Glycoconjugate Journal (1995) **12**: 894–900

Expression of α2,8-sialyltransferase (GD3 synthase) gene in human cancer cell lines: high level expression in melanomas and up-regulation in activated T lymphocytes

SHUJI YAMASHIRO[1], MASAHIKO OKADA[1], MASASHI HARAGUCHI[1], KEIKO FURUKAWA[1], KENNETH O. LLOYD[2], HIROSHI SHIKU[1,3] and KOICHI FURUKAWA[1]*

[1]*Department of Oncology, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki, 852 Japan*
[2]*Immunology Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York 10021, USA*
[3]*Department of Internal Medicine II, Mie University School of Medicine, Edobashi, Tsu, Mie, 514 Japan*

Received 26 April 1995, revised 10 July 1995

GD3 Synthase (α2,8sialyltransferase) (EC 2.4.99.8) cDNA has been cloned by eukaryotic cell expression cloning. Using this cDNA as a probe, the expression level of the gene in human cancer cell lines was analysed by Northern blotting and RT-PCR, then correlated with the ganglioside expression and enzyme activity. Melanoma cell lines showed extremely strong bands in Northern blot and RT-PCR/Southern analysis. The enzyme activity was also very high in melanomas as expected. Neuroblastoma and astrocytoma lines showed relatively low levels of the gene expression, whereas they expressed high levels of GD2. Although the mRNA level of the GD3 synthase gene and enzyme activity in individual cell lines correlated positively, some cell lines showed much higher activity than expected from the mRNA level. Among leukaemia lines, adult T cell leukaemia-associated (HTLV-I$^+$) lines showed fairly high levels of the mRNA. On the other hand, T-ALL lines showed very low levels. In addition, GD3 and GD2 expression and mRNA level of the gene during T lymphocyte activation were analysed. Only GD3 expression was induced by any of the stimulatory reagents used, and corresponding up-regulation of the GD3 synthase gene was shown in RT-PCR/Southern analysis.

Keywords: α2,8-sialyltransferase gene, GD3 synthase gene, melanomas, T lymphocytes

Abbreviations: α2,8S-T, α2,8sialyltransferase; RT-PCR, reverse transcription polymerase chain reaction; HTLV-I, human T lymphotropic virus type I; T-ALL, T cell acute lymphoblastic leukaemia; β1,4GalNAc-T, β1,4-*N*-acetylgalactosaminyltransferase; PMSF, phenylmethylsulfonylfluoride; NeuAc, *N*-acetylneuraminic acid; FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium; mAb, monoclonal antibody; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Con A, concanavalin A, PHA; phytohaemagglutinin; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; ATL, adult T cell leukaemia. Ganglioside nomenclature is based on that of Svennerholm [26].

Introduction

Sialic acid-containing glycosphingolipids, gangliosides, are synthesized by the sequential reactions of multiple glycosyltransferases [1, 2, 3]. In order to analyse the regulatory mechanisms for ganglioside synthesis, we isolated cDNAs of β1,4-*N*-acetylgalactosaminyltransferase (β1,4GalNAc-T) (EC 2.4.1.92) [4] and α2,8-sialyltransferase (α2,8S-T) (EC 2.4.99.8) [5], i.e. GM2/GD2 synthase and GD3 synthase, respectively. β1,4GalNAc-T catalyses the synthesis of GM2/GD2, providing the precursor structures for complex gangliosides of *a* and *b* series [4]. α2,8S-T catalyses GD3 synthesis, resulting in the initiation of the synthesis of the *b* series of gangliosides [4]. Previously, we analysed the genetic and enzymatic basis of GM2/GD2 synthesis by using the cloned *β1,4GalNAc-T* gene as a probe [6], and showed the

*To whom correspondence should be addressed.

importance of the levels of precursor structures in individual cells as well as the expression levels of GM2/GD2 synthase.

In the present study, we have analysed the gene expression of α2,8S-T in human cancer cell lines and correlated this with surface phenotypes and the enzyme activity. Generally, melanoma cell lines showed markedly high levels of the gene expression as well as the gene products. In addition, we have demonstrated the up-regulation of the α2,8S-T gene and GD3 expression during the activation of peripheral T lymphocytes as expected from the previous studies using monoclonal antibodies [7, 8].

Materials and methods

NORTHERN BLOTTING

I. Preparation of RNA

Poly $(A)^+$ RNA was prepared as described previously [4] according to Badley *et al.* [9]. One $\times 10^8$ cells were pelleted in a 50 ml centrifuge tube and lysed in 15 ml of lysis buffer consisting of 0.2 M NaCl, 0.2 M Tris-HCl, pH 7.5, 1.5 mM $MgCl_2$, 2% SDS and 200 $\mu g\,ml^{-1}$ proteinase K, and incubated at 45 °C for 2 h. The NaCl concentration of the lysate was adjusted to 0.5 M by the addition of 0.95 ml of 5 M NaCl. The lysate was poured into a 50 ml centrifuge tube with pre-equilibrated oligo dT cellulose and rocked gently at 37 °C for 1 h. The oligo dT cellulose was washed four times with 10 ml of binding buffer (0.5 M NaCl, 0.01 M Tris-HCl, pH 7.5), resuspended in 1 ml of binding buffer and poured into a small column. It was washed until the OD_{260} of the flow-through was ≤ 0.05. Poly$(A)^+$ RNA was eluted with eluting buffer (0.01 M Tris-HCl, pH 7.5). The RNA was precipitated with 0.15 vol. of 2 M sodium acetate and 2.5 vol. of ethanol at −70 °C. The precipitate was pelleted and washed in 80% ethanol, and then dissolved in 50 μl of DEPC-treated H_2O. Preparation of total RNA was performed as described previously [6].

II. Northern blot analysis

Poly$(A)^+$ RNA (10 μg), or total RNA (25 μg) were electrophoresed on 1.25% formaldehyde agarose gel, blotted onto a nylon membrane (GeneScreen Plus; DuPont/NEN, MA), then fixed by baking for 2 h at 80 °C. Blots were incubated in a hybridization buffer consisting of 50% formamide, 10% dextran sulfate, 1 M NaCl, 1% SDS, 50 mM Tris, pH 8.0 and 100 μl of denatured salmon sperm DNA for 3 h at 42 °C. Hybridization was performed by adding random primed probes at 3×10^5 cpm ml^{-1} at 42 °C for 16 h. The probes were radiolabelled using the Multiprime DNA labelling system (Amersham, Arlington Heights, IL). Blots were washed as described previously [4], then exposed to X-ray film at −70 °C for 48–72 h. The relative amount of each mRNA was determined by an image analyser BAS2000 (Fuji, Tokyo, Japan).

α2,8S-T ASSAY

The membrane fractions of cells were prepared as described by Thampoe *et al.* [10]. Briefly, cells were lysed in ice-cold PBS containing 1 mM PMSF using a nitrogen cavitation apparatus (Parr Instrument Co., Moline, IL) at 400 psi for 30 min. Nuclei were removed by low centrifugation and supernatant was centrifuged at 105 000 × **g** for 1 h at 4 °C. The pellet was resuspended in ice-cold 50 mM cacodylate-HCl buffer, pH 6.0. Enzyme assays were performed as described previously [11]. In a volume of 50 μl the reaction mixture contained: 50 mM sodium cacodylate-HCl, pH 6.0 (Wako Junyaku, Osaka, Japan), 10 mM $MgCl_2$, 0.3% Triton CF-54. (Sigma, St Louis, MO), 325 μM GM3, 400 μM CMP-NeuAc (Sigma), CMP-[^{14}C]NeuAc (3.5×10^5 dpm) (NEN), and membranes containing 200 μg protein. The reaction was allowed to proceed for 2 h in a shaken water bath at 37 °C. The products were separated by a C18 Sep-Pak cartridge (Waters, Millford, MA) and the radiolabelled ganglioside counted in a scintillation counter.

CELLS

Cell lines used in this study (Table 1) were maintained in RPMI1640 containing 10% FBS (leukaemia lines) or in DMEM containing 7.5% FBS (monolayer cells) and cultured in CO_2 in an incubater at 37 °C. The derivation of cell lines were as described previously [6].

FLOW CYTOMETRY

Cell surface expression of gangliosides was examined with a FACScan (Becton-Dickinson, Mountain View, CA) as described previously [4].

RT-PCR

RT

Single-strand cDNA was synthesized with oligo-dT_{14} primer as described [12]. Total RNA (3 μg) was dissolved in 50 μl of 50 mM Tris-HCl, pH 8.3 containing 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM dATP, dGTP, dCTP, dTTP, 0.5 μg oligo-dT_{14}, and 200 U of Moloney murine leukaemia virus reverse transcriptase (GIBCO-BRL, Grand Island, NY), then incubated for 90 min at 37 °C.

PCR

PCR was performed basically according to the previous report [6]. The PCR primers used for the α2,8S-T gene were a pD3T-31 sense primer (α2,8S-T cDNA clone pD3T-31, nucleotides 519–536), 5′-ATCCCAGCATAATTCGGC-3′, and an antisense primer (nucleotides 834–851), 5′-AGAAGGGCCAGAAGCCAT-3′ (see [5]). The primers for the glyceraldehyde-3-phosphate

Table 1. Cell type of the cell lines used in this study

Cell types	*Name of cell lines*
Myelogenous leukaemia	
Megakaryoblastic	MEG-01
Erythroleukaemia	K562
Promyelocytic	HL-60
Myeloma	Ara10
Burkitt lymphoma	Raji
T-ALL	P12/Ichikawa, MOLT-3 CCRF-CEM
ATL-associated	MT-2, HUT102 ATN-1
Neuroblastoma	SK-NSH, IMR-32
Astrocytoma	AS
Melanoma	MeWo, SK-MEL-31 SK-MEL-23, SK-MEL-37 SK-MEL-28

dehydrogenase (GAPDH) gene were a sense primer (mouse GAPDH cDNA, nucleotides 241–260, 5′-TGTCATCAACGGGAAGCCCA-3′, and an antisense primer (nucleotides 521–540), 5′-TTGTCATGGATGACCTTGGC-3′ (see [13]). Five μl of reverse transcription products were diluted in a 50 μl reaction mixture consisting of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, all 4 dNTPs (0.2 mM each), 0.002% gelatin, and PCR was done by adding 0.325 μg each of the sense primer and the antisense primer and 1 U of Taq DNA polymerase (Wako Junyaku). Samples were subjected to 15, 20, 25 and 30 cycles of amplification. Each cycle consisted of three steps; denaturation for 1 min at 94 °C, primer annealing for 1 min at 55 °C and polymerization for 2 min at 72 °C.

SOUTHERN BLOT ANALYSIS

Ten μl of RT-PCR products were electrophoresed on a 10% poly-acrylamide gel, and treated with an alkaline solution (0.4 N NaOH, 0.6 M NaCl) prior to transfer onto nylon filters. Filters were prehybridized in a solution containing 1 M NaCl, 1% SDS, 10% dextran sulfate, 50 mM Tris-HCl, pH 7.5, and 100 μg ml^{-1} of denatured salmon sperm DNA. Samples were hybridized overnight at 65 °C in the same buffer containing 1×10^5 cpm ml^{-1} full length cDNA probes as described previously [6], then exposed to an imaging plate and analysed by using the BAS2000 Image Analyzer.

MONOCLONAL ANTIBODIES

Monoclonal antibodies (mAb) used in this study were as follows. mAb 3F8 (anti GD2; IgG3, [14]) was kindly provided by Dr N.K. Cheung, Sloan-Kettering Cancer Center. mAb R24 (anti GD3; IgG3, [15]) was presented by Dr L.J. Old, Sloan-Kettering Cancer Center. Anti-GD1b mAb GGR12(IgG) and anti-GT1b mAb GMR5(IgM), provided by Dr T. Tai, Metropolitan Institute, Tokyo, have been described previously [16].

STIMULATION OF PERIPHERAL T LYMPHOCYTES

Peripheral mononuclear cells were isolated by Ficoll-conray centrifugation from heparinized whole blood. Separated mononuclear cells were resuspended in RPMI 1640 containing 10% FBS at 1×10^6 ml^{-1} and cultured in a 48-well plate (Corning) in the presence of various stimulatory reagents. Concentration of these reagents was as indicated in Table 2. Anti-CD3 mAb was coated in advance on the plates by diluting ascites 200 times. Cells were collected and pelleted at days 1, 2 and 4, then saved for RT-PCR.

Results

Expression of b series gangliosides in twenty human cancer cell lines

Using specific mAbs, surface expression of GD3, GD2, GD1b and GT1b was analysed. As shown in Fig. 1, all the melanoma lines except for MeWo expressed both GD3 and GD2. Neuroblastoma and astrocytomas expressed mainly GD2, but not GD3. Among leukaemia lines, two out of three HTLV-I$^+$ lines expressed GD2 as reported [17], but not GD3. The NK-like line, YTN17, expressed a high level of GD2 and a low level of GD3. GD1b and GT1b were not definitely expressed on the cell lines examined.

Expression of α2,8S-T gene as determined by RT-PCR and Northern blot

Using total RNA, RT-PCR and Northern blots were performed in order to analyze α2,8S-T gene expression. Generally, RT-PCR/Southern blotting showed stronger bands than Northern blotting especially in the sample with low level expression as shown in Fig. 2. Melanoma lines showed extremely high levels of gene expression, and neuroblastoma, astrocytoma and HTLV-I$^+$ lines showed low levels. Although HUT102 showed a PCR product with the same size as other lines, mRNA detected in Northern blot was much smaller than others, i.e. 1.7 kb. Three T-ALL lines showed very low expression.

Northern blot by poly(A)$^+$ RNA

In order to precisely investigate the size of the α2,8S-T mRNA in HUT102, Northern blot using poly (A)$^+$ RNA was performed. As shown in Fig. 3, HUT102 showed a 1.7 kb band, whereas two melanoma lines and one ATL line showed a 2.6 kb band as reported [5]. This smaller

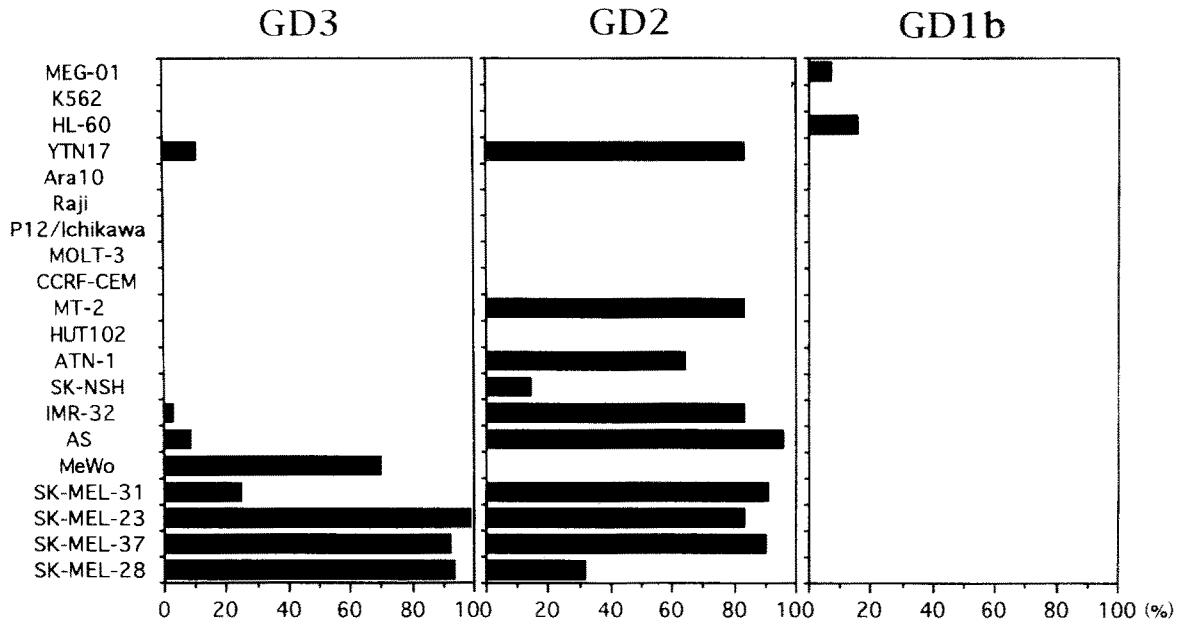

Figure 1. Expression of *b* series gangliosides on human cancer cell lines. Surface expression of gangliosides was analysed with mAb by flow cytometry as described in Materials and methods, and presented as a percentage of positive cells. These data are essentially same as described in [6].

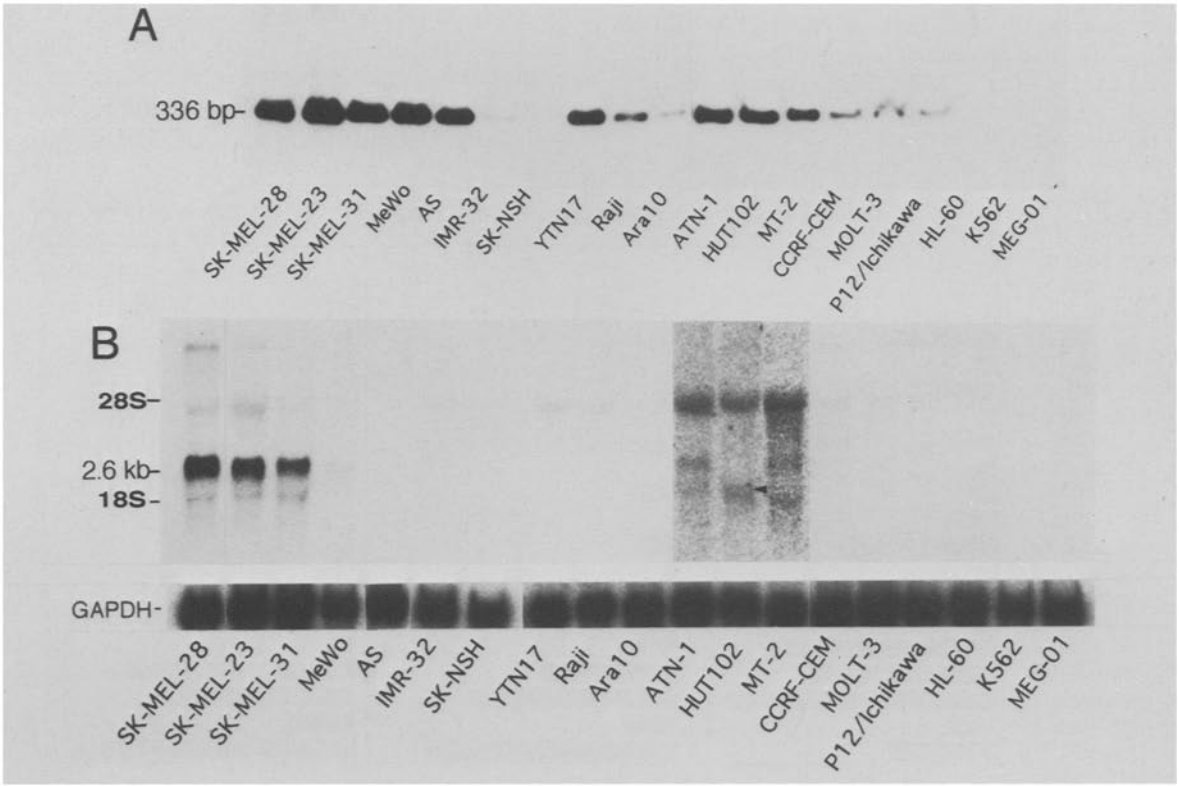

Figure 2. Expression levels of mRNA in the α2,8S-T gene. mRNA levels of the α2,8S-T gene in human cancer cell lines were analysed by RT-PCR (A) and Northern blotting (B). PCR was performed for 20 cycles in these data for both the α2,8S-T and GAPDH genes, and the product was Southern blotted by using the cDNA probes as described in Materials and methods. The results of GAPDH indicated that almost identical amounts of RNA were used (data not shown). Northern blotting was performed by using total RNA (15 μg) extracted from 20 human cancer lines as described in Materials and methods. In the lanes for ATN-1, HUT102 and MT-2, results with longer exposure were shown in order to demonstrate weaker bands. Arrow head indicates a 1.7 kb band in HUT102.

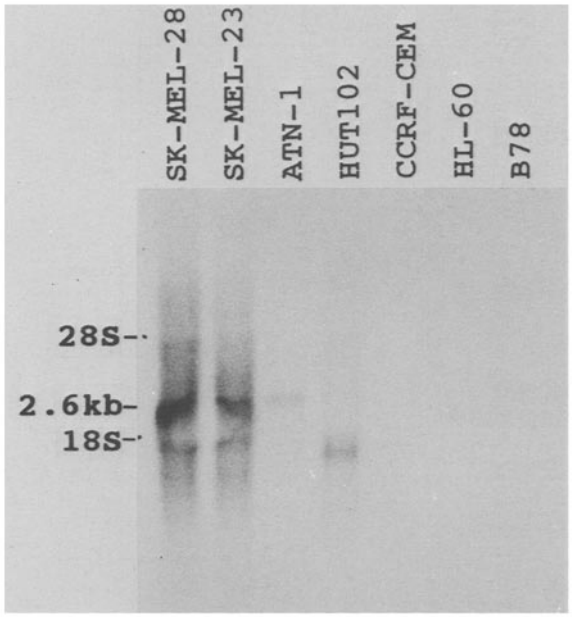

Figure 3. Northern blotting of the *α2,8S-T* gene by poly $(A)^+$ RNA. Using poly $(A)^+$ RNA, Northern blotting was performed. Ten μg each of purified RNA was separated in 1.25% agarose/formaldehyde gel and blotted as described in Materials and methods. Sources of RNA were as follows, SK-MEL-28 and SK-MEL-23 (human melanoma lines); ATN-1 (ATL line); HUT102 (HTLV-I^+ line); CCRF-CEM (human T-ALL); HL-60 (human acute promyelocytic leukaemia line); B78 (a clone of mouse melanoma line B16).

mRNA in HUT102 seemed to be non-functional because no α2,8S-T activity was found in the cell line as indicated in Fig. 4.

α2,8S-T activity in 20 cell lines

α2,8S-T in the individual cell lines was measured and correlated with the gene expression. As summarized in Fig. 4, α2,8S-T activities in these cell lines mostly correlated positively with the levels of gene expression. In astrocytoma AS, the enzyme activity was very high despite the detection of only low mRNA levels.

Up-regulation of the α2,8S-T gene in activated T-lymphocytes

As previously described [7, 8], a small population of human peripheral T lymphocytes express GD3 and the addition of anti-GD3 mAb (R24) induces T-cell activation as measured by cell proliferation and IL-2 production. GD3 and GD2 expression on T lymphocytes stimulated by various reagents was analysed by flow cytometry (Table 2). Almost all reagents or combinations of them, induced elevations in GD3 expression in the 30–90% range, whereas GD2 expression did not change or increased by 2 ~ 5%. As shown in Fig. 5, ConA-stimulated T cells showed elevated levels of α2,8S-T mRNA at day 2 of *in vitro* culture, then decreased gradually. On the other hand, GD3 expression continued

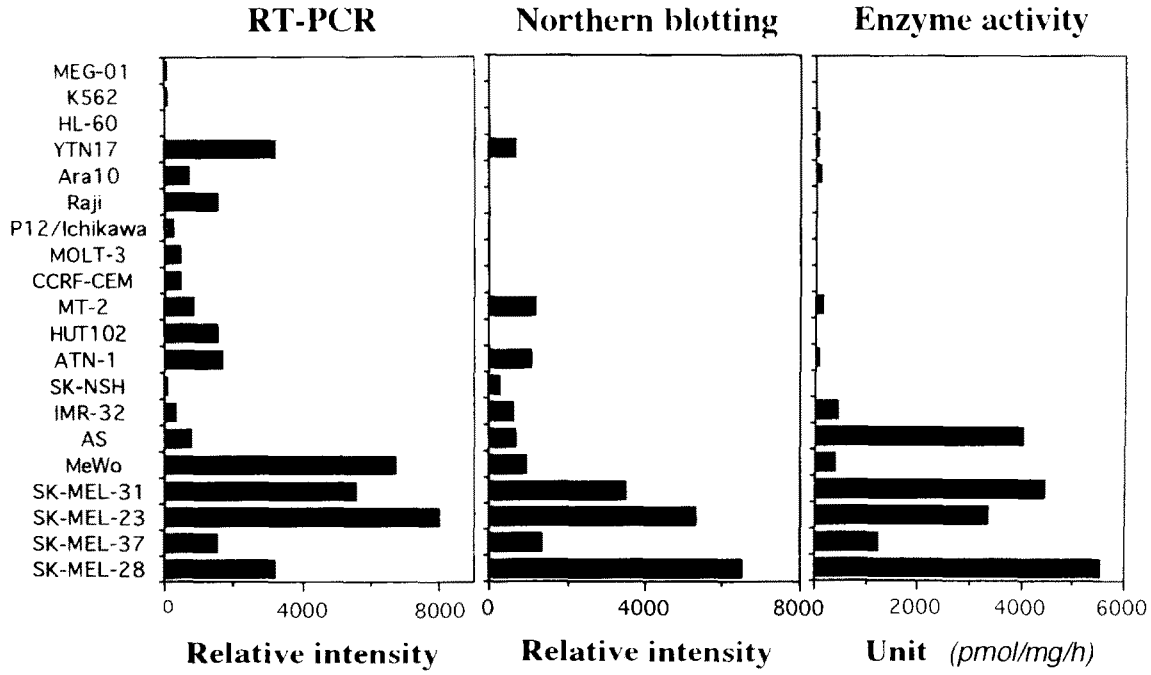

Figure 4. Summary of the α2,8S-T gene expression and the enzyme activity in 20 cell lines. mRNA levels were determined by RT-PCR and Northern blot as described in Materials and methods. They are presented as relative intensities determined by a Bioimaging Analyzer. mRNA levels of the gene were corrected by the intensities of GAPDH bands as shown in Fig. 2. Enzyme activity was determined by using GM3 as an acceptor as described in Materials and methods and presented as specific units (pmol per mg protein h^{-1}).

Table 2. Expression of GD3 and GD2 on PBL treated by various stimulative reagents

Stimulation	*Samples examined*	*% positive cells**	
		GD3	*GD2*
IL-2 (1 U ml^{-1})	(10)	20–30	0
IL-2 (10 U ml^{-1})	(10)	30–50	0
Con A (10 μg ml^{-1})	(6)	70–80	0
PHA (5 μg ml^{-1})	(3)	60–80	2–3
anti-CD3 mAb	(3)	70–80	0
TPA (10 nM)	(3)	50–60	4–5
IL-1 (200 μg ml^{-1}) +anti-CD3 mAb	(3)	80–90	0
IL-1 + PHA	(3)	80–90	0
TPA + anti-CD3 mAb	(3)	80–90	0

*GD3 and GD2 expression was analysed by flow cytometry using mAb R24 and mAb 3F8, respectively. Results on day 6 of culture were shown. Approximate range of % positive obtained from samples in each group was presented.

to increase until day 6 of culture, and was then maintained at a high level.

Discussion

Ganglioside GD3 is highly expressed in human melanoma cell lines and melanoma tissues [15, 18], whereas it is only minimally expressed in normal melanocytes [19, 20]. GD3 has served as a target molecule for immunotherapy in melanoma patients using mAb R24 [21]. Among *b* series gangliosides, GD2 is expressed in many melanoma cell lines, but GD1b or more complex structures are rarely detected (data not shown). Many melanoma lines express both GD3 and GD2, whereas astrocytoma, neuroblastoma and HTLV-I$^+$ lines express mainly GD2. The most outstanding difference between melanomas and other cell types is the extremely high level of α2,8S-T gene expression and GD3 in melanomas. Elucidation of the mechanism by which the α2,8S-T gene is activated during the malignant transformation of melanocytes will be important in understanding the role played by gangliosides in tumour cells and is now being investigated in our laboratory.

Although mRNA levels of α2,8S-T closely correlated with enzyme activities in most cell lines, some exceptions were noted. For example, AS cells showed high activity of the enzyme despite a low mRNA level. A few explanations for these discrepancies may be proposed. First, α2,8S-T activity may be regulated by post-translational modifications such as phosphorylation or glycosylation, as well as at the transcriptional level. Second, another sialyltransferase with similar function but not detectable by the probe used may exist.

HTLV-I$^+$ line HUT102 showed aberrent mRNA, although RT-PCR demonstrated an amplified band of the

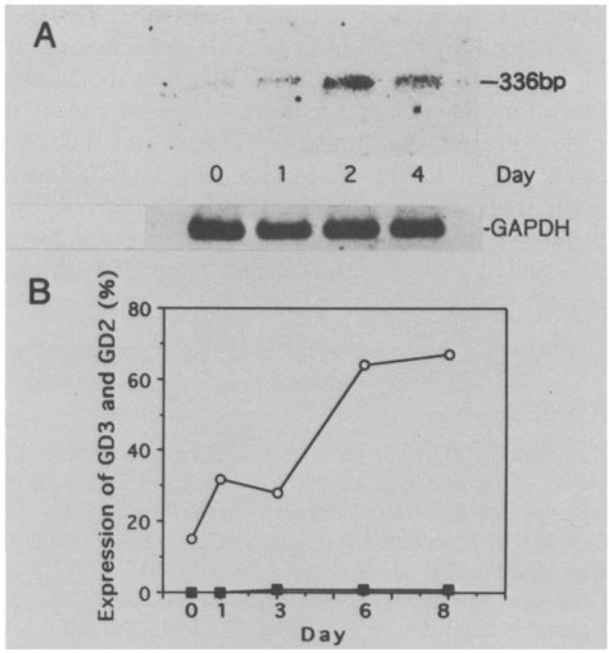

Figure 5. Up-regulation of the α2,8S-T gene during T cell activation. (A) mRNA level of GD3 synthase gene in ConA-stimulated T lymphocytes was analysed by RT-PCR/Southern blot as described in Materials and methods. Results of GAPDH control were added at the bottom. (B) GD3 (○) and GD2 (■) expression as determined by flow cytometry.

expected size. These results suggest that the genome structure of the gene is disrupted in some area containing the coding region. Whether or not this finding is common in cells infected by HTLV-I and is due to the chromosomal instability of this kind of cell [21–25] are now under investigation.

As reported previously [17], GD2 was poorly expressed on the activated T lymphocytes stimulated by various reagents. In contrast, GD3 expression was induced in variable amounts, by stimulation with almost all reagents or their combination. Although up-regulation of α2,8S-T gene was confirmed in RT-PCR using RNA from Con A blasts, the duration of the mRNA elevation was shorter than expected. The time course of the induced pattern of GD3 expression varied among lymphocyte samples. The sensitivity of T lymphocytes for GD3 induction may be different depending on the donors' age or sex. The expression of GM3, the precursor of GD3, may be also simultaneously induced by these reagents and may affect the synthesis and expression of GD3. The regulation of the α2,3S-T gene responsible for the synthesis of GM3 also needs to be analysed.

Acknowledgements

We thank Dr T. Tai (Metropolitan Institute for Medical Science, Tokyo) for mAbs. We also thank Mrs T.

Shimomura for excellent technical assistance. This work was supported by the Grant-in-Aid for Scientific Research on Priority Area No. 05274103 from the Ministry of Education, Science and Culture of Japan, and by the Grant-in-Aid from the Ministry of Health and Welfare of Japan and by the US Public Health Service (N.I.H. grant CA 60). This work was also partly supported by a Grant-in-Aid from the Mizutani Foundation for Glycoscience and by a research grant from the Princess Takamatsu Cancer Research Fund.

References

1. Kaufman B, Basu S, Roseman S (1966) In *Inborn Disorders of Sphingolipid Metabolism* (Aronson SM, Volk BW eds) pp. 193–213, New York: Pergamon Press.
2. Caputto R, Maccioni HJ, Arce A, Cumar FA (1971) *Adv Exp Med Biol* **71**: 27–44.
3. Paulson JC, Colley KJ (1989) *J Biol Chem* **264**: 17615–18.
4. Nagata Y, Yamashiro S, Yodoi J, Lloyd KO, Shiku H, Furukawa K (1992) *J Biol Chem* **267**: 12082–89.
5. Haraguchi M, Yamashiro S, Yamamoto A, Furukawa K, Takamiya K, Lloyd KO, Shiku H, Furukawa K (1994) *Proc Natl Acad Sci USA* **91**: 10455–59.
6. Yamashiro S, Ruan S, Furukawa K, Tai T, Lloyd KO, Shiku H, Furukawa K (1993) *Cancer Res* **53**: 5395–400.
7. Hersey P, Schibeci SD, Townsend P, Burns C, Cheresh DA (1986) *Cancer Res* **46**: 6083–90.
8. Welte K, Miller G, Chapman PB, Yuasa H, Natoli E, Kunicka JE, Cordon-Cardo C, Buhrer C, Old LJ, Houghton AN (1987) *J Immunol* **139**: 1763–71.
9. Badley J, Bishop G, Joho T, Frelinger JA (1988) *Biotechnique* **6**: 114–16.
10. Thampoe IJ, Furukawa K, Vellve E, Lloyd KO (1989) *Cancer Res* **49**: 6258–64.
11. Ruan S, Lloyd KO (1992) *Cancer Res* **52**: 5725–31.
12. Furukawa K, Furukawa K, Shiku H (1991) *FEBS Lett* **295**: 141–45.
13. Sabath DE, Broome HE, Prystowsky MB (1990) *Gene* **91**: 185–91.
14. Cheung NV, Sarrinen UM, Neely JE, Landmeier B, Donovan D, Coccia PF (1985) *Cancer Res* **45**: 2642–49.
15. Pukel CS, Lloyd KO, Travassos LR, Dippold WG, Oettgen HF, Old LJ (1982) *J Exp Med* **155**: 1133–47.
16. Ozawa H, Kotani M, Kawashima I, Ando S, Tai T (1992) *Biochem Biophys Acta* **1123**: 184–90.
17. Furukawa K, Akagi T, Nagata Y, Yamada Y, Shimotohno K, Cheung N-KV, Shiku H, Furukawa K (1993) *Proc Nat Acad Sci USA* **90**: 1972–76.
18. Real FX, Houghton AN, Albino AP, Cordon-Cardo C, Melamed MR, Oettgen HF, Old LJ (1985) *Cancer Res* **45**: 4401–11.
19. Carubia JM, Yu RK, Macaela LJ, Kirkwood JM, Varga JM (1984) *Biochem Biophys Res Commun* **120**: 500–4.
20. Furukawa K, Arita Y, Satomi N, Eisinger M, Lloyd KO (1990) *Arch Biochem Biophys* **281**: 70–75.
21. Houghton AN, Mintzer D, Cordon-Cardo C, Welt S, Fliegel B, Vadhan S, Carswell E, Melamed MR, Oettgen HF, Old LJ (1985) *Proc Natl Acad Sci USA* **82**: 1242–46.
22. Matutes E, Brito-Babapulle V, Swansbury J, Ellis J, Morilla R, Dearden C, Sempere A, Catovsky D (1991) *Blood* **78**: 3269–74.
23. Maruyama K, Fukushima T, Mochizuki S, Kawamura K, Miyauchi M (1992) *Leukemia* **6**: 605–35.
24. Maruyama K, Fukushima T, Mochizuki S, Kawamura K, Koshikawa N, Miyauchi M, Nakano M (1994) *Leukemia* **8**: 536–40.
25. Saggioro D, Majone F, Forino M, Turchetto L, Leszl A, Chieco-Bianchi L (1994) *Leuk Lymph* **12**: 281–86.
26. Svennerholm L (1963) *J Neurochem* **10**: 613–23.