown to be present in a murine cytotoxic T lymphocyte line [17] and recently the cDNA of this enzyme has been cloned [18]. The cDNA of the murine lymphocyte β1,4GalNAc-transferase shares 51% nucleotide sequence identity with a cDNA encoding the human G_{M2}/G_{D2} synthase [18].

This paper records the observation that a soluble form of Sd^a-β-GalNAc-transferase is released by CaCo-2 cells particularly when they were maintained in culture for more than 3 weeks in order to ensure a homogeneous and high degree of differentiation [19]. The substrate specificity of the partially-purified soluble enzyme towards α2,3 sialylated oligosaccharides is also reported as well as the polarized release of the enzyme.

Materials and methods

Materials

UDP-[6^3H]GalNAc (8.7 Ci mmol^{-1}) was from New England Nuclear. Unlabelled UDP-GalNAc, fetuin and dexamethasone were from Sigma. Asialofetuin was prepared by hydrolysis in 0.1 N H_2SO_4 at 80 °C for 1 h. 3′-Sialyllactose, 3′-sialyl-*N*-acetyllactosamine, 3′-sialyl-Lewis^x, 3′-sialyl-Lewis^a and di-sialyl-lacto-*N*-tetraose was from Oxford GlycoSystem. GalNAcβ1,4(NeuAcα2,3)-Galβ1,4Glc was prepared as previously described [2]. Fetuin-Sepharose was prepared by coupling fetuin to CNBr-activated Sepharose-4B (Pharmacia) as recommended by the manufacturers. DEAE-Sephacel was from Pharmacia (Sweden); Blue-Sepharose was from Bio-Rad. Polycarbonate filters for cultures were from Transwell-Costar. Neuraminidase from Newcastle disease virus (NDV) was from Genzyme Corporation, Cambridge, MA, USA, and β-hexosaminidase (jackbean) from Sigma. ALP Kine test (p-Nitrophenylphosphate kinetic method) for the alkaline phosphatase (ALP) determination was from Sclavo Diagnostic, Milan, Italy. All chemicals were of reagent grade.

Cells and culture conditions

CaCo 2-cells were obtained by Dr G. Bises, Istituto Nazionale della Nutrizione, Rome, Italy; they were maintained in DMEM supplemented with 20% fetal calf serum and refed three times a week. Cells were subcultured when they were 80% confluent in 25 cm^2 flasks in complete medium or plated on polycarbonate filters mounted on multiwell plates. In both systems the confluence was usually reached after 3–4 days from the seeding. Starting from the confluence the cells were maintained in culture for an increasing time in DMEM supplemented with 20% fetal calf serum which was changed three times a week. The cells grown on polycarbonate filters for at least 3 weeks formed a monolayer practically impermeable (less than 2%) to the passage of mannitol from the superior to inferior chamber. Usually, 24 h before collecting the conditioned medium, a new serum-free DMEM supplemented with 5 μg ml^{-1} of human transferrin and 5 ng ml^{-1} of selenous acid was added to the cultures.

To prepare a large amount of conditioned medium, cells were cultured in flasks of 175 cm^2 and maintained in culture for 2–3 months after the confluence. Three weeks after the confluence, when domes were observed, the cells were cultured for 2 days a week in a serum-free medium. The conditioned serum-free media collected each week were pooled and maintained at −80 °C.

Affinity chromatography of conditioned medium from CaCo-2 cells

Pooled serum-free media (600 ml maintained at −80 °C) were centrifuged at 5000 × **g** and applied to a column of Blue Sepharose (1 × 12 cm) equilibrated with 0.1 M cacodylate buffer, pH 6.5, containing 0.1 M NaCl. The column was washed with equilibrating buffer, and then eluted with a gradient of 0.15–2.5 M NaCl in 0.1 M cacodylate buffer. Fractions of 5 ml were collected and the optical density of 230 nm monitored. The transferase activity (system A) was measured after exhaustive dialysis against water of each fraction. The active fractions were pooled then applied to a column of fetuin-Sepharose (1 × 10 cm) equilibrated with 0.020 M Tris-HCl buffer, pH 7.5. After a washing with the equilibrating buffer the column was eluted with 2.5 M NaCl and the fractions, after dialysis, were monitored for transferase activity and optical density.

Sd^a-βGalNAc-transferase assay

Assay A. The standard assay mixture had the following final concentrations: 80 mM Tris-HCl buffer pH 7.5, 10 mM $MnCl_2$, 80 μM UDP-[^{3}H]GalNAc (550 dpm pmol^{-1}) and 0.5% Triton X, when cell homogenate was used as enzyme source. Usually 20 μl of cell homogenate, at 3 mg of protein per ml, or 20 μl of serum-free medium were added to give a total volume of 50 μl. The assay was performed in duplicate with 250 μg of fetuin or asialofetuin as acceptors. The incubation (at 37 °C for 1 h) was stopped by the addition of 1 ml of 1% phosphotungstic acid in 0.5 M HCl. The samples were centrifuged, washed and counted for radioactivity as previously described [7]. The transferase activity was calculated by subtracting the radioactivity transferred to fetuin minus that found in asialofetuin.

Assay B. To test the substrate specificity towards low

molecular oligosaccharides, the assay mixture was as detailed before with 20 nmol of the oligosaccharide acceptor. The reaction was stopped by the addition of 1 ml of water and the sample was applied to a DEAE-Sephacel column and eluted with a gradient of pyridine-acetic acid buffer, pH 5, as previously described [20]. Fractions were monitored for radioactivity and those eluted in the elution position of mono-sialylated glycans were pooled, freeze-dried and fractionated by HPLC. When 3′-sialyl-Lewis^x and 3′-sialyl-Lewis^a were used as acceptors a significant amount of [^{3}H]GalNAc was eluted from DEAE-sephacel column as monosialylated glycans. When they were subjected to HPLC analysis the majority of [^{3}H]GalNAc-labelled glycans was eluted at the retention time of the GalNAcβ1,4(NeuAcα2,3)Galβ1,4GlcNAc tetrasaccharide, indicating a contamination of acceptors with NeuAcα2,3-Galβ1,4GlcNAc.

HPLC

A Merck NH_2-Lichrosorb (12.5 × 0.4 cm) column was used for HPLC with 72:28 acetonitrile-15 mM KH_2PO_4 as mobil phase at a speed of 2 ml min^{-1}; 1 ml fractions were collected and counted for radioactivity.

Glycosidase digestions

Digestion with NDV neuraminidase (20 mU ml^{-1}) in 0.1 M cacodylate buffer pH 6.5 was performed at 37 °C for 24 h according to Paulson *et al.* [21] and β-*N*-acetyl-hexosaminidase treatment was as previously described [7].

Analytical methods

Proteins were determined by the Lowry method with BSA as standard [22]. ALP activity was determined by the ALP Kine test (p-nitrophenylphosphate kinetic method) as described by the manufacturer and sucrase as described by Dahlqvist [23].

Results

Identification and characterization of soluble Sd^a-βGalNAc-transferase

Previously we have shown that a soluble α2,6 sialyltransferase towards *N*-acetyllactosamine is released by CaCo-2 cells and that its release parallels the content of cell-bound enzyme [24]. To ascertain if the Sd^a-βGalNAc-transferase is also released in soluble form, we assayed the enzyme activity in medium and homogenate of CaCo-2 cells at different times of post-confluence. Consistent with previous results [14] the cell-associated activity increased with the time of post-confluence, i.e. with the degree of differentiation and, most importantly, a very high transferase activity was found in medium of highly differentiated cells (Fig. 1). At 1 month of post-confluence the enzyme activity detected in 24 h-conditioned medium was more than twice of that cell-associated.

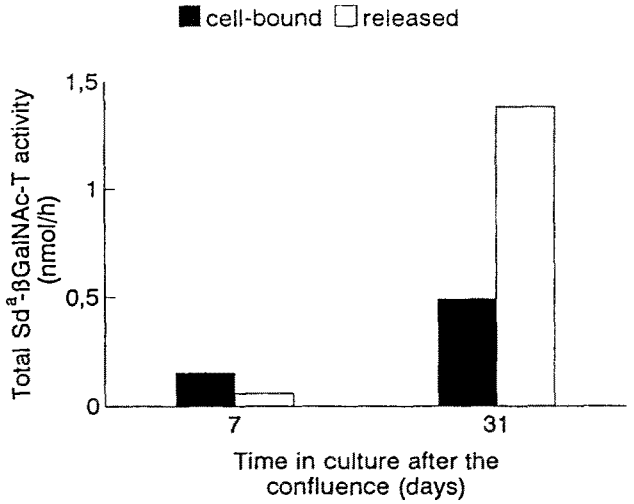

Figure 1. Released and cell-associated Sd^a-βGalNAc-transferase activity in CaCo-2 cells. CaCo-2 cells were maintained in culture after the confluence for the indicated times. The medium was then substituted with new serum-free medium, and after 24 h the transferase activity was assayed (assay A) in medium and cell homogenate as described in the text. The total activity found in cell homogenate from an entire plate is termed 'cell-bound', whereas that found in 24 h conditioned medium from the same flask is termed 'released in medium'. The values are means of two experiments.

To characterize the enzyme with respect to the substrate specificity, a large amount of conditioned medium (serum-free) was obtained by culturing CaCo-2 cells for 2–3 months as described in the Methods section. Previously [4], we found that the soluble Sd^a-βGalNAc-transferase occurring in urine of Sd(a+) individuals could be concentrated and partially purified by Blue-Sepharose chromatography, and α1,3-*N*-acetylgalactosaminyltransferase from plasma of human A-blood group donors has been purified by a similar affinity chromatography [25]. When conditioned-medium of CaCo-2 cells was applied to a Blue-Sepharose column more than 95% of inactive proteins were recovered in the flow through, whereas 65% of the enzyme activity was eluted by a NaCl gradient in fractions retarded with respect to the major peak of retained proteins (Fig. 2). The specific activity of active fractions was 85-fold higher than that of conditioned medium. A ten-fold purification was further achieved by affinity chromatography on fetuin-Sepharose (Fig. 3). This enzyme preparation was used to determine the requirement for the enzyme activity and the acceptor substrate specificity.

In a similar way to the cell-bound enzyme [14], the activity of the soluble form was dependent on Mn^{2+} (optimal concentration, 10 mM) and the optimum pH was 7.5, however as expected for a soluble glycosyltransferase

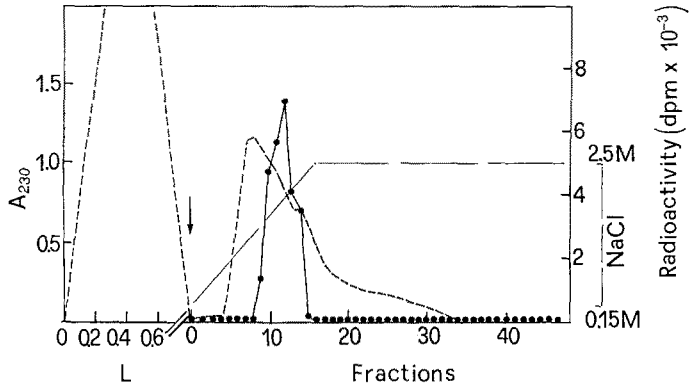

Figure 2. Blue-Sepharose chromatography. Conditioned medium collected as described in the text from differentiated CaCo-2 cells (600 ml) was applied to the column and exhaustively washed with 0.01 M cacodylate buffer, pH 6.5. The arrow indicates the initial elution with the NaCl gradient. Absorbance at 230 nm (- - - -); Sd^a-βGalNAc-transferase activity was detected as described in assay A (●).

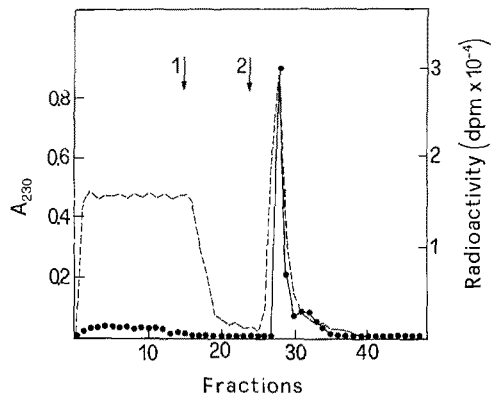

Figure 3. Fetuin-Sepharose chromatography. Active fractions from Blue-Sepharose chromatography were dialysed against water and applied to the column. Arrow 1 indicates the washing with 0.02 M Tris-HCl buffer and arrow 2 the elution with 2.5 M NaCl. Absorbance at 230 nm (- - - -). Sd^a-βGalNAc-transferase activity (●).

the presence of detergents was not required. The enzyme activity determined as a function of UDP-GalNAc concentration using fetuin as an acceptor was linear up to 40 μM. When the values were plotted according to the method of Lineweaver and Burk the K_m for UDP-GalNAc was 64 μM, a value very similar to that of Sd^a-βGalNAc-transferase purified from human urine (66 μM) [4]. The K_m for fetuin, evaluated for the linearity range, was 222 μM.

Table 1 shows the substrate specificity of partially purified enzyme. In complete agreement with previous observations [1, 2, 17, 18], after desialylation fetuin dramatically reduced its capability to serve as an acceptor; we believe that the small activity observed towards asialofetuin, depended on a contamination of incompletely desialylated molecules in the asialofetuin preparation. 3'Sialyl-*N*-acetyllactosamine and 3'-sialyllactose served as good acceptors, whereas lactose and 6'-sialyllactose were completely ineffective, confirming the substrate specificity of Sd^a-enzyme for α2,3-sialylated glycans. In practice, no [^{3}H]GalNAc was transferred to sialyl-Lewis^x and sialyl-Lewis^a, indicating that the fucosylation of 3'sialyl-*N*-acetyllactosamine deeply affected its capability to serve as an acceptor. However, the activity of the enzyme towards a disialylated acceptor such as di-sialyl-lacto-*N*-tetraose was reduced but not abolished. A very similar substrate specificity was found when a microsomal fraction from pig colonocytes, containing in large amount the Sd^a-βGalNAc-transferase [12], was used as source of the enzyme (results not shown).

Table 1. Substrate specificity of Sd^a-βGalNAc-transferase partially purified from conditioned medium of differentiated CaCo-2 cells.

Acceptors	*Sd^a-βGalNAc-transferase activity^a (nmol h^{-1} per mg protein)*
Fetuin	1.74
Asialofetuin	0.26
3'-sialyllactosamine	5.17
3'-sialyllactose	4.51
6'-sialyllactose	0
Lactose	0
Sialyl-Lewis^x	0.03
Sialyl-Lewis^a	0.07
di-sialyl-lacto-*N*-tetraose	2.11

^{a}Medium containing CaCo-2 cells was concentrated and partially purified by Blue-Sepharose and fetuin-Sepharose chromatographies as described in the text. The assay was performed as described under Materials and methods with 250 μg of glycoprotein acceptor or 20 nmol of each oligosaccharide acceptor.

The products of the transferase reaction in which 3'-sialyllactose and 3'-*N*-sialyllactosamine were present as acceptors were eluted in HPLC as authentic samples of GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc and GalNAc-β1,4-(NeuAcα2,3)Galβ1,4GlcNAc. We have shown that GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc, a structure known to be resistant to neuraminidase from *Vibrio cholera*, is partially desialylated by NDV neuraminidase [12]. When [^{3}H]GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc and [^{3}H]GalNAcβ1,4(NeuAcα2,3)Galβ1,4GlcNAc, biosynthesized by soluble Sd^a-βGalNAc-transferase, were subjected to NDV neuraminidase treatment, more than 50% of sialic acid was removed and about 90% of [^{3}H]GalNAc was released from the desialylated compound by β-hexosaminidase treatment.

Release of Sd^a-β-GalNAc-transferase in apical and basolateral medium of differentiated CaCo-2 cells

When CaCo-2 cells were maintained in culture for some weeks on polycarbonate filters mounted in chambers that allow the separate collection of the medium facing the apical and basolateral surface of the monolayer, the polarized secretion of some proteins and enzymes has been found [24, 26, 27]. Using this experimental approach we investigated whether a polarized release of Sd^a-βGalNAc-transferase could be also observed. In a preliminary step, we looked for a reliable marker of polarized release. ALP is a glycosylphosphatidylinositol protein which is selectively sorted to the apical face of epithelial cells [28, 29]. In CaCo-2 cells the ALP is very high and increases as a function of the enterocytic differentiation [14]. Table 2 shows that (i) cell-associated ALP activity increased significantly when the post-confluence of cells was extended from 3 to 5 weeks; (ii) release in the medium, in terms of activity, was very low ranging from 5 to 9% of that cell-associated; (iii) ALP activity was almost entirely recovered in the medium collected from the chamber corresponding to the apical face of the monolayer. Even the sucrase, an enzyme used as a marker of enterocytic differentiation [16, 19], is selectively sorted to the brush border membrane of CaCo-2 cells, but its activity could not be detected in the apical medium or in the basolateral medium (data not shown). Together, these results indicated that in our culture conditions the release of brush border enzymes did not depend on an unspecific proteolysis consequent to a reduced viability of cells and that an effective polarity was acquired by the cells. When conditioned media from 3 or 5-weeks postconfluent cells were examined for Sd^a-βGalNAc-transferase activity, the soluble enzyme was found both in the apical and basolateral compartment. A significant preferential release through the basolateral face occurred only in 5-weeks postconfluent cells (Table 3).

Table 2. Polarized release of alkaline phosphatase (ALP) by CaCo-2 cell monolayer at increasing times of post-confluence.

Days of postconfluence	*Total ALP activity (U)*[a]		
	Cell-bound	*Apical medium*	*Basolateral medium*
21	54 ± 1	4.5 ± 0.40	0.32 ± 0.09
28	195 ± 25	6.0 ± 0.07	0.58 ± 0.17
35	200 ± 22	8.1 ± 0.56	0.78 ± 0.18

[a]CaCo-2 cells were maintained in culture after the confluence on polycarbonate filters and at indicated times the medium was removed from the upper and lower chamber of the well. After four washings of both chambers with serum-free medium, 2.5 ml of serum-free medium was added to each chamber and left for 24 h; then medium from the upper and lower chambers was separately collected and the volumes measured. The cells were scrapped off the filter and homogenized. The ALP activity was measured in each medium and in cell homogenate and expressed in arbitrary units. The total activity termed 'cell bound' corresponds to the activity found in the total cell-homogenate of a single filter, whereas those termed 'apical medium' and 'basolateral medium' correspond to the activity detected in the entire medium of the upper and lower chamber. Values are means ± SD of four batches of cells.

Discussion

Current results indicate that in the medium CaCo-2 cells release a soluble form of Sd^a-βGalNAc-transferase, particularly when they are highly differentiated. In terms of activity, the amount of the enzyme released in the medium by cells maintained in culture after confluence for 1 month was more than twice of that detected in cells. This suggests that the proteolytic cleavage of the NH_2-terminal signal domain, which is believed to anchor glycosyltransferases to membranes of the Golgi apparatus [30], occurs very easily in the case of Sd^a-βGalNAc-transferase. A similar observation has been found by Smith and Lowe [18] for the recombinant form of murine-lymphocyteβ1,4GalNAc-transferase;indeed,when

Table 3. Sd^a-βGalNAc-transferase activity recovered in the apical and basolateral medium of CaCo-2 cells at different times of post-confluence.

Post-confluence (days)	*Medium*	*Volume (ml)*	*Sd^{a}βGalNAc-T activity*[a]	
			(nmol h^{-1} ml^{-1})	*Total activity (nmol h^{-1})*
21	Apical	2.43 ± 0.16	0.110 ± 0.007	0.267
	Basolateral	2.66 ± 0.11	0.095 ± 0.008	0.254
35	Apical	2.40 ± 0.07	0.145 ± 0.020	0.348
	Basolateral	2.69 ± 0.03	0.203 ± 0.007	0.548

[a]Apical and basolateral media were collected as described in the legend of Table 2. The transferase activity was measured as indicated in the text (assay A). Values are means ± SD of four batches of cells.

CHO-Tag cells were transfected with the vector encoding all the peptide sequence of the enzyme (which includes a short cytosolic tail and the transmembrane region) the majority of the enzyme is released into the medium of transfected cells.

The Sd^a-βGalNAc-transferase released by CaCo-2 cells has been concentrated and partially purified by affinity chromatography on Blue-Sepharose and fetuin-Sepharose. The substrate specificity of soluble enzyme is similar to that of Sd^a-enzyme from epithelial cells of colon mucosa: 3′sialyl-*N*-acetyllactosamine was found to be a better acceptor than 3′-sialyllactose, supporting the notion that the catalytic site of the enzyme recognizes a carbohydrate sequence of the acceptor larger than the NeuAcα2,3Galβ-unit which is strictly required for the enzyme activity. This behaviour is reminiscent of that of anti-Sd^a antibodies. Indeed, in the Sd^a serological tests the GalNAcβ1,4(NeuAcα2,3)Galβ1,4GlcNAc is more active than GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc [31]. Sialyl-$Lewis^x$ and sialyl-$Lewis^a$ did not serve as effective acceptors, indicating that the fucosylation of subterminal GlcNAc hinders the transferase activity. The expression of α(1,3/1,4)-fucosyltransferase involved in the biosynthesis of sialyl-$Lewis^x$ and sialyl-$Lewis^a$ has been frequently associated with neoplastic transformation (for references see [32]). Recent studies [33, 34] indicate that the expression of sialyl-$Lewis^x$ antigen in colon cancer cells correlates with the metastatic potential. Contrary to this, biosynthesis of the Sd^a antigen declines in colon cancers [7]. Therefore it may be suggested that the biosynthetic pathway yielding the Sd^a antigen is more effective in well differentiated enterocytes, whereas that producing sialyl-$Lewis^x$ is activated in undifferentiated colonic cells.

Current results show also that soluble Sd^a-βGalNAc-transferase is preferentially released through the basolateral face of highly differentiated cells. Since the cell monolayer obtained by culturing CaCo-2 cells on porous membranes mimics the polarity of the intestinal mucosa, one can propose that *in vivo* a significant amount of the soluble enzyme goes into the blood stream. Considering that the large intestine is the major location of Sd^a-βGalNAc-transferase, it is very likely that its occurrence in human plasma [13] is mainly due to the release by such a tissue. We have previously shown that α2,6 sialyltransferase towards Galβ1,4GlcNAc is also released as a soluble enzyme from differentiated CaCo-2 cells [24], and proposed that colonic cells contribute to its occurrence in the plasma, particularly when α2,6 sialyltransferase expression in colonic cells increases as occurs after neoplastic transformation [35].

The functional significance of soluble glycosyltransferases outside of the cell has not been clarified, but it is unlikely that the transferase activity may be functional because of the very low concentration of sugar-nucleotide donors [36]. The release of α2,6 sialyltransferase from the liver appears to be largely affected by pathological conditions, primarily by inflammatory injury, and Jamieson *et al.* [37] includes such a glycosyltransferase as an acute phase reactant. Recently [38], it has been shown that under cytokine stimulation, CaCo-2 cells release some acute phase proteins which might be involved in a local response to inflammation. In this context, it would be of interest to ascertain if cytokines also affect the release of Sd^a-βGalNAc-transferase from CaCo-2 cells.

Since the release of Sd^a-βGalNAc-transferase correlates with the differentiation degree of intestinal cells and the tissue distribution of the enzyme indicates that it is predominantly present in large intestine mucosa, one can hypothesize that the plasma level should change in conditions that alter both the integrity and differentiation status of the colon mucosa. The screening of Sd^a-βGalNAc-transferase activity in the plasma of patients with different lesions of the large intestine are now in progress to support this hypothesis.

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