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Changes in the glycolipid composition and characteristic activation of GM3 synthase in the thymus of mouse after administration of dexamethasone

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Glycolipids in the thymus of mice after administration of dexamethasone were compared with those in control mice. In parallel with a decrease in the tissue weight due to the disappearance of immature thymocytes in the cortex, the amounts of GlcCer, Gg_4Cer and GM1 decreased from 18 h after intraperitoneal administration of dexamethasone, but those of Gb_4Cer and Forssman glycolipid did not change, indicating the differential distribution of ganglio- and globo-series glycolipids in the thymus, GlcCer, Gg_4Cer and GM1 being on dexamethasone-sensitive cortical thymocytes, and Gb_4Cer and Forssman glycolipid on dexamethasone-resistant cells including thymic stromal cells, respectively. At the same time, a characteristic increase in GM3, whose amount per thymus and concentration per mg of thymus were increased 4-fold and 13-fold compared to those in the control mice, respectively, was observed at the onset of the decrease in tissue weight and was due to the increased activity of LacCer sialyltransferase with the enhanced expression of its gene and the concomitant decrease in cytosolic sialidase activity. One can suggest that endogenous accumulation of GM3 is involved in the dexamethasone-induced apoptosis of cortical thymocytes. On radiolabeling of the thymus with CMP-[^{14}C]-NeuAc, the incorporation of radioactivity into GM3 was preferentially observed in the thymuses of dexamethasone-administered mice, but not in those of control mice, suggesting the possible involvement of plasma membrane-associated sialytransferase in GM3 synthesis in the thymuses of dexamethasone-administered mice.
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Abbreviations: **Neutral glycolipids are abbreviated according to the recommendation of the IUPAC-IUB Commission on Biochemical Nomenclature. Eur J Biochem 79, 11–21 (1977). The ganglioside nomenclature of Svennerholm (1963) is employed throughout, except that GM1b, GM1b-GalNAc, GM1b-GalNAc-Gal, GD1c and GD1α denote IV^{3}NeuAcα-Gg_4Cer, IV^{4}GalNAcβ,IV^{3}NeuAcα-Gg_4Cer, IV^4(Galβ1-3GalNAcβ), IV^{3}NeuAcα-Gg_4Cer, IV^{3}NeuAcα_2-Gg_4Cer and III^{6}NeuAcα, IV^{3}NeuAcα-Gg_4Cer, respectively.**

Introduction

The thymus is an organ in which immature thymocytes derived from bone marrow proliferate and differentiate into functional T-lymphocytes through an interaction with the thymic microenvironment. The process of differentiation of thymocytes is characterized by the expression of cell surface-specific antigens, among which are glycolipids that are differentiation antigens in mouse immunocytes, *i.e.* GM1 and GM1b-GalNAc-Gal in thymocytes [2], GM1b-GalNAc in ConA-stimulated thymocytes [3], GD1c, GD1α and Gb_4Cer in T lymphocytes [4–6], and Gg_4Cer in NK cells and immature thymocytes [7,8]. The differentiation-related glycoconjugates on immunocytes not only act as cell surface markers, but also induce several immune reactions, such as blastogenesis by autologous allogenic responder cells [9], regulation of T cell antigenicity [10], and production of autoantibodies toward lymphocytes in NZB

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mice with the autoimmune status [11]. However, the structures of glycoconjugates in mouse immunocytes are species-specific and are not detected in other animal species [12], indicating that individual immune reactions can not be ascribed to specific carbohydrate structures. For example, although the major glycolipids in mouse thymocytes are of the ganglio-series, ones consisting of lacto-series type 2 chains are preferentially present in rabbit thymocytes, whose ganglioside composition provides a thymus-characteristic profile among several rabbit tissues [13,14]. Also, the sialyl Lewis x structure is expressed on human lymphocytes, and functions as a receptor for the cellular adhesion molecule ELAM-1 [15] for the immune reaction during inflammation, as well as lymphocyte development [16]. The interaction of the carbohydrates in mouse and rabbit lymphocytes with ligands, like ELAM-1, is thought to involve an alternative structure to the sialyl Lewis x one in humans. Although information on the carbohydrate structures of mouse immunocytes has accumulated through comparative analysis with several, carbohydrate-specific probes such as cholera toxin and monoclonal antibodies in combination with well-characterized antigens, such as Thy-1, T-cell receptor (TCR), IL-2 receptor, CD4 and CD8, as described above, the details of the glycolipid compositions of individual immunocytes are not clearly understood yet. Accordingly, the present study aimed at characterization of the glycolipid compositions in steroid-sensitive and steroid-resistant thymocytes in mice after the administration of dexamethasone, an inducer of apoptosis of steroid-sensitive immunocytes, to determine the functional significance of carbohydrates in the mouse immune system.

Materials and methods

Materials

Glycolipids were purified from various sources in our laboratory: GalCer and GM1 from bovine brain, GlcCer, LacCer, Gb_3Cer, Gb_4Cer and GM3 from human erythrocytes, Forssman glycolipid from equine kidney, Gg_3Cer from guinea pig erythrocytes, and GM1b from rat ascites hepatoma cells. Gg_4Cer was prepared by treatment with *Arthrobactor ureafaciens* sialidase [17], and *N*-stearoyl derivatives of GlcCer, LacCer, Gb_3Cer and Gg_3Cer, as standards for quantitation of the respective glycolipids by thin layer chromatography (TLC), were synthesized by deacylation of the purified glycolipids with sphingolipid ceramide *N*-deacylase (*Pseudomonas sp*. TK4), followed by reacylation with stearoyl chloride.GM1b-GalNAc and GM1b-GalNAc-Gal were kindly donated by Dr. K. Horikawa K, The University of Kumamoto, Kumamoto, and Dr. A, Suzuki, Riken, Wako, respectively.

Carbohydrate-specific antibodies

Rabbit polyclonal antibodies toward GM1, Gg_4Cer and Gg_3Cer were generated by immunizing rabbits intradermally with an emulsion prepared with 1 mg of the respective purified glycolipid and 0.5 mg of bovine serum albumin (BSA) in 1 ml of phosphate-buffered saline (PBS), and 1 ml of Freund's complete adjuvant (Sigma, St. Louis, MO, USA), with subsequent monitoring of the antibody titer by means of an enzyme-linked immunosorbent assay, the titers being 1: 100,000 for anti-GM1, 1: 600,000 for anti-Gg_4Cer, and 1: 300,000 for anti-Gg_3Cer antibodies, respectively. No cross reaction with structurally related glycolipid derivatives of individual antigens was observed [18]. Monoclonal antibodies against GM2 (NeuGc) (Pyk-2), and GM2 plus GM1-GalNAc (YHD-06) were provided by Dr. M. Yamasaki, Konica Co., Tokyo [3]. Monoclonal anti-GM3 (M2590) antibody was obtained from Seikagaku Co., Tokyo.

Analysis of glycolipids

Mice (Balb/c, HR-1, female, 5 weeks old) were bred in our animal laboratory, and housed at 25 $\pm$ 1°C and 65–75% humidity. The animal care and experimental protocols were in accord with the guidelines of Kinki University. Dexamethasone (Wako Chemicals, Tokyo) (2.5 mg in 100 μl of PBS) was injected intraperitoneally into mice, and then their thymuses were homogenized in water with a Polytron homogenizer. After lyophilization, total lipids were extracted from the lyophilized powder with chloroform/methanol/water (20:10:1, 10:20:1 and 1:1, by vol.), and then the concentrations of cholesterol and lipid-bound phosphorus in the total lipid extracts were determined by gas liquid chromatography with 5α-cholestane as an internal standard and Bartlett's method, respectively [18]. Then, the lipid extracts were fractionated into neutral and acidic lipids on a DEAE-Sephadex column (A-25, acetate form; Pharmacia, Uppsala, Sweden). The neutral glycolipids were separated from unabsorbed neutral lipid fractions by acetylation, separation of the acetylated derivatives, deacetylation and desalting, whereas the gangliosides were prepared from the absorbed acidic lipid fractions by cleavage of the ester-containing lipids, followed by dialysis. The gangliosides and neutral glycolipids thus obtained were developed on TLC plates with chloroform/methanol/0.5% $CaCl_2$ in water (55:45:10, by vol.) and chloroform/methanol/water (65:35:8, by vol.), and then visualized with resorcinol-HCl and orcinol-H_2SO_4 reagents, respectively. The density of spots was determined at analytical wavelengths of 580 nm for resorcinol-HCl-positive spots and 420 nm for orcinol-H_2SO_4-positive spots, respectively, using a dual-wavelength TLC densitometer (CS-9000; Shimadzu, Kyoto). Standard curves were essentially obtained by using the same glycolipids as those detected in the thymuses: 0.1 to 1.5 μg of GalCer (18:0), LacCer (18:0), Gb_3Cer (18:0), Gg_3Cer, Gb_4Cer, Gg_4Cer, GM3 and GM1, on the same plate. The molecular weights of glycolipids were calculated as ones with N-stearoyl sphingosine.

TLC-immunostaining

Total lipid extracts, gangliosides and neutral glycolipids from the thymuses were applied on plastic-coated TLC plates, which were then developed successively with *n*-hexane/diethyl ether

(4:1, by vol.) and chloroform/methanol/0.5% $CaCl_2$ in water (55:45:10, by vol.). Each plate was incubated with a blocking buffer (1% polyvinylpyrrolidone (PVP) and 1% ovalbumin in PBS) at 4°C overnight and then with anti-carbohydrate antibodies in 3% PVP in PBS at 37°C for 2 h. Mouse monoclonal antibodies were used at the concentration of approximately 0.2 μg/ml, and rabbit antisera were usually diluted to 1: 500 (by vol.). Afterward, the plates were washed 5 times with 0.1% Tween 20 in PBS, and the antibodies bound to the TLC plates were detected using peroxidase-conjugated anti-rabbit IgG + M or anti-mouse IgG + M antibodies (Cappel Laboratories, Cochranville, PA), diluted 1: 1000 (by vol.) with 3% PVP in PBS, and with enzyme substrates H_2O_2 and 4-chloro-1-naphthol, as described previously [2,3]. The density of spots was also determined using 10 to 100 ng of the respective glycolipids as standards for quantitation with a TLC-densitometer as described above, the limit of detection being 5 ng of glycolipids.

Radiolabeling with CMP-[^{14}C]-NeuAc

Thymuses from control mice and mice 48 h after the administration of dexamethasone were put in dishes with 2 ml of RPMI 1640 medium containing 5% fetal calf serum and CMP-[^{14}C]-NeuAc (150 Ci/mol; Amersham), and then the dishes were incubated at 37°C for 16 h. After washing the tissues twice with the medium, lipids were extracted from the tissues, fractionated into gangliosides as above and then developed on a TLC plate with chloroform/methanol/0.5% $CaCl_2$ (55:45:10, by vol.). The radioactivity incorporated into gangliosides was determined with an Image Analyzer (BAS 2000; Fuji Film, Tokyo) and a liquid scintillation counter (Tri-Carb 1500; Packard).

LacCer sialyltransferase and sialidase

Thymuses from control mice and mice 48 h after the administration of dexamethasone were homogenized in 0.25 M sucrose with a Potter-Elvehjm homogenizer to prepare 10% (w/v) homogenates, which were then centrifuged at 1000 × *g* for 10 min at 4°C to remove cell debris, followed by centrifugation at 100,000×*g* for 60 min to obtain cytosol and microsomal fractions. The microsomal fraction was suspended in 0.25 M sucrose by sonication. The protein concentrations in the cytosol and microsomal fractions were measured by the protein dye binding method with BSA as the standard [19].

Microsomal LacCer sialyltransferase was determined using LacCer and CMP-[^{14}C]-NeuAc as the substrates. The standard assay mixture comprised 38 nmol of LacCer, 100 μg of Triton CF 54/Tween 80 (2:1, by vol), 0.5 μmol of $MgCl_2$, 0.37 μM CMP-[^{14}C]-NeuAc (150 Ci/mol), 50 mM cacodylate-HCl buffer (pH 6.5), and microsomes (0.4 mg protein), in a final volume of 100 μl. After incubation at 37°C for 2 h, the reaction was terminated with 400 μl of 0.1 M KCl containing 0.05 M EDTA and 24 μg of egg lecithin (Wako Chemicals, Osaka), and then the solution was applied to a C18-SepPack cartridge (Waters, Milford, MA). The cartridge was washed with 10 ml each of 0.1 M KCl and distilled water, and then the radiolabeled products were recovered with 5 ml of methanol and 10 ml of chloroform-methanol (1:1, by vol.). The products were separated by TLC with chloroform/methanol/0.5% $CaCl_2$ in water (55:45:10, by vol.), and then the radioactivity incorporated into GM3 was determined as described above.

Cytosolic sialidase activity was determined using 4-methylumbelliferyl NeuAc as the substrate [20]. The standard assay mixture comprised 0.6 mM 4-methylumbelliferyl NeuAc, 25 mM cacodylate-HCl buffer, pH 6.5, and cytosol (0.1–0.4 mg protein), in a total volume of 200 μl. After incubation at 37°C for 2 h, 4-methyl umbelliferone produced was determined spectrofluorometrically with excitation and emission wavelengths of 365 and 449 nm, respectively.

RT-PCR

Total RNA was extracted from thymuses by the acid guanidine, thiocyanate-phenol-chloroform method, transcribed to cDNA with reverse transcriptase (ReverTra Ace; TOYOBO, Osaka), and then subjected to PCR with KOD-Plus DNA polymerase (TOYOBO) under the following conditions: LacCer sialyltransferase (GenBank Accession Number AF119416), sense primer, 5′-ttgaggacaggtacagcatc-3′, antisense primer, 5′-gggacttttctgccacttg-3′; GM3 GalNAc transferase (GenBank Accession number L25885), sense primer, 5′-aggggaagtaacaggagtga-3′, antisense primer, 5′-gaatgagtgtccgtagtcga-3′; 35 cycles of 94°C for 15 s, 55°C for 30 sec and 68°C for 1 min. The primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as a control. The resulting PCR products were electrophorased on a 1.2% agarose gel, stained with ethidium bromide, and then examined under a UV transilluminator.

Results

Change in the glycolipid composition in the thymuses of mice on administration of dexamethasone

Thymocytes exposed to dexamethasone are known to undergo apoptosis, resulting in the disappearance of steroid-sensitive thymocytes from the thymus. In fact, the tissue weight of the thymus abruptly decreased from 12 h after the administration of dexamethasone and attained the minimum weight at 18 h (Figure 1). On comparison of sections of the thymus between control mice and mice 48 h after the administration of dexamethasone, the cortical layer was found to become thicker with dexamethasone, indicating that the immature thymocytes in the cortex are highly sensitive to dexamethasone (Figure 2). In accord with the reduced tissue weight, the marker glycolipids for thymocyte differentiation, *i.e.* GM1 and Gg_4Cer, were decreased in amount per thymus. As shown in Figure 3A–D, although the amounts of Gg_3Cer, Gb_4Cer and Forssman glycolipid in the thymuses of dexamethasone-administered mice were similar to those in the

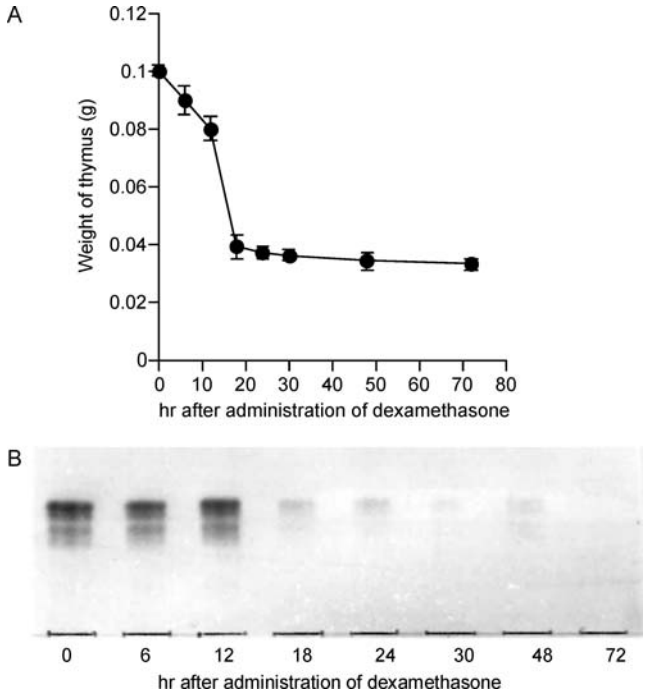

Figure 1. Changes in the tissue weight of the thymus (A) and the amount of GM1 per thymus (B) in mice after administration of dexamethasone. Detection of GM1 was carried out by TLC-immunostaining with anti-GM1 antibodies as described in the text.

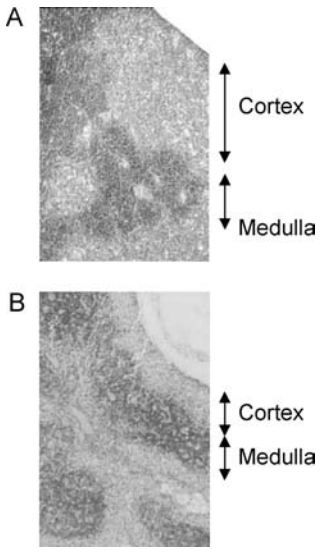

Figure 2. Hematoxylin-eosin staining of the thymuses of a control mouse (A) and a mouse 48 h after administration of dexamethasone (B).

controls, ceramide monohexoside (mainly GlcCer) and Gg_4Cer in the thymuses of dexamethasone-administered mice were greatly decreased, compared to those in the controls. A similar decrease in the amount as that for Gg_4Cer was observed for GM1 (Figures 1B and 3F), indicating that both GM1 and Gg_4Cer are present in immature thymocytes susceptible to dexamethasone. In contrast to the findings that the amounts of ganglio- and globo-series glycolipids in the thymuses of dexamethasone-administered mice were decreased and did not change, respectively, that of GM3 was significantly increased in the thymuses of dexamethasone-administered mice, as shown in Figure 3E. The increase in the amount of GM3 exhibited an inverse relationship to the decrease in the tissue weight, and was observed from 18 h after the administration of dexamethasone.

The amounts of glycolipids in the thymuses of control mice and mice 48 h after the administration of dexamethasone are given in Table 1. The rates of the decreases in the amounts of cholesterol and total phospholipids on the administration of dexamethasone were one-third of those in the controls and were consistent with that in the tissue weight, and their concentrations per mg of thymus were not significantly different between before and after the administration of dexamethasone (Table 1). In the thymuses of control mice, the total amount of neutral glycolipids was16 times those of gangliosides, and ceramide monohexosides comprised 59 molar percentage of the total glycolipids. On the administration of dexamethasone, ceramide monohexoside, Gg_4Cer, GM1, GM1b, GM1b-GalNAc, and GM1b-GalNAc-Gal decreased to one-third or one-fourth of the control levels, while the decreases in their concentrations per mg of thymus were moderate, suggesting their distribution in both steroid-sensitive and resistant thymocytes. On the other hand, no change in the amount was observed for Gb_4Cer or Forssman glycolipid, whose concentrations per mg of thymus in dexamethasone-administered mice were three-fold of those in control mice, paralleling the decrease in the tissue weight, indicating their preferential distribution in dexamethasone-resistant cells including the thymus matrix structure. Whereas GM3 was found to increase on the administration of dexamethasone, being 4-fold in amount and 13-fold in concentration, in comparison to those in the controls. Consequently, although GM3 in the thymuses of control mice comprised 15% of the total gangliosides, in the dexamethasone-administered mice it comprised 71%.

Change in the activity of LacCer sialyltransferase on administration of dexamethasone

As shown in Table 2, although cytosolic sialidase, with 4-methylumbelliferyl NeuAc as the substrate, in the thymuses of dexamethasone-administered mice exhibited lower specific activity than in control mice, the specific activity of LacCer sialyltransferase in the microsomal fraction of dexamethasone-administered mice was significantly higher than that in the controls. In addition, incorporation of radioactivity into GM3 on incubation of thymus tissues with CMP-[^{14}C]-NeuAc was preferentially observed in the thymuses of dexamethasone-administered mice, but not in control mice, although the incorporation of radioactivity into polar gangliosides was the same in control and dexamethasone-administered mice (Figure 4).

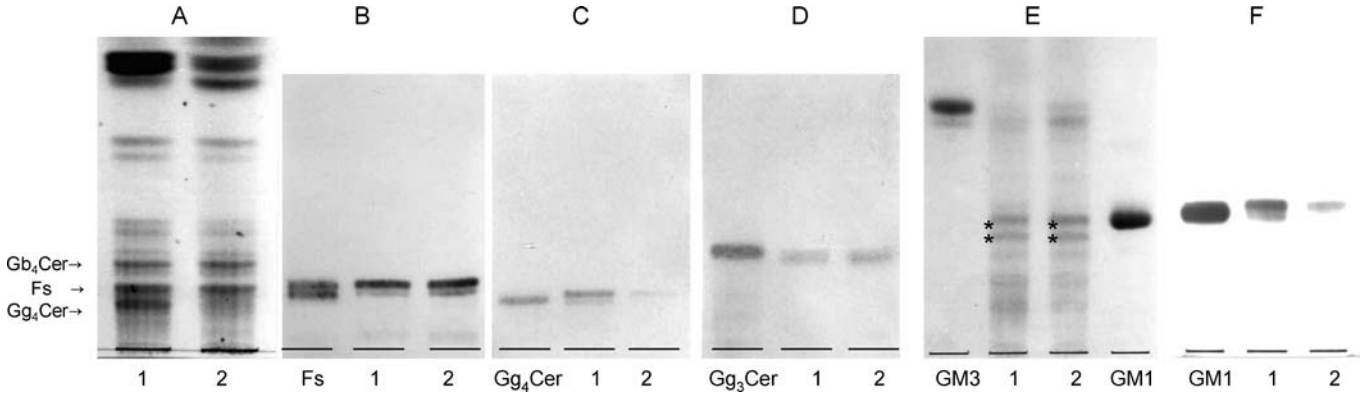

Figure 3. TLC of neutral glycolipids (A–D) and gangliosides (E, F) from the thymuses of control mice (1) and mice 48 h after administration of dexamethasone (2). The glycolipids, corresponding to 10% of the lipids for A and E, and 2% of the lipids for B, C, D and F, were developed on TLC plates with chloroform/methanol/0.5% $CaCl_2$ in water (55:45:10, by vol.), and were detected with orcinol-H_2SO_4 (A), anti-Forssman (Fs) antibody (B), anti-Gg_4Cer antibody (C), anti-Gg_3Cer antibody (D), resorcinol-HCl (E), and anti-GM1 antibody (F). The spots with asterisks in Figure 3E were not resorcinol-positive. Fs, Forssman glycolipid.

Because the plasma membrane is impermeable to CMP-NeuAc, one can suggest that a plasma membrane-associated sialyltransferase is involved in the synthesis of GM3 in the thymuses of dexamethasone-administered mice. Also, the increase in the specific activity of LacCer sialyltransferase in the thymuses of dexamethasone-administered mice was found to be transcriptionally regulated (Figure 5), the LacCer sialyltransferase gene (GM3 synthase gene) being expressed at relatively higher levels in the dexamethasone-administered mice than in the control mice, no difference being observed in the expression of the GM3: GalNAc transferase gene (GM2 synthase gene).

Discussion

As clearly shown in this paper, globo- and ganglio-series glycolipids were distributed in different regions of the thymus, being in the dexamethasone-resistant thymic structure and dexamethasone-sensitive cortical cells, respectively, and this distinct mode of distribution of glycolipids was identical with that in the gastrointestinal tissues of mice [21]. This distribution of glycolipids belonging to the globo- and ganglio-series seemed to be a common property in several tissues of mice. Forssman glycolipid, as the end structure of globo-series glycolipids, was principally found in the stromal tissues and did

Table 1. Concentrations of glycosphingolipids in the thymuses of control mice and mice 48 h after administration with dexamethasone

	Control mice		*Dexamethasone-administered mice*	
	(mg)		(mg)	
Weight of thymus	100.0 ± 5.8		34.1 ± 3.2	
	(μmol/thymus)	(nmol/mg of thymus)	(μmol/thymus)	(nmol/mg of thymus)
Cholesterol	0.3 ± 0.0	3.0	0.1 ± 0.1	3.0
Phospholipids	1.3 ± 0.1	13.0	0.5 ± 0.1	15.0
	(nmol/thymus)	(nmol/mg of thymus)	(nmol/thymus)	(nmol/mg of thymus)
GalCer, GlcCer	20.0 ± 0.5	0.2	5.1 ± 0.2	0.15
LacCer	1.3 ± 0.1	0.01	1.0 ± 0.1	0.03
Gb_3Cer	0.5 ± 0.1	0.01	0.4 ± 0.1	0.01
Gg_3Cer	0.6 ± 0.0	0.01	0.7 ± 0.0	0.02
Gb_4Cer	2.3 ± 0.2	0.02	2.1 ± 0.2	0.06
Gg_4Cer	4.0 ± 0.1	0.04	0.9 ± 0.1	0.03
Forssman	3.1 ± 0.0	0.03	3.1 ± 0.1	0.09
GM3	0.3 ± 0.0	0.003	1.2 ± 0.1	0.04
GM1	0.6 ± 0.1	0.006	0.2 ± 0.0	0.01
GM1b	0.4 ± 0.1	0.004	0.1 ± 0.0	0.003
GM1b-GalNAc	0.3 ± 0.1	0.003	0.1 ± 0.0	0.003
GM1b-GalNAc-Gal	0.4 ± 0.0	0.004	0.1 ± 0.0	0.003

Mean values for five tissues are presented.

Table 2. Activities of LacCer sialyltransferase and sialidase in the thymuses of control mice and dexamethasone-administered mice. The cytosol and microsomal fractions were used for determination of sialidase with 4-methylumbelliferyl NeuAc and LacCer sialyltransferase with CMP-^{14}C-NeuAc, respectively

	Control mice	*Dexamethasone-administered mice*
	(pmol/mg protein/h)	
LacCer sialyltransferase	0.8 ± 0.1	2.7 ± 0.1
Sialidase	36.6 ± 0.4	7.2 ± 0.5

Mean values for three separate experiments are presented.

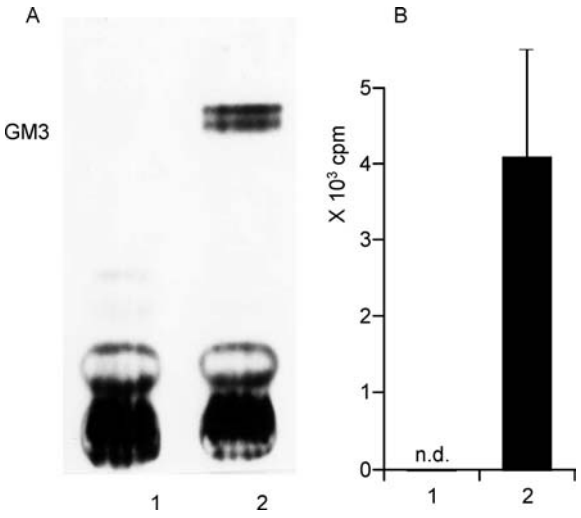

Figure 4. Autoradiogram (A) of gangliosides from thymus tissues labeled with CMP-[^{14}C]-NeuAc from control mice (1) and dexamethasone-administered mice (2), and radioactivity incorporated into GM3 (B). n.d., not detected.

not show a significant change in its relative concentration during development, while modification of the carbohydrate structures of ganglio-series glycolipids or regulation of their expression occurred during acquirement of the cellular function in the epithelium or the upper region of the stromal tissues [21], suggesting that comparison of ganglio-series glycolipids on the basis of the content of Forssman glycolipid is useful for estimating the functional establishment of mouse tissues. Our results also indicated that Forssman glycolipid in the mouse thymus might be distributed in the thymic matrix, which is not affected by dexamethasone. While the ganglio-series glycolipids, which are widely distributed in several tissues of mouse, are also constituents in thymocytes.

In the previous studies, the expression of ganglio-series glycolipids in thymocytes, particularly, in the cortex of the thymus, was revealed to be regulated during the differentiation of thymocytes in experiments involving cholera toxin with affinity to GM1 and anti-Gg_4Cer antibodies [2,8]. During ontogenesis, the anti-Gg_4Cer positive cells were thymocytes without the

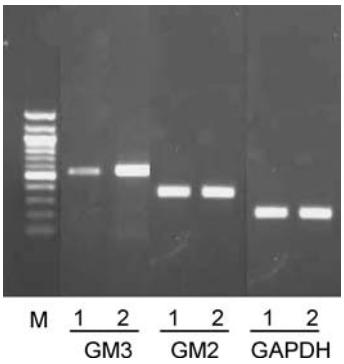

Figure 5. Expression of the LacCer: sialyltransferase (GM3 synthase), GM3: GalNAc transferase (GM2 synthase), and GAPDH genes. The PCR products obtained under the conditions given in the text were electrophoresed on a 1.2% agarose gel, stained with ethidium bromide, and then examined under a UV transilluminator. M, DNA size markers; 1, control mice; 2, mice 48 h after administration of dexamethasone.

Thy-1 antigen, and the cellular population decreased in association with the increasing population of Thy-1-positive cells, indicating that the expression of Gg_4Cer is a marker of immature thymocytes [8]. Also, GM1 was expressed on 62% of thymocytes without the CD4 and CD8 antigens as early as at 13 days of gestation and on 95% of thymocytes with the Thy-1, CD4 and CD8 antigens after 15 days of gestation, indicating that the expression of GM1 changes during the differentiation of mature thymocytes with proteinous antigens [2]. The intensity of the staining with cholera toxin of GM1, and the concomitant expression of Thy-1, TCR, IL-1 receptor, CD4 and CD8 on thymocytes should be useful for differentiating the thymocyte subpopulations [2]. As shown in Table 1, both Gg_4Cer and GM1 were actually present in cortical thymocytes, and the amount of Gg_4Cer in the cells was 7 times that of GM1, but Gg_4Cer was nevertheless unable to react with its antibody. In accord with the previous observations [2,8], the majority of thymocytes at 5 weeks of age were positive with cholera toxin, but not with anti-Gg_4Cer antibodies, regardless of their presence in the thymocytes, as judged on flow cytometric analysis (Iwamori *et al.*, unpublished observations), indicating that Gg_4Cer is cryptic toward its antibody. However, modified structures of Gg_4Cer, such as GM1b, GD1c and GD1α, might become reactive with their antibodies to provide cellular antigens [4–6].

On the other hand, dexamethasone-induced accumulation of GM3 was thought to be critical for the apoptotic death of thymocytes. Although mice with targeted deletion of LacCer sialyltransferase survived for more than one year and did not exhibit a clear change in behavior [22], mice with targeted deletions of GM3: GalNAc transferase (GM2 synthase) and GM3 sialyltransferase (GD3 synthase) all died by 15 weeks of age, due to accumulation of GM3 as a sole ganglioside [23]. Also, the cause of death of patients suffering from GM3 gangliosidosis in the infantile period is probably the accumulation of GM3 [24]. In this connection, a rapid increase in GM3 on enhanced

transcription of LacCer sialyltransferase and a concomitant decrease in sialidase seemed to be involved in the apoptosis of thymocytes, depending on the expression of steroid receptors on the cells. However, the site of GM3 synthesis in the thymus after the administration of dexamethasone remains obscure. As shown in Figure 4, the synthesis of GM3 on incubation of thymic tissues with CMP-[^{14}C]-NeuAc was only detected in the tissues of dexamethasone-administered mice, *i.e*. not in those of control mice. Since sugar nucleotides are known not to pass through the plasma membrane, and thus require carrier proteins [25], one can suggest that the activity detected in Figure 4 is due to a plasma membrane-associated enzyme, like the surface-associated fucosyltransferase, acting as a carbohydrate ligand for cell-to-cell adhesion [26]. Analysis of the distribution of sialyltransferase in the thymuses of dexamethasone-administered mice is required to clarify the possible involvement of GM3 in the apoptotic death of dexamethasone-sensitive thymocytes.

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