Glycosphingolipid composition of rat placenta: changes associated with stage of pregnancy

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The composition of glycolipids and their changes in the placenta were investigated in the normal pregnant rat. Total lipid fractions extracted from the placenta between days 12 and 20 of pregnancy (day 0 = oestrus) were subjected to glycolipid analysis using DEAE-Sephadex chromatography, silica-gel HPLC, silica-gel TLC, TLC/immunostaining, matrix-assisted secondary-ion mass spectrometry in the negative-ion mode and ^{1}H NMR. Glycolipids identified in the rat placenta were: gangliosides G_{M3} (NeuAcLacCer and NeuGcLacCer) and G_{D3} (NeuAcNeuAcLacCer, NeuAcNeuGcLacCer and NeuGcNeuAcLacCer), and neutral glycolipids ceramide monosaccharide (CMH) (GlcCer), ceramide disaccharide (CDH) (LacCer), ceramide trisaccharide (CTH) (Gb_3Cer) and ceramide tetrasaccharide (CQH) (Gb_4Cer). The content of neutral glycolipids was higher than that of gangliosides throughout pregnancy. Of the neutral glycolipids, CMH and CTH predominated and the level of CDH was low at mid-pregnancy. During late pregnancy, CMH and CTH decreased and CDH increased markedly. CQH remained at a low level throughout pregnancy. Of the gangliosides, G_{M3} was predominant on days 12–16 and then decreased, whereas G_{D3}, which was low on day 12, increased slightly on day 16 and maintained the same level thereafter. Immunohistochemical studies indicated that these changes in the expression of major gangliosides from G_{M3} to G_{D3} occurred in labyrinthine trophoblasts. Thus expression of these glycolipids appears to change markedly during pregnancy.

INTRODUCTION

The placenta plays a central role in the maintenance of pregnancy and has various functions including nutrient metabolism, and the control of immunological and endocrinological events, which occur at different stages of pregnancy and fetal development [2]. We have studied the regulation of placental function at different stages of pregnancy, and found it to be controlled by mechanisms involving changes in the maternal endocrine milieu [3,4]. The placenta consists of various cell types including trophoblasts and decidual cells, the proportions of which vary according to the stage of pregnancy [5].

Glycosphingolipids are ubiquitous membrane components of cells, and gangliosides are postulated to be instrumental in regulating membrane physiology. Gangliosides modify certain intrinsic ATP-utilizing plasma-membrane proteins, e.g. protein kinases, epidermal growth factor receptors and $(Na^+ + K^+)$-ATPase [6]. G_{M3} and its catabolic derivatives are reported to be potent modulators of growth-factor receptors and integrin receptors [7–10]. Expression of several growth factors such as epidermal growth factor, basic fibroblast growth factor and transforming growth factor β and relevant receptors in the placenta suggests their biological role in pregnancy [11].

Expression of the stage-specific functions of the placenta is also related to the differentiation of trophoblasts and changes in the cell composition of rat placenta [5]. G_{D2} and G_{D3} have been implicated in the mechanism of cell differentiation through adhesion between cells, or between cells and extracellular matrix proteins [12]. Thus investigation of glycolipids in the placenta is important in this context. The objective of the present study was to determine the major glycolipid components of the placenta and investigate their changes between mid- and late pregnancy in the rat.

MATERIALS AND METHODS

Materials

DEAE-Sephadex A-25 was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden), Florisil (100–200 mesh) from Wako Pure Chemical (Osaka, Japan), DEAE-Toyopearl 650 M (TSK gel) from Tosoh Co. (Tokyo, Japan), Iatrobeads (6RS8060) from Iatron Laboratories (Tokyo, Japan) and high-performance TLC plates (HPTLC; silica gel 60) from E. Merck (Darmstadt, Germany). The enzyme used was neuraminidase (EC 3.2.1.18) from *Arthrobacter ureafaciens* (Nacalai Tesque, Kyoto, Japan). Poly-L-lysine solution was purchased from Sigma Diagnostics (St. Louis, MO, U.S.A.). All other reagents were of ultrapure grade from commercial sources.

Ganglioside standards, G_{M1}, G_{M3} and G_{D1a} (bovine brain), were purchased from Bachem Inc. (Torrance, CA, U.S.A.) and G_{D3} (bovine milk) was purchased from Wako Pure Chemical.

A mouse monoclonal antibody, MECTOLONE-015 (IgM isotype) reactive with G_{D3} (NeuAc-NeuAc-) and moderately cross-reactive with G_{M3} (NeuAc), was a gift from Mect Co. (Tokyo, Japan). A mouse monoclonal antibody, R24, which was produced by immunization with SK-MEL-28 melanoma cells and is reactive with G_{D3} (NeuAc-NeuAc- and NeuAc-NeuGc-) gangliosides [13–15], was purchased from Signet Laboratories (Dedham, MA, U.S.A.). GMR3 monoclonal antibody, which is reactive with NeuGcα2-8NeuGcα2-3Gal- terminal sequences including G_{D3} (NeuGc-NeuGc-) ganglioside [16], was a gift from

Abbreviations used: the nomenclature used for gangliosides is based on the system of Svennerholm [1]; NeuGc, glycollylneuraminic acid; Cer, ceramide; CMH, ceramide monosaccharide; CDH, ceramide disaccharide; CTH, ceramide trisaccharide; CQH, ceramide tetrasaccharide; HPTLC, high-performance TLC; SIMS, matrix-assisted secondary-ion mass spectrometry.

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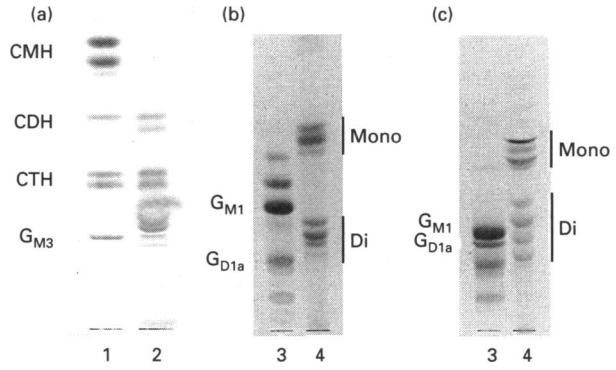

Figure 1 Thin-layer chromatogram of gangliosides and neutral glycosphingolipids isolated from rat placenta

(**a**) Lane 1, mixture of standard neutral glycosphingolipids (pig erythrocyte); lane 2, neutral glycosphingolipid fraction from rat placenta. (**b** and **c**) Lane 3, mixture of standard gangliosides (bovine brain); lane 4, ganglioside fraction from rat placenta. The separation was performed on an HPTLC glass plate developed in chloroform/methanol/0.5% $CaCl_2$ (60:35:8, by vol.) (**a**), (55:45:10, by vol.) (**b**), chloroform/methanol/2.5 M NH_4OH (60:35:8, by vol.) (**c**), and spots were visualized with orcinol/H_2SO_4 (**a**) and resorcinol/HCl (**b** and **c**) reagents.

Dr. T. Tai (Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan).

Animals

Adult female Wistar–Imamichi rats (8 weeks old) were purchased from Imamichi Institute for Animal Reproduction (Ibaragi, Japan). They were kept under a lighting schedule of 14 h light/10 h dark (lights on 05:00–19:00 h) and fed with commercial rat chow and tap water *ad libitum*. Female rats that had shown three consecutive 4-day cycles were housed with a fertile male on the evening of pro-oestrus. The first day on which a leucocytic vaginal smear was observed (the day after confirmation of sperm in the vagina) was designated day 1 of pregnancy. Placental tissues were collected from days 12 to 20 as follows. The antimesometrial wall of the uterus was cut and the decidua was shelled out of the uterus with forceps. After removal of the embryos with forceps, the remaining tissues (decidual cells and trophoblasts) were frozen quickly and stored at −80 °C until extraction of glycolipids.

Extraction of glycolipids from rat placental tissues

Each frozen sample (3–35 g wet weight) was homogenized with an ULTRA-TURRAX T25 blender (Ika-Labortechnik, Staufen, Germany). Crude glycolipids were extracted twice from the homogenized tissues successively with chloroform/methanol/water (1:1:0.2, by vol.) and propan-2-ol/hexane/water (55:25:20, by vol.). The combined extract was then evaporated to dryness.

Separation of gangliosides and neutral glycolipids

The ganglioside fraction was separated as reported previously [17] with modifications. Briefly, the total lipid extract obtained above was dissolved in solvent A [chloroform/methanol/water (30:60:8, by vol.)] and applied to a DEAE-Sephadex A-25 column. The column was washed successively with solvent A and methanol, and the ganglioside fraction was eluted with solvent B (0.45 M ammonium acetate in methanol) and then dialysed against water. After incubation of the fraction with pyridine/acetic anhydride (3:2, v/v) at room temperature overnight, it was applied to a Florisil column [18], and acetylated gangliosides were eluted with dichloroethane/methanol (9:1, v/v) and dichloroethane/acetone (1:1, v/v). The eluate was deacetylated at 4 °C with 0.5 M NaOH in methanol for 1 h and neutralized with 0.5 vol. of 0.5 M acetic acid in methanol. The gangliosides were applied to a DEAE-Toyopearl column (CIG TB-TBA-23000; 22 mm internal diameter × 30 cm; Kusano Kagakukikaki Co., Tokyo, Japan) equipped for HPTLC with a Waters 600 HPLC system (Millipore; Milford, MA, U.S.A.). The neutral fraction was eluted successively with solvent A and methanol, and the gangliosides with a linear gradient of methanol/solvent B (100%).

The elution pattern was monitored by HPLC developed with solvent C [chloroform/methanol/0.5% $CaCl_2$ (55:45:10, by vol.)]. Glycolipids were visualized with orcinol/H_2SO_4 reagent and gangliosides were visualized with resorcinol/HCl reagent.

Further purification of individual gangliosides was accomplished by HPLC using a silica-gel column (SSC-Aquasil-SS-352N; 4.6 mm internal diameter × 25 cm) (Senshu Scientific Co., Tokyo, Japan) and eluted with solvent D (chloroform/methanol/2.5 M NH_4OH). Separation was performed by programmed linear gradient elution first with a solvent mixture of solvent D (80:20:2, by vol.) and second a solvent mixture of solvent D (50:40:5, by vol.). The total solvent elution volume was 40 ml, and 0.5 ml fractions were collected. The elution pattern was monitored by TLC developed with solvent C.

Enzyme treatment

The ganglioside fraction separated by DEAE-Sephadex column chromatography was treated with neuraminidase (*A. ureafaciens*) by previously reported methods [17]. The evaporated fraction (10–20 ng) was added to the reaction mixture [0.1 unit of the enzyme in 120 μl of 0.01 M phosphate buffer (pH 7.3) and 150 μg of sodium taurodeoxycholate] and incubated at 37 °C for 16 h.

Negative-ion matrix-assisted secondary-ion mass spectrometry (SIMS)

SIMS of the gangliosides was performed in the negative-ion mode using a TSQ-700 triple-stage quadrupole mass spectrometer (Finnigan MAT). Triethanolamine was used as the matrix. The primary beam for the bombardment was 15.0 keV caesium.

^{1}H-NMR spectroscopy

NMR spectra were obtained with a JEOL JNM-GX270 spectrometer (JEOL, Tokyo, Japan) at 60 °C. Sample (100 μg) was dissolved in 0.4 ml of [^{2}H]dimethyl sulphoxide containing 2% ^2H_2O, and t-butyl alcohol was used as the internal standard for chemical shift.

TLC/immunostaining

Enzyme immunostaining of gangliosides on TLC plates was performed as previously described [19] with slight modification. Briefly, aliquots of gangliosides were spotted on a plastic TLC plate (Polygram SIL G; Macherey-Nagel, Postfach, Germany) and developed with solvent D (55:45:10, by vol.). The plate was dried and soaked in blocking solution (Block Ace; Snow Brand, Tokyo, Japan). MECTOLONE-015 monoclonal antibody was applied to the chromatogram, which was then kept at room temperature with shaking for 2 h. Then the chromatogram was soaked in blocking solution for 15 min and re-incubated with horseradish peroxidase-conjugated goat anti-mouse immuno-

globulin (Cappel, Cochranville, PA, U.S.A.) for 2 h. After several successive washes with PBS, the chromatogram was exposed to the substrate solution (4-chloro-1-naphthol and H_2O_2).

Quantitative study of glycolipids

Changes in the amount of glycolipids in rat placenta were investigated during pregnancy. Some 10–12 placental tissue samples were collected from each pregnant rat on days 12, 14, 16, 18 and 20, and the pooled samples were kept frozen until extraction of the glycolipid. Glycolipid of each frozen placenta (1.0–1.2 g wet weight) from an individual dam ($n = 3$) was extracted as described above. After separation on DEAE-Sephadex A-25, an aliquot of these glycolipid fractions was examined by HPTLC with solvent A (60:35:8, by vol.) (neutral glycolipid) and solvent C (55:45:10, by vol.) (ganglioside). These glycolipids were visualized by spraying with orcinol/H_2SO_4 reagent and then scanned with a fluorescence analysis system (Toyobo, Osaka, Japan). GlcCer (neutral glycolipid) and G_{M3} (ganglioside) were used as standards. Data were expressed as pmol/mg wet weight of tissue.

Immunohistochemistry

The distribution of gangliosides in frozen sections of rat placenta was determined by immunofluorescence [20]. Serial sections (10 μm thick) were cut with a cryostat microtome and thaw-mounted on poly-L-lysine-coated glass slides. The mounted sections were air-dried for 2 h and fixed with acetone at −20 °C for 10 min. The sections were washed with PBS three times at room temperature for 10 min and then incubated with 3% BSA in PBS for 30 min at room temperature. After one wash with PBS, they were incubated overnight at 4 °C with the antibody, diluted 1:10 in the case of MECTOLONE-015 and 1:20 for R24, in PBS containing 3% BSA. The preparation was washed with PBS and incubated with fluorescein isothiocyanate-conjugated $F(ab')_2$ goat anti-mouse immunoglobulin (Cappel) (diluted 1:100 in PBS) and incubation was carried out for 1 h. After a wash with PBS, the sections were mounted with a coverslip. The slides were viewed and photographed with a Zeiss photomicroscope.

RESULTS

Gangliosides and neutral glycosphingolipids in rat placenta

The placental composition of gangliosides and neutral glycosphingolipids on day 20 of pregnancy was analysed by HPTLC. By comparison with standard glycolipids, the major neutral glycolipids were identified as LacCer, Gb_3Cer and Gb_4Cer (Figure 1a). This result was confirmed by SIMS (results not shown) and ^{1}H-NMR spectroscopy (Table 1). From their chemical shifts and coupling constants, they were identified as the anomeric protons of β-GalNAc, α-Gal, β-Gal and β-Glc respectively [ceramide tetrasaccharide (CQH)].

Gangliosides corresponding to G_{M3} and G_{D3} were detected (Figure 1b). Treatment of these ganglioside fractions with neuraminidase resulted in only one resorcinol-negative orcinol-positive band showing mobility similar to that of LacCer (Figure 2). The amounts of G_{M3} and G_{D3} were estimated to be 54.8 and 103.8 ng/mg of tissue wet weight respectively.

Table 1 Summary of glycosphingolipids present in rat placenta

Data were obtained at 270 MHz and 60 °C in [^{2}H]dimethyl sulphoxide containing 2% ^2H_2O, referenced to internal Me_4Si. Values are anomeric proton chemical shifts (p.p.m.) and those in parentheses are proton–proton coupling constants ($J_{1,2}$ Hz).

Neutral glycosphingolipids		Gangliosides	
CMH	Glcβ1-Cer	G_{M3}	NeuAcα2-3Galβ1-4Glcβ-Cer
CDH	Galβ1-4Glcβ1-Cer		NeuGcα2-3Galβ1-4Glcβ-Cer
CTH	Galα1-4Galβ1-4Glcβ1-Cer 4.81(3.3) 4.28(7.3) 4.18(7.9)	G_{D3}	NeuAcα2-8NeuAcα2-3Galβ1-4Glcβ-Cer NeuAcα2-8NeuGcα2-3Galβ1-4Glcβ-Cer
CQH	GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-Cer 4.54(8.3) 4.83(3.6) 4.29(7.6) 4.18(7.9)		NeuGcα2-8NeuAcα2-3Galβ1-4Glcβ-Cer

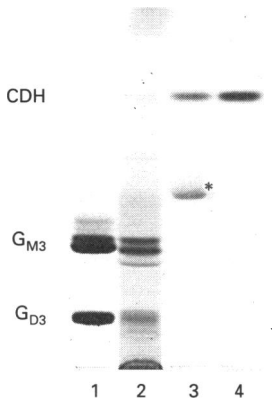

Figure 2 HPTLC pattern after neuraminidase treatment of disialoganglioside

Lane 1, mixture of standard gangliosides (G_{M3} from dog erythrocyte and G_{D3} from bovine milk); lane 2, ganglioside fraction from rat placenta; lane 3, after neuraminidase treatment of isolated disialoganglioside; lane 4, authentic LacCer. The plate was developed with chloroform/methanol/0.5% $CaCl_2$ (60:35:8, by vol.) and the spots were visualized with orcinol/H_2SO_4 reagent. The spot denoted by * is due to sodium taurodeoxycholate, which was added to the enzyme incubation mixture.

Negative-ion SIMS analysis

Two bands of G_{M3} and four bands of G_{D3} were separated by chloroform/methanol/2.5 M NH_4OH (55:45:10, by vol.), suggesting that these ganglioside fractions contained glycol- and acetyl-neuraminic acids (NeuGc and NeuAc) (Figure 1c). The structures of the placental gangliosides were analysed by negative-ion SIMS. The data are shown in Figure 3. Negative-ion SIMS of the upper band from monosialogangliosides showed that G_{M3} contained five ceramide species and *N*-acetylneuraminic acid (Figure 3a). Negative-ion SIMS of the lower band showed that G_{M3} contained mainly one ceramide and *N*-glycollylneuraminic acid. The ion at m/z 1167 is G_{M3} (NeuAc) including a ceramide bearing a hydroxy group (Figure 3b).

The fraction containing the three upper bands of G_{D3} was analysed, and three deprotonated ganglioside molecules were detected. Two ceramide species with NeuAc-NeuAc- and NeuAc-NeuGc- were identified in the fraction (Figure 3c). Analysis of the lowest band (Figure 1c) revealed that the G_{D3} contained NeuAc-NeuGc- and NeuGc-NeuAc- with one ceramide species. There was some contamination of G_{D3} (NeuAc-NeuAc-) including a ceramide bearing a hydroxy group (Figure 3d). Ganglioside G_{D3} consisting of NeuGc-NeuGc- was not detected in these SIMS. This was confirmed by the negative result of TLC/immunostaining with GMR3 monoclonal antibody (anti-

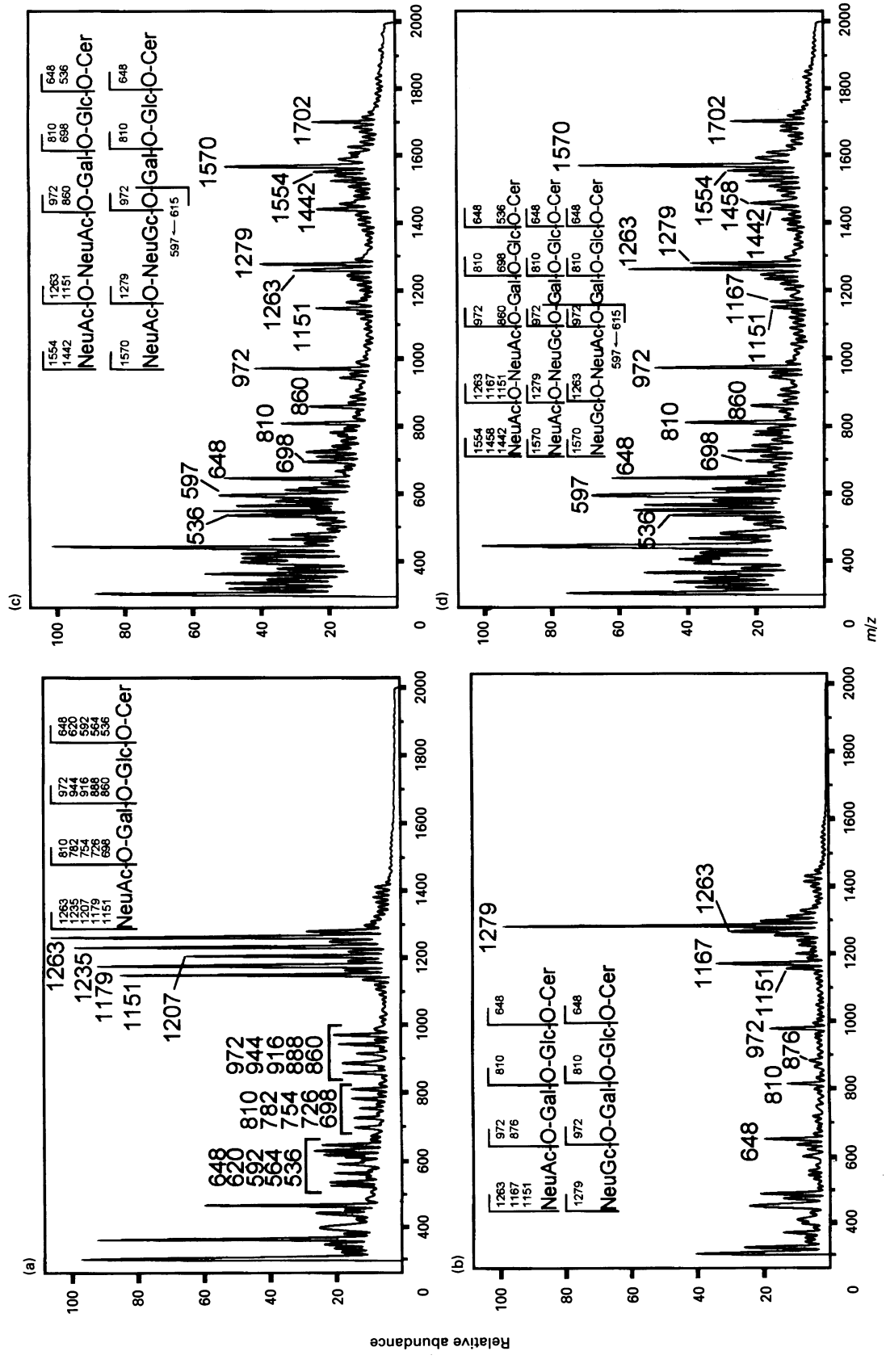

Figure 3 Negative-ion SIMS of mono- and di-sialogangliosides

(**a**) Monosialoganglioside upper fraction; (**b**) monosialoganglioside lower fraction; (**c**) disialoganglioside upper fraction; (**d**) disialoganglioside lower fraction. Observed *m/z* values are offset for nominal masses.

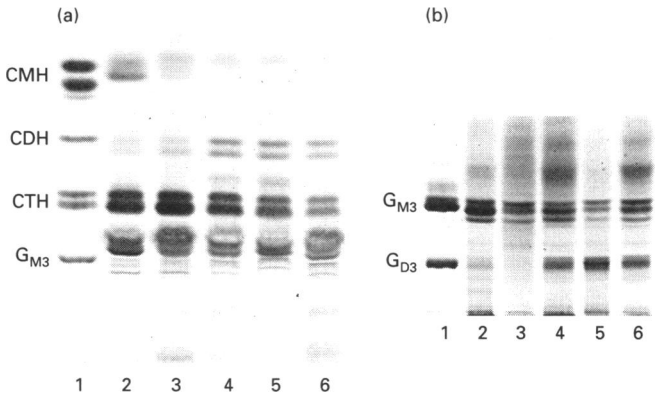

Figure 4 Thin-layer chromatogram of rat placenta glycolipid components during pregnancy

The plates were developed with chloroform/methanol/0.5% $CaCl_2$ (60:35:8, by vol.) (**a**) and chloroform/methanol/0.5% $CaCl_2$ (55:45:10, by vol.) (**b**), and the spots were visualized with orcinol/H_2SO_4 reagent. Lane 1, standard mixture; lane 2, pregnancy day 12; lane 3, day 14; lane 4, day 16; lane 5, day 18; lane 6, day 20. In each lane, extract from 5 mg (**a**) and 10 mg (**b**) wet weight of tissue was applied.

GD3 NeuGc-NeuGc-) (results not shown). The ion at m/z 1702 was a cluster negative ion between the sample molecule and the matrix triethanolamine (Figures 3c and 3d).

Changes in placental glycosphingolipid composition during pregnancy

G_{M3} was prevalent on days 12–16, but then decreased (Figures 4 and 5). In contrast, the level of G_{D3} was low until day 14 and then increased slightly on day 16, maintaining this level thereafter.

GlcCer content was high on days 12 and 14, and then decreased gradually toward the end of pregnancy. Gb_3Cer was predominant throughout pregnancy, although there was a tendency for a decrease toward the end. The content of LacCer was low until day 14 and then markedly increased after day 16. The content of Gb_4Cer remained low throughout pregnancy.

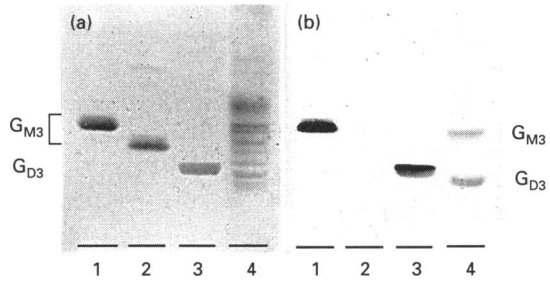

Figure 6 TLC/immunostaining with anti-G_{M3} and -G_{D3} monoclonal antibody (MECTOLONE-015)

(**a** and **b**) Lane 1, authentic G_{M3} (NeuAc); lane 2, authentic G_{M3} (NeuGc); lane 3, authentic G_{D3} (NeuAc-NeuAc); lane 4, ganglioside fraction from rat placenta. The plates were developed with chloroform/methanol/0.5% $CaCl_2$ (60:35:8, by vol.). (**a**) Detection with orcinol/H_2SO_4 reagent; (**b**) immunostaining with MECTOLONE-015.

TLC/immunostaining and immunohistochemical analysis

The antibody employed (MECTOLONE-015) reacted with the G_{D3} (NeuAc-NeuAc-) and G_{M3} (NeuAc) standards, but not with G_{M3} (NeuGc) (Figure 6). The antibody did not recognize G_{D3} (NeuAc-NeuGc- and NeuGc-NeuAc-) (results not shown), but reacted with the placental gangliosides, suggesting that the placenta contains G_{M3} (NeuAc) and G_{D3} NeuAc-NeuAc-). MECTOLONE-015 reacted with some types of G_{M3} and G_{D3}, and R24 reacted with G_{D3}, but not with G_{M3}.

Ganglioside distribution in the placenta was examined using these antibodies (Figure 7). On day 12, trophoblasts in the labyrinthine zone were positively stained with MECTOLONE-015 but not with R24, indicating that G_{M3} was localized in this zone (Figures 7c and 7d). Junctional trophoblasts and decidual cells were unreactive with these antibodies. On day 16, both antibodies reacted strongly with trophoblasts in the labyrinthine zone (Figures 7g and 7h). In sections of both tissues, giant trophoblasts were unreactive with these antibodies. Therefore the major gangliosides (G_{M3} and G_{D3}) present in the placenta were localized mainly in growing-phase trophoblasts, and not in finally differentiated trophoblasts.

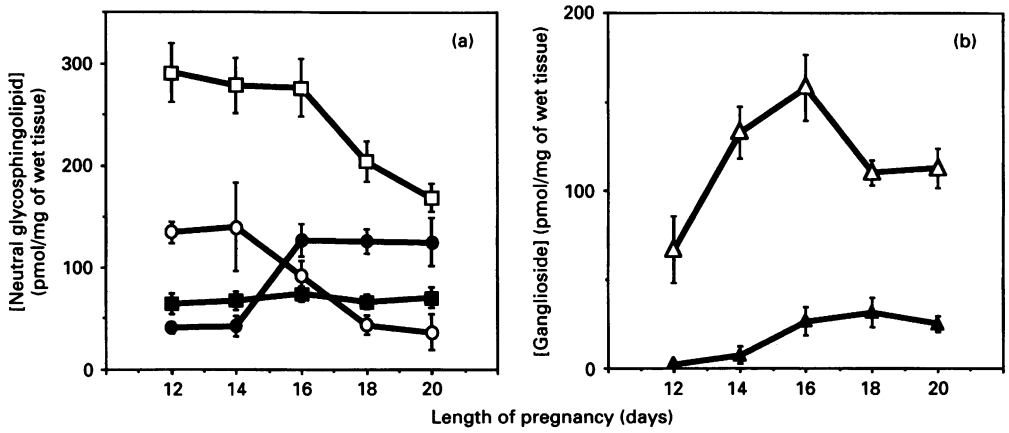

Figure 5 Changes in glycosphingolipid composition of rat placenta during pregnancy

(**a**) Changes in neutral glycosphingolipid; (**b**) changes in ganglioside. ○, GlcCer; ●, LacCer; □, Gb_3Cer; ■, Gb_4Cer; △, G_{M3}; ▲, G_{D3}. Error bars indicate the S.E.M. derived from three individual dams.

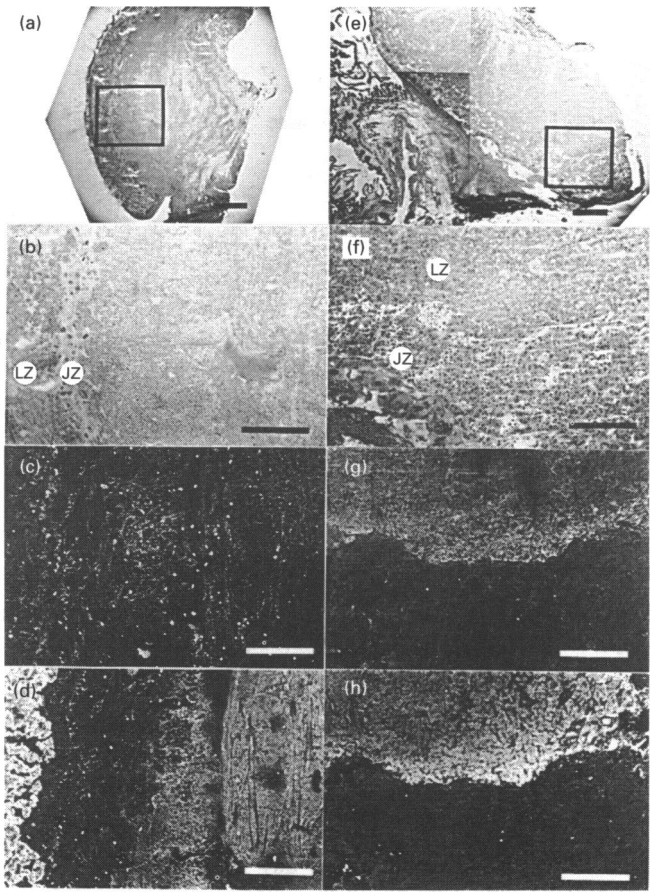

Figure 7 Indirect immunofluorescence analysis of ganglioside G_{M3} and G_{D3} expression in rat placenta

Sequential sections were immunostained with monoclonal antibodies using fluorescein isothiocyanate-labelled goat anti-mouse immunoglobulin antibody. (**a**, **b**, **c** and **d**) Rat placenta obtained on day 12 of pregnancy; (**e**, **f**, **g** and **h**) rat placenta obtained on day 16 of pregnancy. (**a**, **b**, **e** and **f**) Haematoxylin–eosin stain; C and G, R24 [anti-G_{D3}(NeuAc-NeuAc- and NeuAc-NeuGc-)]; D and H, MECTOLONE-015 [anti-G_{M3} (NeuAc) and -G_{D3} (NeuAc-NeuAc-)]. (**b**, **c**, **d**, **f**, **g** and **h**) high-magnification views of the square areas in (**a**) and (**e**). JZ, junctional zone; LZ, labyrinthine zone. Bars represent 1 mm (**a** and **e**) and 0.5 mm (**b**, **c**, **d**, **f**, **g** and **h**).

DISCUSSION

The major gangliosides present in the rat placenta were identified as G_{M3} and G_{D3}, and neutral glycosphingolipids as GlcCer, LacCer, Gb_3Cer and Gb_4Cer, as summarized in Table 1. We also found that the composition of the gangliosides changed markedly according to the stage of pregnancy.

The glycolipid composition of various tissues is known to vary depending on the animal species, except for the brain. On the other hand, brain tissue from various animals commonly expresses G_{M1}, G_{D1a}, G_{D1b}, G_{T1b} and G_{Q1b} [21,22]. In fetal brain, G_{M3} and G_{D3} are prevalent during development but change to gangliosides of more complex structure in various species [21,22]. The gangliosides expressed commonly among animal species are thought to play essential roles in brain function. Interestingly, the composition of major gangliosides in rat placenta seems to be similar to that in human placenta [23–26]. Neutral glycolipids, Gb_3Cer and Gb_4Cer, were also expressed throughout pregnancy in rat placenta, and these have been found in human term placenta and mouse placenta [25,27–30]. Thus the marked changes in glycolipid composition and their conserved expression in placenta among these animal species imply a specific biological role for gangliosides in placental function.

Both NeuAc and NeuGc were found in G_{M3} extracted from rat placenta (Table 1). As the expression of NeuGc-containing gangliosides in tissues is well correlated with the activity of CMP-NeuAc hydroxylase [31,32], and a sialyltransferase specific for CMP-NeuGc has not been isolated, a single species of sialyltransferase must be responsible for transfer of CMP-NeuAc and CMP-NeuGc. However, NeuGc was identified by SIMS in only one ceramide species in spite of the presence of five different ceramides bearing NeuAc. This uneven distribution of NeuGc suggests that the formation of G_{M3} with NeuGc is strictly regulated, and that another mechanism must be considered.

Gb_3Cer was found to be dominantly expressed throughout pregnancy in the rat placenta. Although LacCer is an obligate precursor of various series of neutral glycolipids including the globo-, neolacto- and ganglio- series, neutral glycolipids in the placenta were all of the globo- series [29,30] and the activity of galactosyl-transferase is dominant in the placenta [29]. Taken together with the fact that the only gangliosides were G_{M3} and G_{D3} and that gangliosides with longer sugar chains could not be detected, GalNAc transferase activity seems to be negligible in the placenta.

Expression of these gangliosides may change the response of trophoblasts to growth factors. Data on autocrine/paracrine networks involving various growth factors or cytokines in the placenta have accumulated in relation to the growth and differentiation of trophoblasts [11,33]. G_{M3}, which is predominant at mid-pregnancy, has been implicated as a modulator of growth factors including epidermal and fibroblast growth factors [7–10,34]. Also G_{D3}, which is expressed in late pregnancy, is critical for epithelial development, being involved in epithelial–mesenchymal interactions in the mouse. Therefore expression of gangliosides can theoretically perturb various events, including the intraplacental regulation system [35]. Changes in gangliosides composition occur in labyrinthine trophoblasts. The placenta expresses placental lactogen-α, placental lactogen-I and placental lactogen-I specifically at mid-pregnancy, whereas other placental lactogens are reported to be expressed between day 16 and term [3,4,36]. Further aspects of the relationship between changes in ganglioside composition and trophoblast differentiation remain to be clarified.

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REFERENCES

1 Svennerholm, L. (1963) J. Neurochem. **10**, 613–623
2 Soares, M. J., Faria, T. N., Roby, K. F. and Deb, S. (1991) Endocrinol. Rev. **12**, 402–423
3 Furuyama, N., Shiota, K. and Takahashi, M. (1991) Endocrinol. Jpn. **38**, 533–540
4 Shiota, K., Furuyama, N. and Takahashi, M. (1991) Endocrinol. Jpn. **38**, 541–549
5 Davies, J. and Glasser, S. R. (1968) Acta Anat. **69**, 542–608
6 Hannun, Y. A. and Bell, R. M. (1989) Science **243**, 500–507
7 Bremer, E. G., Hakomori, S.-I., Bowen-Pope, D. F., Raines, E. and Ross, R. (1984) J. Biol. Chem. **259**, 6818–6825
8 Hanai, N., Dohi, T., Nores, G. A. and Hakomori, S.-I. (1988) J. Biol. Chem. **263**, 6296–6301
9 Song, W., Vacca, M. F., Welti, R. and Rintoul, D. A. (1991) J. Biol. Chem. **266**, 10174–10181

10 Zheng, M., Fang, H., Tsuruoka, T., Tsuji, T. Sasaki, T. and Hakomori, S.-I. (1993) J. Biol. Chem. **268**, 2217–2222
11 Shiota, K., Hirosawa, M., Hattori, N. et al. (1994) Endocrine J. **41**, S43–S56
12 Cheresh, D. A., Pierschbacher, M. D., Herzig, M. A. and Mujoo, K. (1986) J. Cell Biol. **102**, 688–696
13 Houghton, A. N., Mintzer, D., Cordon-Cardo, C. et al. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 1242–1246
14 Pukel, C. S., Lloyd, K. O., Travassos, L. R., Dippold, W. G., Oettgen, H. F. and Old, L. J. (1982) J. Exp. Med. **155**, 1133–1147
15 Tai, T., Kawashima, I., Furukawa, K. and Lloyd, K. O. (1988) Arch. Biochem. Biophys. **260**, 51–55
16 Ozawa, H., Kawashima, I. and Tai, T. (1992) Arch. Biochem. Biophys. **294**, 427–433
17 Hidari, K., Itonori, S., Sanai, Y., Ohashi, M., Kasama, T. and Nagai, Y. (1991) J. Biochem. (Tokyo) **110**, 412–416
18 Saito, T. and Hakomori, S.-I. (1971) J. Lipid Res. **12**, 257–259
19 Itonori, S., Hidari, K., Sanai, Y., Taniguchi, M. and Nagai, Y. (1989) Glycoconjugale J. **6**, 551–560
20 Kotani, M., Kawashima, I., Ozawa, H., Terashima, T. and Tai, T. (1993) Glycobiology **3**, 137–146
21 Rosenberg, A., Sauer, A., Noble, E. P., Gross, H.-J., Chang, R. and Brossmer, R. (1992) J. Biol. Chem. **267**, 10607–10612
22 Yu, R. K., Macala, L. J., Taki, T., Weinfeld, H. M. and Yu, F. S. (1988) J. Neurochem. **50**, 1825–1829
23 Levery, S. B., Nudelman, E. D., Salyan, M. E. K. and Hakomori, S.-I. (1989) Biochemistry **28**, 7772–7781
24 Svennerholm, L. (1965) Acta Chem. Scand. **19**, 1506–1507
25 Rueda, R., Tabsh, K. and Ladisch, S. (1993) FEBS Lett. **328**, 13–16
26 Taki, T., Matsuo, K.-I., Yamamoto, K., Matsubara, T., Hayashi, A., Abe, T. and Matsumoto, M. (1988) Lipids **23**, 192–198
27 Borne, A. E. G. K., Bos, M. J. E., Joustra-Maas, N., Tromp, J. F., Bois, R. W.-D. and Tetteroo, P. A. T. (1986) Br. J. Haematol. **63**, 35–46
28 Hansson, G. C., Wazniowska, K., Rock, J. A. et al. (1988) Arch. Biochem. Biophys. **260**, 168–176
29 Lampio, A., Airaksinen, A. and Maaheimo, H. (1993) Glycoconjugate J. **10**, 165–169
30 Svejcar, J., Ehrlich-Rogozinski, S., Riedel, D., Müthing, J. and Sharon, N. (1993) Glycoconjugate J. **10**, 247–250
31 Kawano, T., Kozutsumi, Y., Takematsu, H., Kawasaki, T. and Suzuki, A. (1993) Glycoconjugate J. **10**, 109–115
32 Shaw, L., Schneckenburger, P., Carlsen, J., Christiansen K. and Schauer, R. (1992) Eur. J. Biochem. **206**, 269–277
33 Mitchell, M. D., Trautman, M. S. and Dudley, D. J. (1993) Placenta **14**, 249–275
34 Bremer, E. G. and Hakomori, S.-I. (1982) Biochem. Biophys. Res. Commun. **106**, 711–718
35 Sariola, H., Aufderheide, E., Bernhard, H., Henke-Fahle, S., Dippold, W. and Ekblom, P. (1988) Cell **54**, 235–245
36 Hirosawa, M., Miura, R., Min, K.-S., Hattori, N., Shiota, K., Ogawa, T. (1994) Endocrinol. Jpn. **41**, 387–397

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