Composition and synthesis of glycolipids in megakaryocytes and platelets: differences in synthesis in megakaryocytes at different stages of maturation

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Abstract The composition and synthesis of megakaryocyte and platelet glycolipids were compared since these lipids are thought to be important for biologic activities such as adhesion and maturation. Highly purified guinea pig megakaryocytes at different stages of maturation and platelets were studied. Glycolipids and gangliosides were extracted, separated by thin-layer chromatography, and the carbohydrate content was analyzed by gas–liquid chromatography (GLC). Synthesis of ceramides and glycolipids was determined by the incubation of megakaryocytes with [^{14}C]acetate, [^{3}H]palmitic acid, and [^{3}H]galactose. A major neutral glycolipid present in guinea pig megakaryocytes and platelets was identified as asialoGM2 by selective enzymatic hydrolysis with β-N-acetylhexosaminidase, α-galactosidase and endo-β-galactosidase, and carbohydrate analysis by GLC. Trace amounts of asialoGM1 were detected immunologically. The cells also contained glucosyl ceramide and lactosyl ceramide. Several gangliosides were detected of which one was identified as GM1 by its reaction with the beta-subunit of cholera toxin and by the identification of an asialoGM1 core with anti-asialoGM1 antibody after desialylation. The synthesis of ceramides from palmitic acid and acetate was 5 and 10 times greater, respectively, in megakaryocytes than in platelets. Ceramide and glycolipid synthesis from palmitic acid occurred primarily in immature megakaryocytes while synthesis from acetate occurred primarily in more mature megakaryocytes. The glycosylation of ceramides from galactose was 42 times greater in megakaryocytes than in platelets. Thus, ceramides and glycolipids are primarily synthesized in megakaryocytes, but platelets retain the capacity to synthesize significant amounts of free ceramides. The glycosylation of free ceramides occurs almost exclusively in megakaryocytes and only in trace amounts in platelets. These data indicate that megakaryocytes determine the composition of glycolipids in platelets and that there is considerable compartmentalization of glycolipid synthesis and membrane assembly at various stages of megakaryocytes development.—**Schick, P. K., and X. He.** Composition and synthesis of glycolipids in megakaryocytes and platelets: differences in synthesis in megakaryocytes at different stages of maturation. *J. Lipid Res.* 1990. **31:** 1645–1654.

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Glycolipids are considered to be important for receptor-mediated activities, adhesion, and cellular maturation (1). Several studies have characterized glycolipids in human platelets and have found that GM3, hematoside, is the predominant ganglioside and that lactosyl ceramide is the primary neutral glycolipid present in these cells (2–4). There is evidence that thrombin induces a change in the organization of glycolipids on the platelet surface (4) and that platelet gangliosides are involved in the binding of serotonin (3, 5, 6). Megakaryocyte glycolipids have not been investigated prior to this study. Megakaryocytes have extensive capacity for protein and lipid synthesis while platelets have limited synthetic capacities. Therefore, megakaryocytes most likely determine the lipid composition of platelets (7–10). Thus, it was important to delineate the glycolipid composition and the capacity for the synthesis of these lipids in megakaryocytes to begin to define the role of glycolipids in megakaryocyte maturation and the mechanisms for establishing the glycolipid content of platelets. There is considerable evidence that gangliosides and other glycolipids are involved in maturation and differentiation. For example, there are differences in the glycolipids in normal and transformed or malignant cells (1). Also, glycolipids can modulate the effects of growth factors (1).

The guinea pig is the ideal source of megakaryocytes for biochemical studies because the yield, purity, and viability of isolated megakaryocytes from guinea pigs are su-

Abbreviations: HPTLC, high performance thin-layer chromatography; GLC, gas–liquid chromatography; PBS, phosphate-buffered saline.

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perior to those from other animal species and humans (7–11). In this study we have used methods suitable for evaluating glycolipids and gangliosides in small amounts of tissues. The study identified the neutral glycolipid and ganglioside content in guinea pig megakaryocytes and platelets and detected marked differences in the capacity for the synthesis of ceramides in megakaryocytes and platelets. The synthesis of glycolipids was shown to differ in megakaryocytes at various stages of maturation. The structure of neutral glycolipids and gangliosides relevant to the study are shown in **Table 1**.

TABLE 1. Relevant glycolipid structures

Neutral glycolipids	
Glucosyl ceramide	Glcβ1→Cer
Lactosyl ceramide	Galβ1→Glcβ1→Cer
AsialoGM2	GalNAcβ1→4Galβ1→4Glcβ1→Cer
AsialoGM1	Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→Cer
Gangliosides	
Hemato (GM3)	NeuAcα2→3Galβ1→4Glcβ1→Cer
Ganglio series (GM1)	Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→Cer 3 α ↓ 2 NeuAc

The gangliosides in guinea pig megakaryocytes and platelets were shown to belong to the Ganglio series.GM3, the major ganglioside in human platelets, belongs to the Hemato series. The core structure of Ganglio series gangliosides is asialoGM2. Glc, glucose; Gal, galactose; GalNac, N-acetylgalactosamine; NeuAc, N-acetylneuraminic acid.

MATERIALS AND METHODS

Preparation of cells

Platelet rich plasma was prepared from blood collected in acid-citrate-dextrose by cardiac puncture. Platelets were washed twice in calcium- and magnesium-free Hanks' balanced salt solution pH 6.5 (7–11). Guinea pig megakaryocytes were isolated to about 85% purity by cell number and greater than 98% by cell volume with an albumin density gradient followed by two velocity gradients as previously described (7–11). Platelet counts were determined with a Serono-Baker MK-4/HC platelet counter (J. T. Baker Diagnostics, Allentown, PA), and megakaryocyte counts were determined under phase-contrast microscopy in a hemocytometer. Contamination of isolated megakaryocytes with other bone marrow cells and isolated platelets with other peripheral blood cells was determined under phase contrast microscopy. Contamination of washed platelets with erythrocytes and leukocytes was less than 0.0001%.

Protein determinations

The protein content of washed platelets and megakaryocytes was determined by the method of Lowry et al. (12).

Isolation of megakaryocytes at different stages of maturation

Megakaryocyte populations at different stages of maturation were isolated by a recently introduced Celsep procedure (13) which separates megakaryocytes by size. Four population groups were prepared: *1*) Group I contained 75% immature megakarocytes; *2*) Group II contained about 50% immature megakaryocytes; *3*) Group III contained about 80% mature megakaryocytes; and *4*) Group IV contained 95% mature megakaryocytes.

Isolation and purification of glycolipids

Neutral and acidic glycolipids were extracted and isolated by a modification of the method of Ariga et al. (14). Briefly, total lipids were extracted with chloroform–methanol 1:1, 2:1, and 1:2 (v/v), respectively, and the extracts were combined. A DEAE-Sephadex A-25 (acetate form) column was used to separate neutral glycolipids from gangliosides and sulfatides, acidic glycolipids. The first fraction was eluted with chloroform–methanol–water 30:60:8 (v/v/v) and contained neutral glycolipids, neutral lipids, and phospholipids. The neutral glycolipids in this fraction were purified by mild alkaline hydrolysis to remove phospholipids and saponifiable neutral lipids, and subsequently by silicic acid column chromatography to remove nonsaponifiable neutral lipids (4, 14). The second fraction was eluted from the DEAE-Sephadex A-25 column with chloroform–methanol–0.8 N sodium acetate 30:60:8 (v/v/v) and contained gangliosides, sulfatides, and acidic phospholipids. Gangliosides present in the second fraction were purified by: *1*) mild alkaline hydrolysis to remove acidic phospholipids (15); *2*) Sephadex LH-20 column to remove salts; and *3*) Iatrobeads (6RS-8060) column chromatography to purify gangliosides by removing sulfatides (15). Iatrobeads were obtained from Iatron Industries, Inc., Tokyo, Japan.

High performance thin-layer chromatography (HPTLC)

Neutral glycolipids were separated by neutral and basic HPTLC solvent systems: (A) chloroform–methanol–water 60:35:8 (v/v/v) (16); and (B) chloroform–methanol–2.5 N ammonia 60:35:8 (v/v/v) (16), respectively. Gangliosides were separated by neutral and basic HPTLC solvent systems: (C) chloroform–methanol–0.5% $CaCl_2 \cdot 2H_2O$ 50:45:10 (v/v/v) (17); and (D) chloroform–methanol–2.5 N ammonia 60:40:9 (v/v/v) (17), respectively. Gangliosides were also separated by two-dimensional HPTLC solvent system with chloroform–methanol–0.5% $CaCl_2 \cdot 2H_2O$ 50:40:10 (v/v/v) in the first dimension and chloroform–methanol–2.5N ammonia 50:40:10 (v/v/v) in the second dimension (17). Silica gel 60 chromatoplates (E.

Merck HPTLC Fertigplatten Kieselgel 60 #5641, E-M Sciences, Cherry Hill, NJ) were used. Neutral glycolipids were visualized by orcinol sulfuric acid reagent (18) and gangliosides were visualized by either resorcinol–HCl reagent (15) or orcinol sulfuric acid reagent.

Densitometry

Neutral glycolipids that had been visualized by orcinol and gangliosides that had been stained with resorcinol–HLC reagent were quantitated by microdensitometry (19, 20) with a Camag TLC Scanner and a SP49020 Integrator (Camag Scientific, Inc., Wrightsville Beach, NC). Authentic neutral glycolipid standards glucosyl ceramide (Sigma Chemical Co., St. Louis, MO) and trihexosylceramide (Supelco, Co., Bellefonte, PA), were used in the study. Human brain ganglioside standards, asialo-GM1 and asialo-GM2, were kindly provided by Dr. Robert K. Yu, University of Virginia, Richmond, VA. Different concentrations of authentic glycolipids were run on the HPTLC chromatoplate in each experiment and were used to obtain standard curves of the relationship of absorbance to glycolipid concentration. From 0.4 to 2.0 μg of the standards was applied and the absorbances were linear to the glycolipid concentration. The standard curves were similar to those of previous studies (20).

Enzymatic hydrolysis

The neutral glycolipid band that is shown in Fig. 1 and had an R_f value that was slightly lower than trihexosyl ceramide was analyzed by digestion with three enzymes: *1*) β-N-acetylhexosaminidase from jack beans; *2*) α-galactosidase from *Aspergillus niger*; *3*) endo-β-galactosidase from *Bacteroides fragilis*. Lactosyl ceramide was subjected to hydrolysis with β-galactosidase from jack beans. These enzymes were purchased from Sigma Co., St. Louis, MO. The conditions for hydrolyzing glycolipids by α-galactosidase from *Aspergillis niger*, β-galactosidase from jack beans and β-N-acetylhexosaminidase from jack beans were similar to those described by Hakomori and Siddiqui (21). The incubation conditions with endo-β-galactosidase have been described by Scudder et al. (22, 23). The following were the major steps in the hydrolysis of purified glycolipids with β-N-acetylhexosaminidase and α-galactosidase. *1*) Glycolipids were dissolved in 100 μl of 0.05 M sodium citrate buffer containing 100 μg of sodium taurodeoxycholate in conical vessels in an ultrasonic bath; *2*) the enzymes were then added and the hydrolysis was carried out at 37°C for 18 h; the pH for incubations with α-galactosidase, β-galactosidase, and β-N-acetylhexosaminidase was maintained at 4.4, 4.0, and 5.0, respectively. The hydrolysis of glycolipids with endo-β-galactosidase was performed at pH 5.8 at 37°C for 2 h.

Gas–liquid chromatographic analysis of carbohydrates

Glycolipids were reduced and acetylated to produce alditol acetate derivatives (24) and analyzed by GLC with a 3-foot column with 3% SP-2340 on 100/120 Supelcoport. Authentic alditol acetate derivatives were obtained from Supelco (Bellefonte, PA). Recovery was variable. Thus the data were not used for quantitation but they were suitable for the identification of carbohydrates in guinea pig platelet and megakaryocyte neutral glycolipids.

Mild acid hydrolysis

The isolated gangliosides were subjected to mild acid hydrolysis at 80°C for 30 min under conditions that resulted in the hydrolysis of all sialic acid residues (25, 26).

HPTLC immunostaining in situ

HPTLC-immunostaining was performed according to the method of Saito, Kasai, and Yu (27). Glycolipids were separated by HPTLC. Neuramidase from *Arthrobacter ureafaciens* (Calbiochem-Behring, San Diego, CA) in 0.1 M sodium acetate, pH 4.8, was layered onto the separated lipids and hydrolysis was achieved by the incubation of the chromatoplate at room temperature for 2 h. The chromatoplate was washed with phosphate-buffered saline (PBS), pH 7.3, and air dried. Polyvalent anti-asialoGM1 serum, diluted 1:8 in 0.3% gelatin-PBS, was layered onto the chromatoplate, and the enzyme-treated glycolipids were incubated for 1 h. The preparation, purification, and characterization of the anti-asialoGM1 antibody has been described, and the antibody has been shown to be highly specific to asialoGM1 (28, 29). After washing with PBS, a ^{125}I-labeled protein A (Dupont-New England Nuclear Research Products, MA) solution with 500,000 cpm/ml 0.3% gelatin–PBS was applied to each lane and the plate was incubated for 1 h at room temperature. The plate was then washed with PBS containing 0.1% Triton X-100 and exposed to Kodak XAR film at −70°C for 6 h.

Identification of GM1 with the β-subunit of cholera toxin [which selectively binds to GM1 (30)] by the HPTLC overlay method (31)

Gangliosides were separated by HPTLC as described above and FITC-β-subunit of cholera toxin (5 μg per ml PBS, pH 7.2, containing 0.3% gelatin) was layered over the chromatoplate and the binding of cholera toxin β-subunit was visualized under UV light.

Glycolipid synthesis in megakaryocytes at different stages of maturation

Megakaryocyte populations separated by the Celsep procedure were incubated with 10 μCi/ml [^{14}C]acetate (56 mCi/mmol), 20 μCi/ml [^{3}H]palmitic acid (30 Ci/ml), and

10 μCi/mmol [^{3}H]galactose (46 Ci/mmol) (Dupont-New England Nuclear Research Products, Boston, MA) for 18 h at 37°C. Megakaryocyte lipids were extracted by the Bligh-Dyer method and glycolipids were purified by silicic acid column chromatography and alkaline hydrolysis as previously described (4). Ceramides and glycolipids were separated by TLC with solvent system (A) and in some experiments using a TLC system that isolates ceramides A and B (chloroform–methanol–acetic acid 90:2:8), and the synthesis of lipids was determined by measuring radioactivity in scraped lipid bands by scintillation spectrometry.

Analysis of incorporated radioactive galactose, palmitate, and acetate

To determine whether glycosyl or galatosyl ceramide had been synthesized, glucosyl and galatosyl ceramides were separated by sodium borate-impregnated HPTLC chromatoplate with a solvent system of chloroform–methanol–water–15 M NH_4OH 280:70:6:1 (v/v/v/v) (32). To determine whether radioactive palmitic acid and acetate had been incorporated into the sphingosine, fatty acid, or carbohydrate moieties of ceramides and glycolipids, ceramides and glycolipids were subjected to acid methanolysis (4). Fatty acid methyl esters were partitioned into hexane, sphingosine was partitioned into diethyl ether, and carbohydrate was partitioned in the methanol phase, and the relative amounts of radioactivity in these moieties were determined by scintilliation spectrometry.

RESULTS

About 800,000 megakaryocytes and 4×10^9 platelets can be isolated from two guinea pigs, which corresponds to a yield of 0.8 mg and 3 mg protein, respectively. In order to obtain sufficient neutral glycolipids and gangliosides for an individual experiment it was necessary to obtain megakaryocytes from three to six guinea pigs. Two factors made it difficult to extensively characterize glycolipids in megakaryocytes: *1*) the availability of small numbers of cells; and *2*) glycolipids are minor components of platelets, megakaryocytes, and other tissues. Analysis of carbohydrates by GLC was limited to neutral glycolipids. Thus, the identification of the small amounts of tissue glycolipids and gangliosides in our study was achieved by selective enzymatic hydrolysis and immunological detection and some of these studies were performed in situ on HPTLC chromatoplates. However, the experimental approach permitted the identification of the major neutral glycolipids and the demonstration of several gangliosides in guinea pig megakaryocytes and platelets. One ganglioside was identified as GM1.

Neutral glycolipids present in human and guinea pig platelets and megakaryocytes are shown in **Fig. 1**. The predominant neutral glycolipid in human platelets is lactosyl ceramide (2, 4). AsialoGM2 is the predominant neutral glycolipid in the guinea pig cells based on evidence presented below and in Figs. 1 and 2.

The identity of the guinea pig megakaryocyte and platelet neutral glycolipids was determined by several methods. The predominant guinea pig neutral glycolipid had an R_f similar to that of asialoGM2 (GalNAcβ 1-4Galβ 1-4Glcβ 1-Cer), globotrihexosyl ceramide (Galα 1-4Galβ 1-4Glcβ 1-Cer), and lactotriaosyl ceramide (GlcNAcβ 1-3Gal β1-4Glcβ 1-Cer). The glycolipids can be selectively hydrolyzed by β-N-acetylhexosaminidase, alpha galactosidase and endo-β-galactosidase, respectively, and these enzymes have been used to identify these glycolipids (21–23,

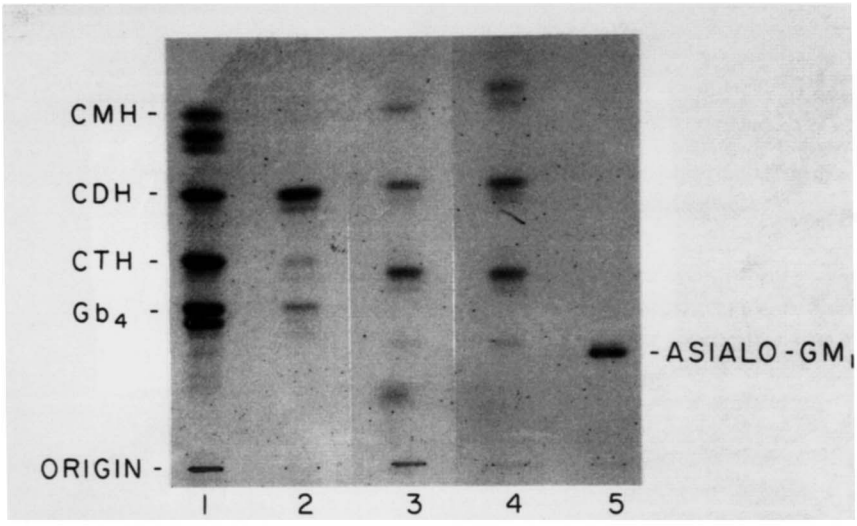

Fig. 1. HPTLC of neutral glycolipids. Lane 1, authentic standards; lane 2, extracts from human platelets; lane 3, extracts from guinea pig platelets; lane 4, neutral glycolipids from guinea pig megakaryocytes; lane 5, asialo-GM1 standard. The chromatoplate was developed in solvent system A and visualized by spraying with orcinol-sulfuric acid reagent. CMH, glucosyl ceramide; CDH, lactosyl ceramide; CTH, trihexosyl ceramide; Gb4, globoside.

31). The predominant glycolipid was hydrolyzed to lactosyl ceramide by β-N- acetylhexosaminidase as shown in **Fig. 2A** but was not hydrolyzed by α-galactosidase (Fig. 2B) or endo-β-galactosidase (Fig. 2C), indicating that the glycolipid was asialoGM2. In control experiments, authentic bovine brain asialoGM2 comigrated with the major guinea pig glycolipid and was only hydrolyzed by β-N-acetylhexosaminidase and not by the other two enzymes. Thus, the study indicated that the predominant neutral glycolipid in guinea pig platelets and megakaryocytes was asialoGM2. The identification of lactosyl ceramide was verified by its susceptibility to hydrolysis to glucosyl ceramide by β-galactosidase as shown in Fig. 2D. The identification of glycosyl ceramide and asialoGM1 were verified by their R_f values in both neutral and basic HPTLC systems A and B, respectively. AsialoGM1 was also identified by immunostaining with a specific anti-asialoGM1 antibody (29).

The identity of lactosyl ceramide and asialoGM2 was verified by the analysis of hexoses by GLC. Lactosyl ceramide contained galactose and glucose, and asialoGM2 contained N-acetylgalactosamine, galactose, and glucose.

Megakaryocytes and platelets contained the same species of neutral glycolipids. A 1/1000 ratio of the number of megakaryocytes to platelets were compared to compensate for the differences in the protein content and volume of the cells as previously described (7–11). Accurate quantitation of glycolipids in these cells was not possible due to small amounts of available material. However, there was approximately 1.3 μg neutral glycolipids per mg protein in platelets. There were equivalent amounts of neutral glycolipids in megakaryocytes and platelets. The percent distribution of glucosyl ceramide, lactosyl ceramide, and asialo-GM2 was 15.3 ± 1.5, 31.5 ± 3.2, and 53.1 ± 1.4, respectively, in platelets; and 34.5 ± 1.6, 33.3 ± 0.3, and 32.1 ± 1.9, respectively, in megakaryocytes (mean ± SD) (n = 4). The data confirm that asialoGM2 is the major neutral glycolipid in guinea pig platelets and that there is substantially less asialoGM2 but greater amounts of a less complex glycolipid, glucosyl ceramide, in guinea pig megakaryocytes.

Gangliosides present in human and guinea pig megakaryocytes and platelets are shown in the HPTLC plate developed in a neutral solvent system C (**Fig. 3A**). GM3 is the primary ganglioside in human platelets while several gangliosides are present in guinea pig cells, which were tentatively called gangliosides a, b, c, d and e. These guinea pig platelet glycolipids reacted with resorcinol-HCl reagent which confirmed that they contain sialic acid and thus were gangliosides. Guinea pig cell gangliosides were separated by preparative HPTLC with solvent system C, and gangliosides a–e eluted. Gangliosides b and c were coeluted since they were not well separated by preparative TLC. The eluted gangliosides were assessed by two HPTLC systems. Fig. 3C demonstrates that the gangliosides a, c, and e comigrated with authentic GT1a, GD1a, and GM1, respectively, when separated by the neutral HPTLC system C; Fig. 3D demonstrates the separation of the eluted gangliosides in the basic HPTLC system D and that gangliosides a, c, and e comigrated with authentic GT1a, GD1a, and GM1 standards, respectively. Fig. 3B demonstrates the separation of the five bands by a two-dimensional HPTLC solvent system and that gangliosides a, c, and e, the three major bands, comigrated with authentic standards GT1a, GD1a, and GM1, respectively; d and b, which were minor bands, mostly likely are doublets of e and c, respectively. Doublets of gangliosides species can be seen on TLC and can be due to differences in the content of fatty acid or sialic acid species (14, 31, 33).

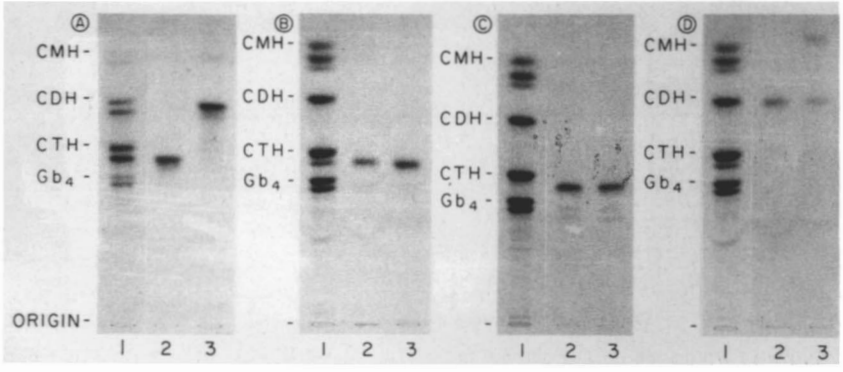

Fig. 2. Enzymatic hydrolysis of guinea pig platelet neutral glycolipid. The separation of authentic neutral glycolipid standards is shown in lanes 1 in Fig. 2A–D. The guinea pig neutral glycolipid shown in Fig. 1 that had an R_f value slightly lower than trihexosyl ceramide was purified by elution from a preparative thin-layer chromatoplate and subjected to enzymatic hydrolysis. Fig. 2A demonstrates the migration of this glycolipid without (lane 2) and with treatment with β-N-acetylhexosaminidase (lane 3). Fig. 2B demonstrates the migration of this glycolipid without (lane 2) and with treatment with α-galactosidase (lane 3). Fig. 2C demonstrates the migration of this lipid without (lane 2) and with treatment with endo-β-galactosidase (lane 3). Fig. 2D demonstrates the migration of purified guinea pig lactosyl ceramide without (lane 2) and with treatment with β-galactosidase (lane 3). CMH, glucosyl ceramide; CDH, lactosyl ceramide; CTH, trihexosyl ceramide; Gb4, globoside.

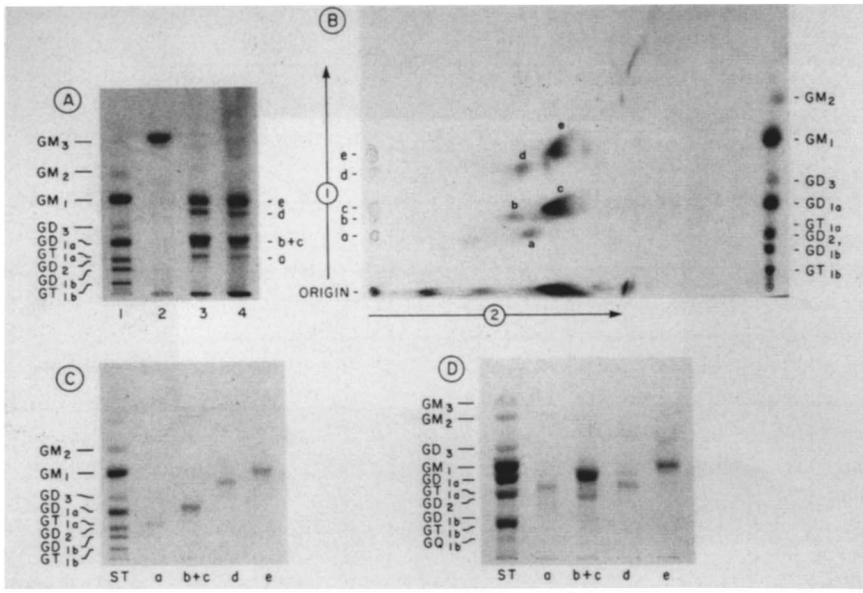

Fig. 3. HPTLC of gangliosides. Fig. 3A demonstrates the separation of human brain gangliosides in lane 1; lane 2, human platelet gangliosides; lane 3, guinea pig platelet gangliosides; lane 4, guinea pig megakaryocyte gangliosides. The chromatoplate in Fig. 3A was developed with neutral solvent system C, and the bands were visualized with orcinol-sulfuric acid reagent. Fig. 3B demonstrates the separation of guinea pig platelet gangliosides in a two-dimensional HPTLC system. The spots were visualized with orcinol-sulfuric acid reagent. Human brain gangliosides served as standards for the identification of the separated guinea pig platelet gangliosides. Figs. 3C and 3D demonstrate the separation of the five guinea pig gangliosides that had been purified by preparative TLC. Fig. 3C represents the separation with neutral HPTLC system C, and Fig. 3D represents the separation with basic HPTLC system D. Lanes ST demonstrate the separation of human brain ganglioside standards and lanes a–e represent the separation of the five guinea pig platelet gangliosides purified by preparative TLC. The bands were visualized with resorcinol-HCl reagent. ST, standards; a–e, designations for the five guinea pig platelet gangliosides.

Gangliosides e and d reacted with the β-subunit of cholera toxin and thus e and d were identified as GM1 and d was considered to be GM1 with a different fatty acid content. The desialylation of Ganglio series gangliosides yields a common core (25, 26), asialoGM1 (Gal-GalNAc-Gal-Glc-Cer). **Fig. 4A** demonstrates that the five bands that had been desialylated by treatment with neuramidase from *Arthrobacter ureafaciens* had reacted with the anti-asialoGM1 antibody. Thus, these experiments indicated that bands e and d contained an asialoGM1 core after desialylation and thus provided further evidence that these bands represented GM1. It is interesting that the other bands also contained the asialoGM1 core and thus belong to the Ganglio series.

The data obtained from microdensitometry from three experiments could only be analyzed as relative amounts of ganglioside species present in each of the two cells due to the small amounts of available material. There were equivalent amounts of gangliosides in megakaryocytes and platelets when the two cells were compared at a 1/1000 ratio. There were no significant differences in the species of gangliosides or the percent distribution of these species in megakaryocytes compared to platelets. Gangliosides a, c, and e represented about two-thirds of guinea pig megakaryocyte and platelet gangliosides. As mentioned above, ganglioside e was identified as GM1. Gangliosides b and d represented about one-third of gangliosides.

Megakaryocytes and platelets were compared for the ability to synthesize ceramides and glycolipids from [^{14}C]acetate, [^{14}C]palmitate, and [^{3}H]galactose and the data are shown in **Fig. 5**. Megakaryocytes were incubated for 17 h while platelets were incubated for 4 h. Despite the differences in incubation times, the data are valid because maximal synthesis of phospholipids and neutral lipids (8) and glycolipids occurred within 1 h in platelets. It is not feasible to maintain platelet viability during incubations longer than 6 h. Megakaryocytes and platelets were compared at a ratio of 1/1000 to normalize for protein content and volume (7–11). Fig. 5a demonstrates that the synthesis of ceramides from palmitic acid is about 5 times greater in megakaryocytes than in platelets and synthesis from acetate is 10 times greater in megakaryocytes than in platelets. Fig. 5b shows that platelets have virtually no capacity to synthesize neutral glycolipids, most likely due to a markedly decreased capacity for glycosylation in comparison to megakaryocytes.

The synthesis of ceramides and glycolipids was studied in megakaryocyte subpopulations at different phases of maturation. Four megakarocytes populations at different

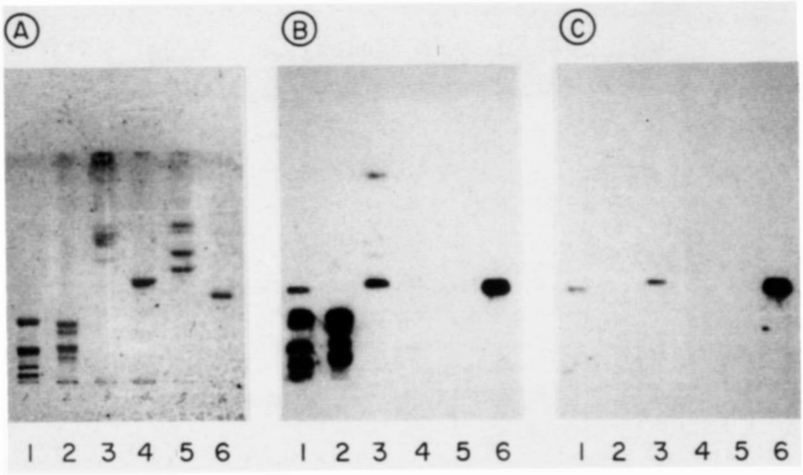

Fig. 4. HPTLC-immunostaining of gangliosides and neutral glycolipids with anti-asialo GM1 antibody. Fig. 4A demonstrates the separation of glycolipids by a neutral HPTLC system C, and the bands were visualized with orcinol; lane 1 represents bovine brain gangliosides; lane 2, guinea pig platelet gangliosides; lane 3, guinea pig platelet neutral glycolipids; lane 4, human platelet gangliosides; lane 5, human platelet neutral glycolipids; and lane 6, asialo-GM1 standard. Figs. 4B and 4C demonstrate the results of autoradiographic immunostaining of chromatoplates that contained the same samples and had been developed in the same solvent as that in Fig. 4A; the lipids in Fig. 4B were treated with *A. ureafaciens* neuraminidase before exposure to the anti-asialo GM1 antibody and the lipids in Fig. 4C were not treated with neuramidase prior to exposure to anti-asialo GM1 antibody. ^{125}I-labeled protein A was used in the autoradiographic procedure to visualize the lipids that had reacted with the anti-asialoGM1.

stages of maturation were isolated by the Celsep procedure and incubated with the radiolabeled acetate for 18 h. Group I contained primarily immature megakaryocytes, groups II and III contained megakaryocytes of intermediate maturity, and group IV contained primarily mature megakaryocytes. The data shown in **Fig. 6** represent the sum of both ceramide and glycolipid syntheses from radiolabeled acetate and palmitic acids. Palmitic acid was primarily incorporated into immature megakaryocytes while significantly more acetate was incorporated in mature than immature cells. About 75 ± 3.4% (mean ± SD) (n = 5) of acetate and palmitic acid was incorporated into ceramides and 24.9 ± 3.1% (mean ± SD) (n = 5) into neutral glycolipids in megakaryocytes at all phases of maturation.

The assessment of the incorporation of radiolabeled galactose into ceramide species separated by TLC revealed that galactose was only detected in glucosyl cera-

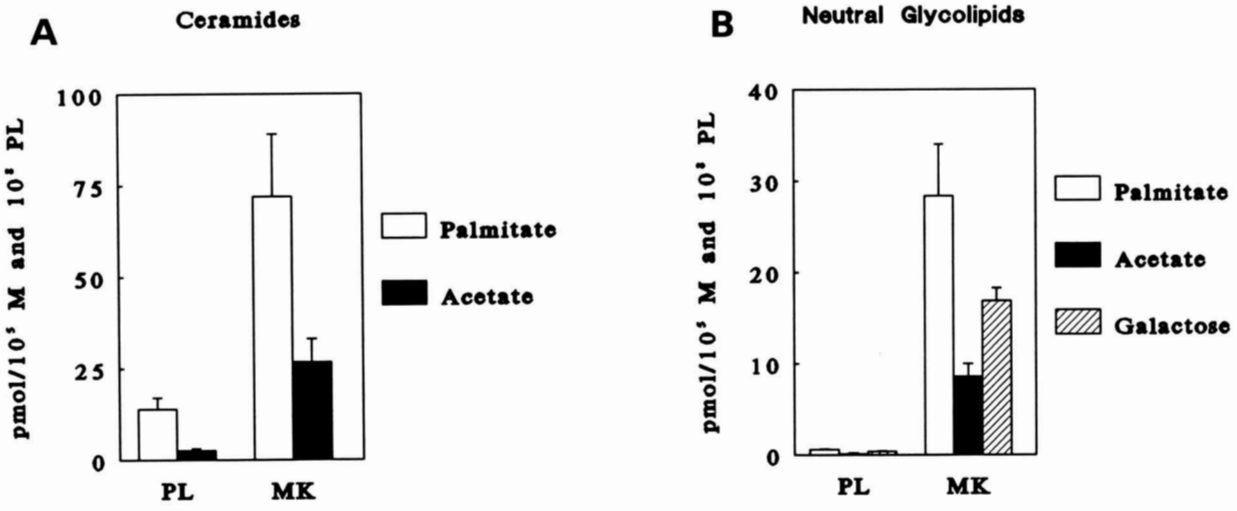

Fig. 5. Synthesis of ceramides and glycolipids in megakaryocytes and platelets. Isolated megakaryocytes were incubated with [^{14}C]acetate, [^{3}H]palmitic acid, and [^{3}H]galactose for 17 h; ceramides and glycolipids were extracted and purified and separated by TLC, and radioactivity in ceramides and glycolipids was determined by scraping bands and by scintillation spectrometry. Data represent the total of ceramides and neutral glycolipids. Fig. 5A represents ceramide synthesis and Fig. 5B represents glycolipid synthesis.

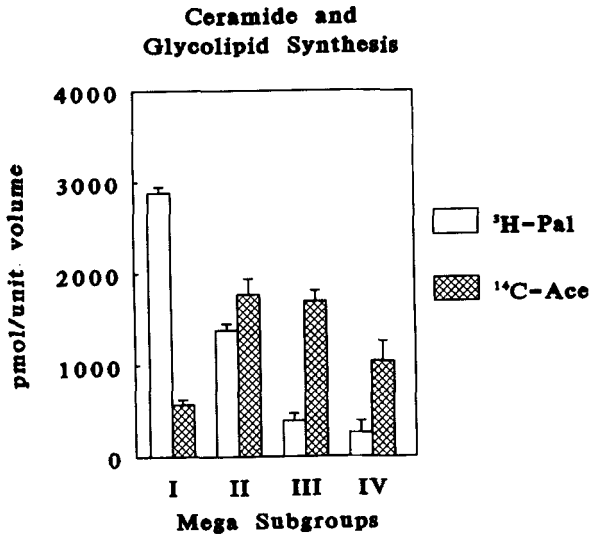

Fig. 6. Synthesis of ceramides and glycolipids in megakaryocyte subpopulations at different phases of maturation. Megakaryocytes were separated into four population groups: Group I contained primarily immature megakaryocytes; Groups II and III contained megakaryocytes of intermediate maturity; and Group IV contained predominantly large mature megakaryocytes. The four megakaryocyte groups were incubated with [^{14}C]acetate and [^{3}H]palmitic acid for 17 h; ceramides and glycolipids were extracted and purified and separated by TLC, and radioactivity in ceramides and glycolipids was determined by scraping bands and by scintillation spectrometry.

mide and thus galactose had been converted to glucose. The incorporation of radiolabeled acetate and palmitic acid into sphingosine, fatty acid, and carbohydrate moieties of ceramides and glycolipids was determined after acid methanolysis of these lipids. This analysis revealed that 72.2 ± 11.4% (mean ± SD) (n = 5) of the radiolabeled palmitic acid could be detected in the fatty acid and the remainder in the sphingosine component of ceramides and glycolipids; 95.9 ± 1.8% (mean ± SD) (n = 4) of radiolabeled acetate was detected in fatty acid and 10% sphingosine component of ceramides and glycolipids. Neither palmitic acid or acetate had been incorporated into the glycolipid carbohydrates.

DISCUSSION

This is the first study to identify the neutral glycolipids and gangliosides that are present in guinea pig megakaryocytes and platelets. AsialoGM2 was found to be a major neutral glycolipid in guinea pig megakaryocytes and platelets, and these cells were found to contain GM1 and several other gangliosides. There is substantially less asialoGM2 but greater amounts of a less complex glycolipid, glucosyl ceramide, in guinea pig megakaryocytes than in platelets.

There are significant differences between the glycolipid composition of guinea pig, porcine, and human platelets. Lactosyl ceramide is the major neutral glycolipid in human platelets and is present in substantial amounts in porcine and guinea pig platelets. AsialoGM2 and asialoGM1 are not present in human platelets. Guinea pig and human platelets differ in that only human platelets contain globoside and trihexosyl ceramide. Porcine platelets contain trihexosyl ceramide but, unlike human platelets, do not contain globoside. Pig platelets were found to contain at least eight gangliosides but their identity was not well characterized (33). It appears that both guinea pig and porcine platelets have a greater number and diversity of gangliosides than human platelets since the latter contains primarily GM3 ganglioside (3, 4).

It is known that there is diversity of glycolipids in tissues such as the nervous system in various animal species (20). Thus, it would be important to reconcile the heterogeneity of glycolipids in animal species with the proposed common functions of glycolipids such as adhesion and the regulation of maturation. There is evidence that different species of glycolipids can mediate the same physiological activity. For example, gangliosides GM1 and GM3 can modulate the response to growth factors (35), gangliosides GM3 and GD3 can bind serotonin (3, 5) and gangliosides GM2 and GD3 and GD1a are important for cell adhesion (34). However there are differences to the extent that these gangliosides influence these cellular activities (5, 35, 36).

There is fragmentary evidence for the role of glycolipids in platelet function. Platelet GM3 ganglioside is thought to bind serotonin (3, 5, 6). Substantial amounts of GM3 ganglioside become exposed on the surface of thrombin-activated platelets (4). Anti-glycolipid antibodies have been detected in patients with Idiopathic Thrombocytopenia Purpura (37). Guinea pig and human platelets are similar in that both aggregate in response to adenosine diphosphate and other aggregating agents, both can secrete adenine nucleotides and serotonin and can produce thromboxane A2 (38, 39), but there are quantitative differences in their response to these agents. Most likely, different gangliosides are involved in similar physiological activities in human and guinea pig platelets. The differences in the sensitivity of human and guinea pig platelets to aggregating agents such as thrombin and collagen may be influenced by the differences in the glycolipid composition of human and guinea pig platelets.

Two aspects of glycolipid synthesis were studied, the synthesis of ceramides and the glycosylation of ceramides. The synthesis of ceramides was 5 times greater from palmitic acid and 10 times greater from acetate in megakaryocytes than in platelets. These data indicate that ceramides are primarily synthesized in megakaryocytes but platelets retain a significant capacity to synthesize free ceramides. However, the glycosylation of ceramides occurs almost exclusively in megakaryocytes and only in trace amounts in platelets. There are considerably more free ceramides in platelets than in other cells but the function of ceramides is not known.

The glycosylation of ceramides for the synthesis of glycolipids was studied by the incorporation of galactose into glycolipids. Galactose had been converted to glucose, most likely due to the activity of an epimerase, since the radiolabeled cerebrosides were shown to be glucosyl ceramides. Glucosyl ceramide was the principal glycolipid synthesized from radiolabeled galactose. The utilization of galactose for the synthesis of glucosyl ceramide could be easily demonstrated in megakaryocytes while this activity was markedly decreased in platelets, about 1/42 of that in megakaryocytes. In megakaryocytes, ceramides serve as intermediates in glycolipid metabolism. It appears that platelets have virtually no ability to glycosylate ceramides and that ceramides synthesized from palmitic acid or acetate remain primarily as free ceramides in platelets.

We have previously shown that megakaryocytes and platelets have different capacities for lipid synthesis. Cholesterol can be synthesized in megakaryocytes but not in platelets while platelets are capable of de novo phospholipid and fatty acid synthesis (40). Only megakaryocytes can acylate all pools of arachidonic acid and are capable of desaturating eicosatrienoic acid for the synthesis of arachidonic acid (9). Now we have provided evidence that ceramide and glycolipid syntheses, like cholesterol synthesis, occur primarily in megakaryocytes. Thus the glycolipid composition of platelets is determined in the megakaryocyte and platelets cannot restructure the membrane content of these lipids.

The study also defined differences in the synthesis of ceramides and glycolipids in megakaryocytes at different stages of maturation. The synthesis of ceramides and glycolipids from palmitic acid occurred primarily in the most immature megakaryocytes while mature megakaryocytes have little capacity to utilize palmitic acid for ceramide and glycolipid synthesis. The synthesis of ceramides and glycolipids from acetate, however, occurred primarily in megakaryocytes of intermediate maturity with very little synthesis in the most immature megakaryocytes. In the course of ceramide synthesis, palmitate was incorporated into both the sphingosine and fatty acid moieties of ceramides, while acetate was almost exclusively incorporated into the fatty acid moiety of ceramides. Thus, there is considerable compartmentalization of glycolipid synthesis in megakaryocytes at different phases of maturation. We have previously reported that most aspects of phospholipid and neutral lipid synthesis occur primarily in immature megakaryocytes while de novo fatty acid synthesis occurs predominantly in mature megakaryocytes (40). It appears that various aspects of lipid synthesis are active at different phases of megakaryocyte maturation. Thus, membrane synthesis and assembly differ at various phases of megakaryocyte development, and these differences most likely are relevant for megakaryocyte maturation.

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