Glycoconjugate Journal (1995) **12**: 787–794

Effects of dibutyryl cAMP and bromodeoxyuridine on expression of *N*-acetylglucosaminyltransferases III and V in GOTO neuroblastoma cells

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Received 3 April 1995, revised 22 April 1995

The sugar chain structures of the cell surface change dramatically during cellular differentiation. A human neuroblastoma cell line, GOTO, is known to differentiate into neuronal cells and Schwannian cell-like cells on treatments with dibutyryl cAMP and bromodeoxyuridine, respectively. We have examined the expression of UDP-*N*-acetylglucosamine: β-D-mannoside β-1,4*N*-acetylglucosaminyltransferase III (GnT-III: EC 2.4.1.144) and UDP-*N*-acetylglucosamine: α-6-D-mannoside β-1,6*N*-acetylglucosaminyltransferase V (GnT-V: EC 2.4.1.155), two major branch forming enzymes in *N*-glycan synthesis, in GOTO cells on two distinct directions of differentiation.

In neuronal cell differentiation, GnT-III activity showed a slight increase during initial treatment with Bt_2cAMP for 4 days and decreased drastically after the fourth day, but the mRNA level of GnT-III did not show a decrease but in fact a slight increase. GnT-V activity increased to approximately two- to three-fold the initial level with increasing mRNA level after 8 days, and lectin blot analysis showed an increase in reactivity to *Datsura stramonium* (DSA) of the immunoprecipitated neural cell adhesion molecule (NCAM). In Schwannian cell differentiation, the activity and mRNA level of GnT-III showed no significant change on treatment with BrdU. GnT-V activity also showed no change in spite of the gradual increase in the mRNA level. These results suggest that the activation of GnT-V during neuronal cell differentiation of GOTO cells might be a specific change for branch formation in N-glycans, and this affects the sugar chain structures of some glycoproteins such as NCAM.

Keywords: glycosyltransferase, *N*-acetylglucosaminyltransferase, neuroblastoma, cell differentiation, cAMP, bromodeoxyuridine

Abbreviations and trivial names: GnT, *N*-acetylglucosaminyltransferase; Bt_2cAMP, N^6, O^6-dibutyryl cAMP; BrdU, bromodeoxyuridine; DSA, *Datsura stramonium*; NCAM, neural cell adhesion molecule; PAGE, polyacrylamide gel electrophoresis.

Introduction

The oligosaccharide structures of the cell surface undergo significant changes during embryogenesis, differentiation and malignant transformation [1, 2]. These structural changes are closely related to the changes in and balance of glycosyltransferase and glycosidase activities. UDP-*N*-acetylglucosamine: β-D-mannoside β-1,4*N*-acetylglucosaminyltransferase III (GnT-III) and UDP-*N*-acetylglucosamine: α-6-D-mannoside β-1,6*N*-acetylglucosaminyltransferase V (GnT-V) play important roles in the biosynthesis of N-glycan branches. GnT-III makes a bisecting-GlcNAc structure in the tri-mannose cores of N-glycans [3], and is implicated in cell differentiation and malignancy-associated changes of N-glycans [4–7]. GnT-V makes a β1-6 branch on the α1-6 mannosides of N-glycans [8], and the β1-6 branch structure is thought to be highly associated with metastatic potential [6, 7, 9]. Both the GnT-III [10, 11] and GnT-V [12, 13] genes have already been cloned. These branching patterns of N-glycans directly influence subsequent processing of sugar chain structures.

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The relation between cell differentiation and glycosyltransferase activities has been investigated by several groups. GnT-II to V activities significantly increased upon the differentiation of human colonic adenocarcinoma CaCo-2 cells [14]. The elevation of GnT-III activity in differentiated CaCo2 cells suggested that this enzyme might be partly responsible for the decreased synthesis of polylactosamino-glycans upon differentiation, because GnT-III activity is known to inhibit further branching and galactosylation [15, 16]. On the other hand, the differentiation of murine embryonal carcinoma F9 cells was closely associated with the induction of multiple glycosyltransferase activities, the most pronounced increases being in GnT-V and Core2GnT, which control the levels of β1-6GlcNAc-branched N- and O-linked oligosaccharides, respectively, which are good substrates for polylactosamine elongation [17]. These observations suggest the importance of the branch-forming glycosyltransferases (i.e. GnT-III, IV and V), because the branch-formation of N- and O-linked oligosaccharides is markedly influenced upon differentiation. The glycosyltransferase changes during cell differentiation, however, seem to differ with each specific cell type.

To investigate the changes of GnT-III and GnT-V expression during neural cell differentiation, we examined GnT-III and V activities, and mRNA expression of cultured human neuroblastoma GOTO cells [18], that are known to differentiate into neuronal cells with Bt_2cAMP [19], and into Schwannian cell-like cells with BrdU [20].

Materials and methods

Cell culture

Human neuroblastoma GOTO cells were obtained from the Japanese Cancer Research Resources Bank (Tokyo). GOTO cells were cultured in 1:1 (v/v) RPMI1640 and Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and kanamicin (0.1 mg ml^{-1}) under a humidified atmosphere of 95% air and 5% CO_2. The cells were plated at 0.5–1 $\times$ 10^5 cells per ml cm^{-2}. After 24 h subculture, the cells were induced by adding 1 mM Bt_2cAMP [19] or 5 μg ml^{-1} BrdU [20]. Both cultures were refed with fresh medium containing the same reagents every 48 h. The cells were cultured in the presence of the differentiating agents for 8–10 days and then harvested for analyses.

Glycosyltransferase assays

Cultured cells were harvested by scraping, washed twice with phosphate-buffered saline (PBS), pH 7.2, and then resuspended in 0.1 ml PBS. After sonication, GnT-III and GnT-V activities in cell lysates were determined. The GnT-III and GnT-V assays were carried out as described previously using a fluorescence-labelled sugar chain, GlcNAcβ1 - 2Manα1 - 6(GlcNAcβ1 - 2Manα1 - 3)Manβ1 - 4GlcNAcβ1-4GlcNAc-PA (pyridylamino) as a substrate [21]. Protein concentrations were determined with a BCA protein assay kit (Pierce) using bovine serum albumin as a standard.

Northern blot analysis

Total RNA was prepared from the cells according to the method of Chomczynski and Sacchi [22]. Twenty μg of RNA was electrophoresed on a 1% agarose gel and then transferred onto a Zeta-probe membrane (BioRad) by capillary action. The membranes were hybridized for 18 h with [^{32}P]-labelled human GnT-III cDNA, a fragment lying between *Sac* I sites [11] or human GnT-V cDNA [13] as a probe at 42 °C in a prehybridization solution comprising 50% formamide, 6XSSC (1XSSC, 15 mM sodium citrate and 150 mM NaCl, pH 7.0), 5 $\times$ Denhardt's solution, 0.5% SDS and 10% polyethyleneglycol 8000. The membranes were rinsed for 30 min at 55 °C in 2XSSC and 0.1% SDS, then rinsed once more for 10 min at room temperature in 2XSSC without SDS, and then exposed to X-ray film (Kodak) with an intensifying screen at −80 °C for 2–7 days. The densitometric measurements representing GnT-III or GnT-V were normalized as to those of the respective β-actin or 28S rRNA band.

Immunoprecipitation of NCAM (neural cell adhesion molecule)

Cultured cells were harvested, washed twice with ice cold PBS, and then incubated for 30 min on ice with 0.5 ml of the lysis solution, i.e. 20 mM Tris/HCl (pH 8.0) containing 133 mM NaCl, 10% glycerol, 1% NP-40, and aprotinin (1 μg ml^{-1}). The lysates were centrifuged at 15 krpm for 15 min at 4 °C. The supernatants were precleared using normal rabbit serum and protein-G-Sepharose 4FF (50% suspension, Pharmacia). After preclearing, the lysates were incubated for 3 h at 4 °C with an anti-human NCAM (CD56) monoclonal antibody (Becton Dickinson): 10 μg. Protein-G-Sepharose 4FF was added, and then the mixture was incubated for 1 h at 4 °C. After centrifugation at 2000 rpm for 5 min, the Sepharose was washed with the lysis buffer four times. The immunoprecipitated NCAM protein was eluted by boiling in Laemmli's sample solution (62.5 mM Tris/HCl (pH 6.8), 2% SDS, 10% glycerol and 0.002% bromophenol blue) including 5% 2-mercaptoethanol. The eluted samples were subjected to 6% SDS-PAGE and then analysed by lectin blotting as described below.

Lectin blot analysis

NCAM proteins were immunoprecipitated from control GOTO cells and GOTO cells treated with Bt_2cAMP (8 days) or BrdU (10 days) as described above. The eluted NCAM samples were electrophoresed on a 6% SDS-PAGE gel. After electrophoresis, the gel was soaked in

the blotting buffer (80 mM Tris, 350 mM glycine and 20% (v/v) methanol). The proteins were electroblotted onto a nitrocellulose membrane (Schleicher & Schuell) using a semi-dry electroblotting system according to the manufacturer's protocol (Hoefer Scientific Inst.). The membrane was incubated for 2 h in PBS containing 3% BSA and 0.05% Tween-20 for blocking. The membrane was washed once with PBS containing 0.1% Tween-20 and then incubated for 4 h with 2 $\mu g\,ml^{-1}$ of biotin-conjugated *Datsura stramonium* (DSA) (Honen Corp. Tokyo) in PBS containing 1% BSA. It was washed four times with PBS, 0.1% Tween-20, and then incubated for 1 h with 1 $\mu g\,ml^{-1}$ of streptoavidin-conjugated horseradish peroxidase. After washing four times with PBS, 0.1% Tween-20, DSA reactive bands were detected using an ECL kit (Amersham) according to the manufacturer's instructions. After detection with the ECL kit, stripping the membrane was performed by washing the membrane for 2 h at 55 °C with 62 mM Tris/HCl (pH 6.8) containing 100 mM 2-mercaptoethanol and 2% SDS. To remove sialic acids from the sugar chains of the proteins blotted onto the membrane, the membrane was soaked for 1 h in 10 mM HCl at 80 °C after stripping membrane. This membrane was reanalysed using DSA as described above.

Results

Cell growth associated changes of GnT-III and GnT-V expression in GOTO cells

GOTO cells were plated at 0.5–1 × 10^5 cells per ml. As shown in Fig. 1A, while the culture was subconfluent, GnT-III activity showed gradual increase but GnT-V activity did not show any significant change during cell growth. Once the culture became confluent, however, GnT-III activity appeared to decrease markedly, i.e. to half the initial level. Despite the decrease in GnT-III activity, the transcriptional level of GnT-III mRNA did not show a significant change. However, GnT-V activity did not show any decrease at the confluent state but in fact a slight increase in proportion to cell growth. In the case of GnT-V, these changes in activity seemed to be compatible with the changes in the transcriptional level (Fig. 1B).

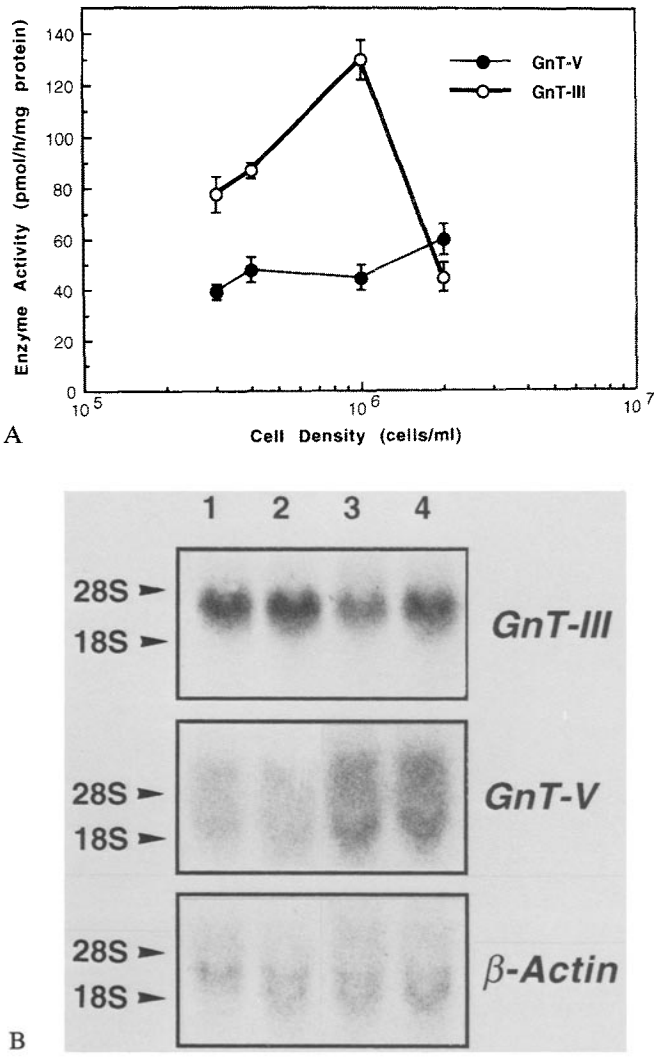

Figure 1. GnT-III and V expression in GOTO cells during cell growth. GOTO cells were plated at 0.5 × 10^5 cells per ml. The medium was changed every 24 h, and cells were harvested and counted, followed by glycosyltransferase assays and Northern blot analyses as described under Materials and methods. (A) GnT-III and V activities were assayed at each indicated cell density. Results shown are means ± SD from three experiments. (B) GnT-III, V and β-actin mRNA levels were examined by Northern blot analyses at each cell density. Lane 1, 3 × 10^5 cells per ml; 2, 5 × 10^5 cells per ml; 3, 1 × 10^6 cells per ml; 4, 2 × 10^6 cells per ml.

Activities and mRNA levels of GnT-III and GnT-V on Bt_2cAMP treatment

After treatment with 1 mM Bt_2cAMP for 4 days, GOTO cells started to form small neurites characteristic of neuronal cells, and then the proportion of neurite-forming cells increased, as shown in Fig. 2. Northern blot analysis of GnT-III and V mRNAs during neuronal cell differentiation of GOTO cells was performed (Fig. 3). GnT-III and V activities were simultaneously assayed in Bt_2cAMP-treated GOTO cells. GnT-III activity showed a slight increase for the initial 4 days, but decreased markedly after the fourth day. However, the mRNA level of GnT-III did not show any change with Bt_2cAMP treatment (Fig. 4A). In contrast, GnT-V activity showed a gradual increase with Bt_2cAMP treatment. After treatment with Bt_2cAMP for 7 days, GnT-V activity increased to approximately two- to three-fold the initial level. Northern blot analysis also showed a gradual increase in the GnT-V mRNA level (Fig. 4B). These observations implied a direct correlation between GnT-V activities

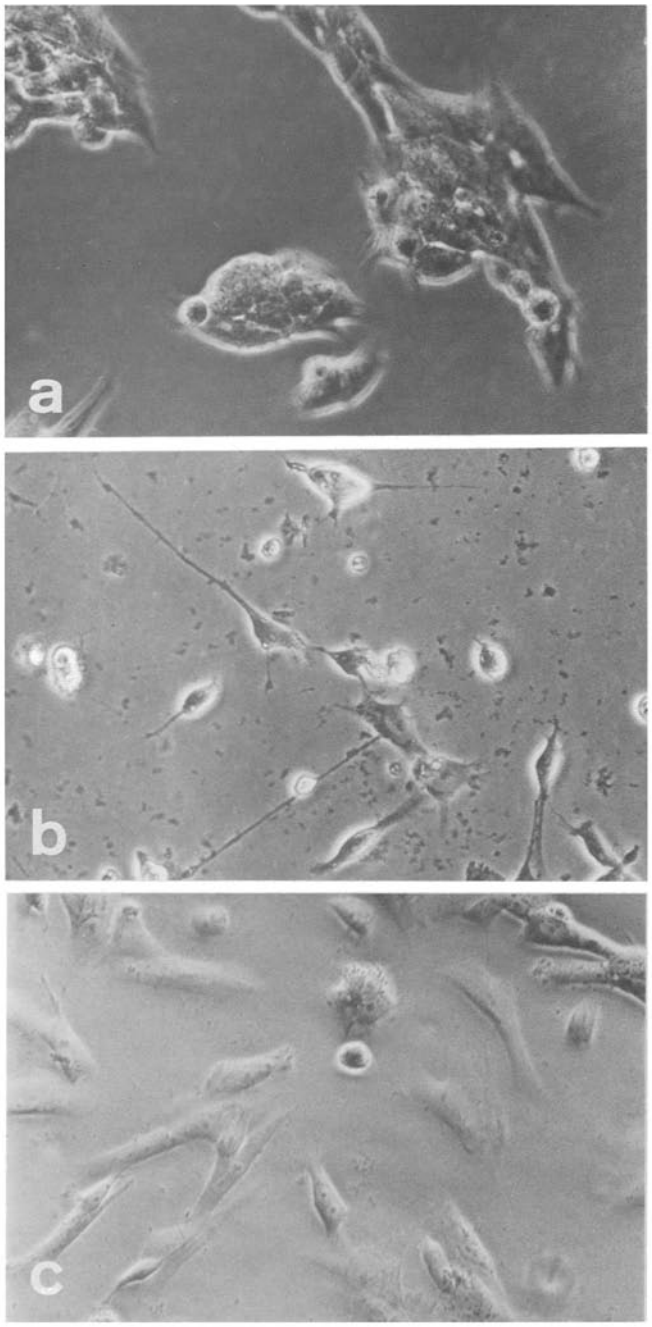

Figure 2. Morphological changes of GOTO cells on Bt_2cAMP or BrdU treatment. (a) untreated cells (× 400); (b) cells grown for 8 days in the presence of 1 mM Bt_2cAMP (× 200); (c) cells grown for 10 days in the presence of 5 $\mu g\,ml^{-1}$ BrdU (× 200).

and mRNA levels on Bt_2cAMP treatment of GOTO cells. To determine whether or not this increase in GnT-V activity, as well as that in the mRNA level, was due to a direct effect of Bt_2cAMP on the mRNA transcriptional level, the mRNA stability of GnT-V was examined by means of time course experiments involving Northern blot analysis with actinomycin D treatment.

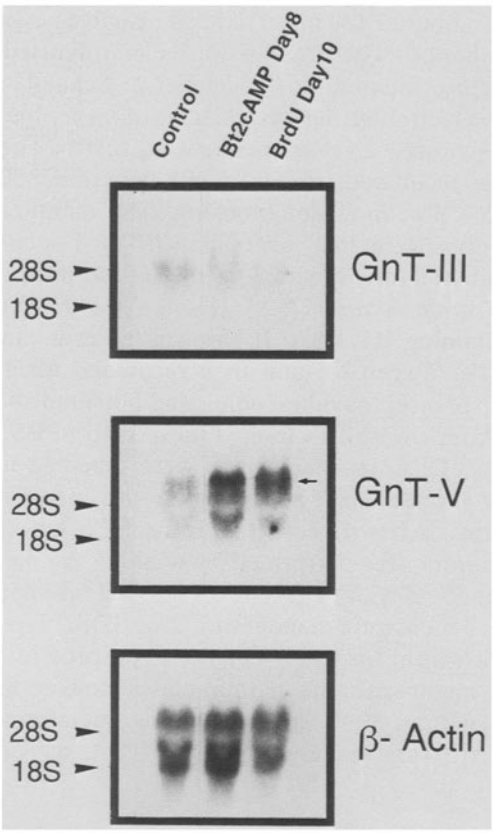

Figure 3. Northern blot analysis of GnT-III and V mRNAs during two distinct modes of differentiation of GOTO cells. The differentiation of GOTO cells into neuronal cells was induced by 8 days treatment with 1 mM Bt_2cAMP, and into Schwannian cell-like cells by 10 days treatment with 5 $\mu g\,ml^{-1}$ BrdU, and the cells were harvested and subjected to Northern blot analysis as described under Materials and methods.

The time course of the decrease in the GnT-V mRNA level after inhibition of RNA synthesis with actinomycin D is shown in Fig. 5. After treatment with Bt_2cAMP for 4 days, cells were treated simultaneously with actinomycin D and Bt_2cAMP. The GnT-V mRNA level with Bt_2cAMP treatment remained high for 24 h, as compared with the decreased GnT-V mRNA level without Bt_2cAMP. This suggested that Bt_2cAMP acted indirectly through the transcription of some intermediate products that stabilized the mature form of GnT-V mRNA.

Activities and mRNA levels of GnT-III and GnT-V on BrdU treatment

Following 5 days exposure to 5 $\mu g\,ml^{-1}$ BrdU, 40% of the GOTO cell population became flat-epithelial-like in shape (Fig. 2). These cells were found to account for more than 60% of the total cells after 10 days exposure. GnT-III and V activities were assayed in BrdU-treated GOTO cells (Fig. 6). GnT-III activity showed no significant change for the initial 7 days. But it decreased to

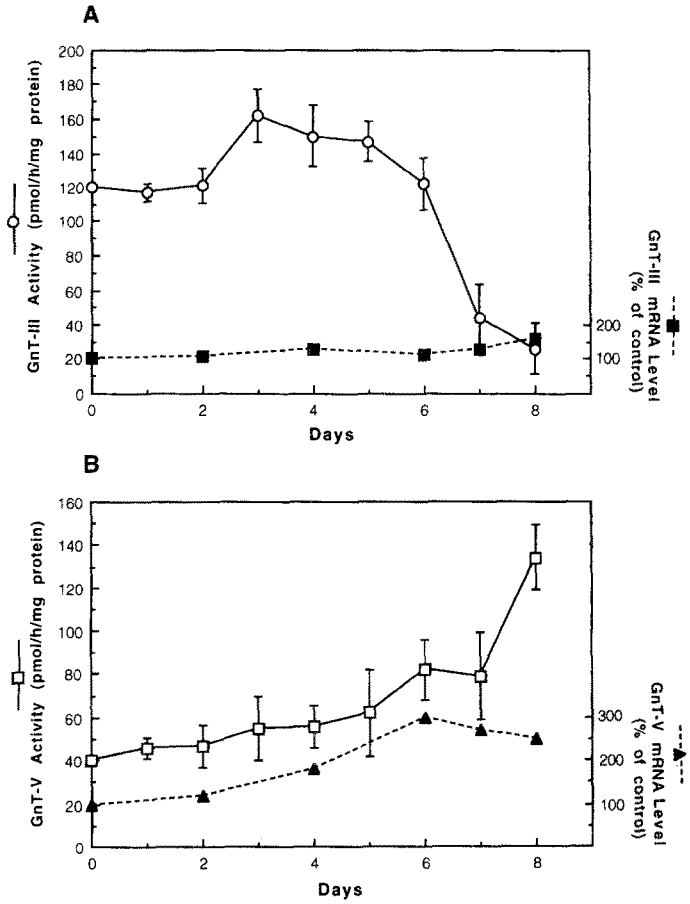

Figure 4. GnT-III and V expression in GOTO cells on Bt_2cAMP treatment. The differentiation of GOTO cells into neuronal cells was induced by 8 days treatment with 1 mM Bt_2cAMP, and the enzyme activities and mRNA levels for GnT-III (A) and GnT-V (B) were measured as described under Materials and methods. GnT-III activity (○), GnT-III mRNA level (■), GnT-V activity (□), GnT-V mRNA level (▲). Each value represents the average of two to three experiments.

approximately 60% of the initial level after 7 days, and remained at this level till the 10th day. This change seemed to be compatible with that in the GnT-III mRNA level, as shown in Fig. 6A. On the other hand, despite the gradual increase in the GnT-V mRNA level, no change was observed in the GnT-V activity after treatment for 10 days with BrdU (Figs 3 and 6B). In this case, the total intensity of GnT-V transcript bands actually increased, but no bands without a 9 kb band were enhanced for GOTO cells treated with BrdU (Fig. 3, arrow).

Lectin blot analysis of NCAMs from differentiated GOTO cells

To investigate qualitative changes of the carbohydrate moiety of a particular glycoprotein during the differentiation of GOTO cells, NCAM was chosen. NCAM is

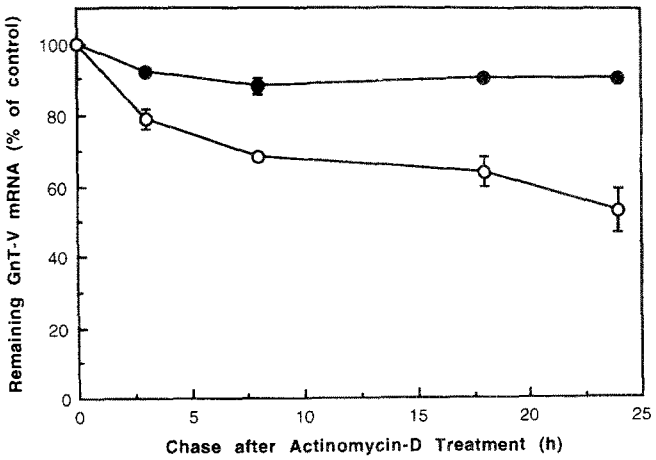

Figure 5. Stability of GnT-V mRNA in GOTO cells on Bt_2cAMP treatment. GOTO cells were cultured with 1 mM Bt_2cAMP for 4 days, followed by incubation for 0–24 h in the presence of actinomycin D (●). Control cells were cultured without Bt_2cAMP (○). GnT-V mRNA levels were measured as described under Materials and methods, and plotted. Each time course was conducted three times, with similar results.

known to exist abundantly on neural related tissues and cell lines [23], and the same is true on the surface of GOTO cells during differentiation, as judged on FACS analyses using an anti-NCAM monoclonal antibody (data not shown). NCAMs were immunoprecipitated from control, Bt_2cAMP-treated (8 days), and BrdU-treated (10 days) GOTO cells with a specific monoclonal antibody, and then analysed for binding to *Datsura stramonium* (DSA) after SDS-PAGE and blotting. As shown in Fig. 7, bands with about 180 kDa and 140 kDa of NCAM subtypes were relatively enhanced in Bt_2cAMP-treated GOTO cells as compared with control cells, especially after mild acid treatment of the membrane. Mild acid treatment was performed to remove sialic acids from blotted NCAM because sometimes NCAM is known to have unique polysialic acids in the N-glycans and these sialic acids might affect DSA reactivity. In the case of BrdU-treated GOTO cells, however, no DSA reactive NCAM bands were observed (Fig. 7).

Discussion

GnT-III and GnT-V are glycosyltransferases involved in N-linked oligosaccharide branch formation. It is known that these enzymes compete for the same substrate sugar chain structure and cooperative expression of these enzymes determines the branch structures. Recently, the roles of GnT-III and GnT-V have been emphasized because the branching structures of carbohydrate moieties are known to change remarkably during the embryogenesis, differentiation and malignant transformation of cells [4–7, 9, 14]. Although GnT-III and

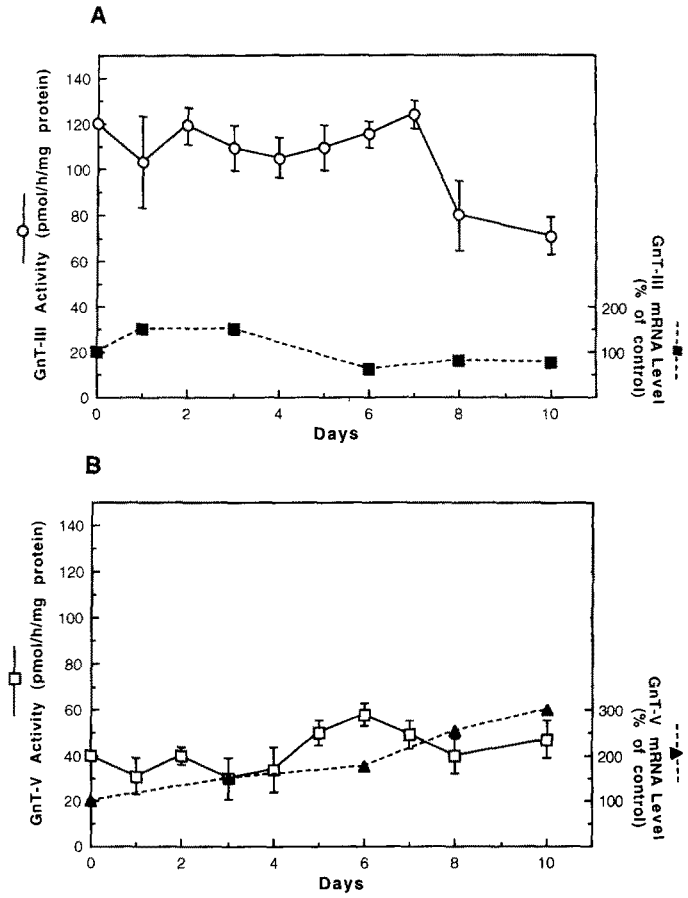

Figure 6. GnT-III and V expressions in GOTO cells on BrdU treatment. The differentiation of GOTO cells into Schwannian-like cells was induced by 10 days treatment with 5 $\mu g\,ml^{-1}$ BrdU, and the enzyme activities and mRNA levels for GnT-III (A) and GnT-V (B) were measured as described under Materials and methods. GnT-III activity (○), GnT-III mRNA level (■), GnT-V activity (□), GnT-V mRNA level (▲). Each value represents the average for two to three experiments.

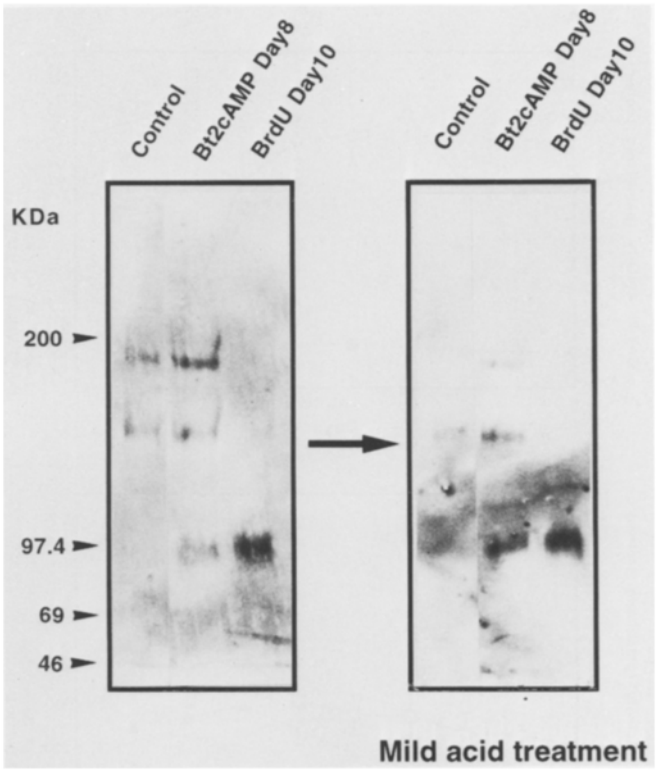

Figure 7. Lectin blot analysis of immunoprecipitated-NCAM in differentiated GOTO cells using DSA. After differentiation of GOTO cells into neuronal cells by 8 days treatment with 1 mM Bt_2cAMP, and into Schwannian-like cells by 10 days treatment with 5 $\mu g\,ml^{-1}$ BrdU, the cells were harvested. From each cell lysate, NCAMs were immunoprecipitated, and then subjected to 6% SDS-PAGE and lectin blot analysis using DSA. Lectin blots before reduction of sialic acids (left) were compared with after mild acid treatment (right), as described under Materials and methods. Arrow shows position of 140 kDa NCAM band.

GnT-V are expressed in several restricted tissues or organs, neural tissues such as cerebrum and cerebellum of rats commonly express abundant levels of GnT-III and GnT-V [24, 25]. N-Glycans possessing bisecting-GlcNAc structures, products of GnT-III, have also been reported to be abundant in mouse whole brain [26] and neuroblastoma cell lines [27]. In this respect, we focused on the relation between glycosyltransferases (GnT-III and V) and differentiation of neuroblastoma cells in the present study. A neuroblastoma cell line, GOTO, is known to differentiate into a neuronal cell phenotype with several inducers (i.e. Bt_2cAMP and retinoic acid, either alone or in combination, GQ1b [28]) or a Schwannian cell phenotype with BrdU. In terms of the ability to differentiate in two distinct ways, GOTO cells could be an interesting experimental model. Today, cell lines are thought to be useful tools for biological analyses of glycoconjugates, but glycoconjugate research involving cell lines is sometimes confronted with difficulty, because even cell growth without any induction can affect the metabolism of glycoconjugate biosynthesis and result in changes of sugar chain structures [29]. We therefore assayed GnT-III and V activities, and the respective transcriptional levels, in GOTO cells during normal cell growth. GnT-III showed marked inactivation at a high cell density, which was not due to a direct effect of mRNA transcription of GnT-III. This suggests that some unknown post-translational modifications of GnT-III proteins, such as phosphorylation, may regulate GnT-III enzyme activity. On the other hand, GnT-V did not show any significant changes during cell growth. In the case of the hepatoma cell line, HepG2, GnT-V activity was 3.2-fold lower in confluent than in subconfluent cultures, and this seemed to affect the sugar chain structures of transferrin [29]. Taken together, our results demonstrated that cell line-specific and growth-associated changes of glycosyltransferases could be important factors for the cell biological analysis of glycoconjugates.

We then examined changes of GnT-III and V following Bt_2cAMP treatment which induced neurite formation. In this system, no short-term effect or induction of GnT-III and V within 6 h was observed, and GnT-IV and β1-4 galactosyltransferase activities showed no significant changes on Bt_2cAMP treatment (data not shown). In the case of GnT-III, the activity increased during 4 days of treatment but significantly decreased after the fourth day, despite no change in the mRNA level. This discrepancy between enzyme activity and the mRNA transcriptional level seems to be similar to that associated with cell growth, and also suggests the occurrence of enzyme regulation by post-translational modification. On the other hand, increase in GnT-V activity was accompanied by an increase in the mRNA level, with enhanced stability of mRNA, which implies a direct correlation between GnT-V activities and mRNA levels in GOTO cells on Bt_2cAMP treatment. Glycosyltransferase activity sometimes seems to be affected by mRNA stability, because the stability of GnT-V mRNA is known to be enhanced in mouse melanoma B16 cells on TGF-β treatment [30] and the mRNA stability of α2-6 sialyltransferase is known to decrease in Hep G2 cells on butyrate treatment [31]. Branching β1-6 GlcNAc transferases such as GnT-V and Core2GnT have been well characterized in endodermal differentiated F9 cells on retinoic acid treatment by Heffernan *et al.* [17]. In this case, GnT-V activity was not appreciably affected by Bt_2cAMP, and retinoic acid caused elevation of many glycosyltransferase activities. But in the case of Chinese hamster ovary cells, Core2GnT was not affected by retinoic acid, and was induced at the gene transcription/translation level by butyrate [32]. Therefore, induction of GnT-V expression by several agents may differ in specific cell types.

In neuronal cell differentiation of GOTO cells on Bt_2cAMP treatment, DSA reactivity to NCAM increased. These results implied a possible correlation between the elevated GnT-V activity and the increase in the DSA reactive carbohydrate moiety of NCAM in Bt_2cAMP treated-GOTO cells. Because DSA recognizes the Galβ1-4GlcNAc structure [33], the reactivity to DSA suggests the existence of branching or a polylactosamine structure of the carbohydrate moieties of glycoproteins. Although GnT-V may be one of the likely candidates that is affected on neuronal differentiation of GOTO cells by Bt_2cAMP, a long-term effect of cAMP is also known to cause enhancement of the synthesis of the dolichol pyrophosphate core oligosaccharide [34], which suggests that the regulation of sugar chain structures by cAMP treatment is not simple.

In Schwannian cell-like differentiation of GOTO cells on BrdU treatment, GnT-III and V activities showed no significant changes, but the mRNA level of GnT-V showed a gradual increase. BrdU itself did not affect or suppress the enzyme activity of GnT-V in our assay system (data not shown). GnT-V has more than two transcripts and its gene expression is regulated by a multi-promoter system in several cells (Saito *et al.*, unpublished data). In the case of BrdU treatment, only the 9 kb transcript of GnT-V was enhanced on 10 days treatment, which suggested that the 9 kb transcript was not responsible for active GnT-V expression. Further investigation is required to explain the discrepancy between GnT-V activity and the mRNA level on BrdU treatment.

Very recently, GD3 synthase was transfected into Neuro2a cells, where it caused induction of cholinergic differentiation [35], and the important role of specific gangliosides was emphasized in neural cell differentiation. Moreover, no *GnT-I* gene targeted transgenic mouse survived, and development of neural tube formation was abolished, which shows that complex type N-glycans are required for morphogenic processes such as neural tube formation [36, 37]. Both GnT-III and GnT-V are responsible for determination of the branch formation in complex type N-glycans, and are considered to be important enzymes following the catalytic reaction of GnT-I. Although it is difficult to emphasize the importance of GnT-V expression during neuronal cell differentiation on the basis of this limited study, transgenic experiments will clarify the biological meaning of GnT-V expression and its products during differentiation in specific cell types.

Acknowledgements

We are grateful to Dr Hiroyuki Saito and Dr Masafumi Yoshimura for technical assistance, and Mr N.J. Halewood for editing and correcting this manuscript. This work was supported in part by Grant-in-Aid for Scientific Research on Priority Areas, No. 05274103, from the Ministry of Education, Science and Culture, Japan.

References

1. Feizi T (1985) *Nature* **314**: 53–57.
2. Rademacher TW, Parekh RB, Dwek RA (1988) *Annu Rev Biochem* **57**: 785–838.
3. Narasimhan S (1982) *J Biol Chem* **257**: 10235–42.
4. Koenderman AHL, Wijermans PW, Van den Eijnden DH (1987) *FEBS Lett* **222**: 42–46.
5. Narasimhan S, Schachter H, Rajalakshmi S (1988) *J Biol Chem* **263**: 1273–81.
6. Easton EW, Blokland I, Geldof AA, Rao BR, Van den Eijnden DH (1992) *FEBS Lett* **308**: 46–49.
7. Miyoshi E, Nishikawa A, Ihara Y, Gu J, Sugiyama T, Hayashi N, Fusamoto H, Kamada T, Taniguchi N (1993) *Cancer Res* **53**: 3899–902.
8. Cummings RD, Trowbridge IS, Kornfeld S (1982) *J Biol Chem* **257**: 13421–27.

9. Dennis JW, Laferte S, Waghrne C, Breitman ML, Kerbel RS (1987) *Science* **236**: 582–85.
10. Nishikawa A, Ihara Y, Hatakeyama M, Kangawa K, Taniguchi N (1992) *J Biol Chem* **267**: 18199–204.
11. Ihara Y, Nishikawa A, Tohma T, Soejima H, Niikawa N, Taniguchi N (1993) *J Biochem* **113**: 692–98.
12. Shoreibah M, Perng G-S, Adler B, Weinstein J, Basu R, Cupples R, Wen D, Browne JK, Buckhaults P, Fregien N, Pierce M (1993) *J Biol Chem* **268**: 15381–85.
13. Saito H, Nishikawa A, Gu J, Ihara Y, Soejima H, Wada Y, Sekiya C, Niikawa N, Taniguchi N (1994) *Biochem Biophys Res Commun* **198**: 318–27.
14. Brockhausen I, Romero PA, Herscovics A (1991) *Cancer Res* **51**: 3136–42.
15. Schachter H (1986) *Biochem Cell Biol* **64**: 163–81.
16. Gu J, Nishikawa A, Tsuruoka N, Ohno M, Yamaguchi N, Kangawa K, Taniguchi N (1993) *J Biochem* **113**: 614–19.
17. Heffernan M, Lotan R, Amos B, Palcic M, Takano R, Dennis JW (1993) *J Biol Chem* **268**: 1242–51.
18. Sekiguchi M, Oota T, Sakakibara K, Inui N, Fujii G (1979) *Jpn J Exp Med* **49**: 67–83.
19. Murakami T, Ohmori H, Gotoh S, Tsuda T, Ohya R, Akiya S, Higashi K (1991) *J Biochem* **110**: 146–50.
20. Tsunomoto K, Todo S, Imashuku S, Kato K (1988) *Cancer Res* **48**: 170–74.
21. Taniguchi N, Nishikawa A, Fujii S, Gu J (1989) *Methods Enzymol* **179**: 397–408.
22. Chomczynski P, Sacchi N (1987) *Anal Biochem* **162**: 156–59.
23. Edelman GM (1986) *Annu Rev Cell Biol* **2**: 81–116.
24. Nishikawa A, Gu J, Fujii S, Taniguchi N (1990) *Biochim Biophys Acta* **1035**: 313–18.
25. Perng G-S, Shoreibah M, Margitich I, Pierce M, Fregien N (1994) *Glycobiology* **4**: 867–71.
26. Shimizu H, Ochiai K, Ikenaka K, Mikoshiba K, Hase S (1993) *J Biochem* **114**: 334–38.
27. Motoyoshi F, Kondo N, Orii T (1993) *Tumor Biol* **14**: 334–37.
28. Tsuji S, Arita M, Nagai Y (1983) *J Biochem* **94**: 303–6.
29. Hahn TJ, Goochee CF (1992) *J Biol Chem* **267**: 23982–87.
30. Miyoshi E, Nishikawa A, Ihara Y, Saito H, Uozumi N, Hayashi N, Fusamoto H, Kamada T, Taniguchi N (1995) *J Biol Chem* (in press).
31. Shah S, Lance P, Smith TJ, Berenson CS, Cohen SA, Horvath PJ, Lau JTY, Baumann H (1992) *J Biol Chem* **267**: 10652–58.
32. Datti A, Dennis JW (1993) *J Biol Chem* **268**: 5409–16.
33. Yamashita K, Totani K, Ohkura T, Takasaki S, Goldstein IJ, Kobata A (1987) *J Biol Chem* **262**: 1602–7.
34. Konrad M, Merz WE (1994) *J Biol Chem* **269**: 8659–66.
35. Kojima N, Kurosawa N, Nishi T, Hanai N, Tsuji S (1994) *J Biol Chem* **269**: 30451–56.
36. Ioffe E, Stanley P (1994) *Proc Natl Acad Sci USA* **91**: 728–32.
37. Metzler M, Gertz A, Sarkar M, Schachter H, Schrader JW, Marth JD (1994) *EMBO J* **13**: 2056–65.