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Biosynthesis of Glycosphingolipids by Human Myeloid Leukemia Cells†

Joanne Buehler,‡ Eileen Qwan,‡ Michael W. DeGregorio,§ and Bruce A. Macher*,‖

Cancer Research Institute and Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, California 94143, and Children's Cancer Research Institute, Pacific Presbyterian Medical Center, San Francisco, California 94115

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ABSTRACT: We have performed comparative studies of the neutral glycosphingolipids synthesized by three human myeloid leukemia cell lines, K562, KG1, and HL-60, which were metabolically labeled with [^{14}C]galactose, to evaluate changes in neutral glycosphingolipid synthesis with myeloid cell differentiation. Individual neutral glycosphingolipids containing one to four sugars were purified by a combination of the following methods: diethylaminoethyl-Sephadex column chromatography, acetylation–Florisil column chromatography, and high-performance liquid chromatography using an Iatrobead column. Compounds with one sugar were analyzed by thin-layer chromatography on borate plates. This analysis showed that HL-60 cells synthesize only glucosylceramide, whereas K562 and KG1 cells synthesize predominately glucosylceramide, but also a small amount of galactosylceramide. Compounds with two to four sugars were characterized by treatment with exo- and endoglycosidases. The results showed that K562 and KG1 cells are similar to cells from patients with acute leukemia in expressing two series (globo and neolacto) of natural glycosphingolipids, whereas the HL-60 cells are similar to mature human myeloid cells in expressing only one series (neolacto). Therefore, human myeloid leukemia cells blocked at different stages of differentiation vary in their ability to synthesize neutral glycosphingolipids.

A number of biochemical changes are known to occur in leukocytes undergoing differentiation including altered expression of cell surface components. Our own work has been directed toward gaining information on the changes which occur in one class of cell surface glycoconjugates (glycosphingolipids) of human leukocytes. In the past few years, we have purified and structurally characterized the glycosphingolipids of normal human peripheral blood leukocytes and leukemia cells (Table I). These studies have demonstrated a number of differences in the glycosphingolipid composition of different populations of normal and leukemic leukocytes. With respect to neutral glycosphingolipids, we found that well-differentiated forms of human lymphoid cells [normal peripheral blood lymphocytes and chronic lymphocytic leukemia (CLL)[1] cells] contain only globo-type compounds, whereas well-differentiated forms of human myeloid cells (normal peripheral blood granulocytes and CML cells) contain neolacto- and gala-type compounds. Therefore, these two classes of well-differentiated human leukocytes, which are derived from a common stem cell precursor, express completely different classes of neutral glycosphingolipids. Our characterization of neutral glycosphingolipids from poorly differentiated human leukocytes (acute leukemia cells) has shown that, regardless of cell lineage, these cells all contain both globo and neolacto compounds. From these results, we have concluded that poorly differentiated leukocytes contain both neolacto and

Table I: Glycosphingolipid Structures

abbreviation	structure	name
GalCer	Galβ1→1Cer	galactosylceramide
GlcCer	Glcβ1→1Cer	glucosylceramide
Gal_2Cer	Galα1→4Galβ1→1Cer	galabiosylceramide
LacCer	Galβ1→4Glcβ1→1Cer	lactosylceramide
Gb_3Cer	Galα1→4Galβ1→4Glcβ1→-1Cer	globotriaosylceramide
Lc_3Cer	GlcNAcβ1→3Galβ1→-4Glcβ1→1Cer	lactotriaosylceramide
Gg_3Cer	GalNAcβ1→4Galβ1→-4Glcβ1→1Cer	gangliotriaosylceramide
Gb_4Cer	GalNAcβ1→3Galα1→-4Ga1β1→4Glcβ1→1Cer	globotetraosylceramide
nLc_4Cer	Galβ1→4GlcNAcβ1→-3Galβ1→4Glcβ1→1Cer	neolactotetraosylceramide

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* Address correspondence to this author at the Cancer Research Institute, University of California, San Francisco.
‡ Cancer Research Institute.
§ Children's Cancer Research Institute.
‖ Cancer Research Institute and Department of Pharmaceutical Chemistry.

[1] Abbreviations: Cer, ceramide; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; DCE, dichloroethane; DEAE-Sephadex, diethylaminoethyl-Sephadex; GL_1, monohexosylceramide; GL_2, dihexosylceramide; GL_3, trihexosylceramide; GL_4, tetrahexosylceramide; GM_3, NeuAcα2→3Galβ1→4Glcβ1→1Cer; GM_2, GalNAcβ1→4(NeuAcα2→3)Galβ1→4Glcβ1→1Cer; GD_3, NeuAcα2→-8NeuAcα2→3Galβ1→4Glcβ1→1Cer; PBS, phosphate-buffered saline; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; PPO, 2,5-diphenyloxazole.

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globo neutral glycosphingolipids but as these cells mature they loose the ability to express one or the other of these classes of neutral glycosphingolipids depending on their cell lineage (i.e., myeloid cells express only neolacto compounds, and lymphoid cells express only globo compounds).

In order to further characterize the relationship between human leukocyte differentiation and neutral glycosphingolipid expression, we require populations of human leukocytes which are blocked at specific stages of differentiation. Such cells are available in the form of human leukemia cell lines. We have chosen to study the neutral glycosphingolipids of three human myeloid cell lines which have characteristics of cells blocked at three distinct stages of differentiation. K562 is a line of undifferentiated blast cells which were obtained from a CML patient in blast crisis (Lozzio & Lozzio, 1975). KG1 is a line of myeloblastic cells obtained from the bone marrow of a patient with erythroleukemia (Koeffler & Golde, 1978). HL-60 is a promyelocytic cell line obtained from a patient with acute promyelocytic leukemia (Collins et al., 1977). Rosenfelder et al. (1982) have demonstrated that K562 and HL-60 can incorporate ^{14}C-labeled sugars into the neutral glycosphingolipids of their cells; however, the structures of these compounds have not been determined (Ziegler et al., 1981). In the present study, we have purified and structurally characterized the neutral glycosphingolipids synthesized by these three human myeloid leukemia cell lines from [^{14}C]galactose. The results from these studies suggest that alterations occur in the biosynthesis of neutral glycosphingolipids in myeloid cells between the myeloblastic and promyelocytic stages of differentiation.

Materials and Methods

Cell Cultures. The three cell lines used in our study were KG1, K562, and HL-60. They were grown in suspension [(2–7) × 10^5 cells/mL, 40 mL/flask] in a medium consisting of RPMI-1640, 10% fetal calf serum (15% for KG1 cells), 2 mM glutamine, and 10 units/mL penicillin and streptomycin. During logarithmic growth, each culture was incubated with 30 μCi of D-[1-^{14}C]galactose (50 mCi/mmol, Amersham, Arlington Heights, IL) and incubated at 37 °C under 7% CO_2 for 48 h. The cells were washed with PBS and resuspended in chloroform/methanol (2:1 v/v) to begin the extraction process.

Standard Glycosphingolipids. Gb_3Cer and Gb_4Cer were purchased from Supelco (Bellefonte, PA). GlcCer, GalCer, LacCer, Lc_3Cer, and nLc_4Cer were purified from human myeloid leukemia cells by standard methods including HPLC and structurally characterized by chemical and enzymatic methods, as previously described (Macher & Klock, 1980).

Extraction and Purification of Neutral Glycosphingolipids. The detailed procedure for isolation of glycosphingolipids can be found in our previous publications (Klock et al., 1981; Lee et al., 1981; Macher et al., 1981; Macher & Klock, 1980). Briefly, glycosphingolipids were extracted at room temperature with 20 volumes of each of the following chloroform/methanol mixtures: 2:1, 1:1, and 1:2 (v/v). The total lipid extract was dried in vacuo, resuspended in chloroform/methanol/water (30:60:8 v/v), and applied to a DEAE-Sephadex anion-exchange column (Sigma, St. Louis, MO; 40–120 μm, 1 × 20 cm). After 30 min, the neutral fraction was eluted with chloroform/methanol/water (30:60:8) (40 mL).

Acetylation–Florisil Column Chromatography–Deacetylation. Purification of the neutral glycosphingolipid fraction from total neutral lipids was done by acetylation and Florisil column chromatography followed by deacetylation (Saito et al., 1971). The dry sample was resuspended in pyridine/acetic anhydride (1.5:1 v/v) and kept at room temperature for 18 h. Toluene was added and the sample dried in vacuo. The acetylated sample was resuspended in hexane/DCE (1:4 v/v) and applied to a Florisil column (1 × 20 cm) (60–100 mesh; Fisher Scientific Co., Fair Lawn, NJ). Four fractions were generated by elution with 100 mL each of the following: (A) hexane/DCE (1:4 v/v); (B) DCE alone; (C) DCE/acetone (1:1 v/v); (D) DCE/methanol/water (2:8:1 v/v). The four fractions were analyzed by TLC (HPTLC, 10 × 10 cm silica gel 60 plates; EM Laboratories, Inc., Cincinnati, OH) using a solvent system containing chloroform/methanol (90:10 v/v) and visualized by autoradiography (see below). The neutral glycosphingolipid-containing fraction (C) was deacetylated as previously described (Lee et al., 1982).

Enrichment of the Individual Neutral Glycosphingolipids by Silicic Acid Column Chromatography. The total neutral glycosphingolipid fraction was dissolved in chloroform and applied to a column containing silicic acid (1 × 20 cm) (Unisil, 100–200 mesh; Clarkson Chemical Co. Inc., Williamsport, PA). The column was eluted successively with (60 mL) (a) chloroform, chloroform/methanol mixtures of (b) 90:10, (c) 80:20, or (d) 75:25, (e) chloroform, (f) acetone/methanol (90:10), and (g) methanol. Fractions b and c contained predominantly GL_1 and GL_2, respectively. Fraction d contained predominantly GL_3, and most of the GL_4 was eluted in fraction f.

High-Performance Liquid Chromatography. Individual neutral glycosphingolipid fractions were purified by HPLC using a Du Pont 850 liquid chromatography system with an Iatrobead column (25 cm × 4.6 mm) (6RS8010 Iatron Chemical Products, Tokyo, Japan). A 30-min linear gradient solvent system of 2-propanol/hexane/water [A (55:44:1) and B (55:35:10) v/v] (0–100% B) was used followed by a 5-min hold at 100% B at a flow rate of 2 mL/min. One-milliliter fractions were collected, and an aliquot of each was assayed for radioactivity by liquid scintillation analysis (Beckman, LS-100C).

Thin-Layer Chromatography and Autoradiography. Purification of the neutral glycosphingolipids was monitored by TLC on silica gel 60 plates (HPTLC; EM Laboratories, Inc., Cincinnati, OH) using chloroform/methanol/water (60:35:8 v/v) as the solvent system. Following chromatography, the plate was air-dried and soaked with a solution of 2,5-diphenyloxazole (PPO) (Fisher) in toluene/hexane (1:1 v/v), dried, incubated with the X-ray film (Kodal XAR-2) at 4 °C, and subsequently developed. To quantify the neutral glycosphingolipids separated on the TLC, individual spots were scraped into liquid scintillation vials and assayed for radioactivity.

Separation of glucosylceramide from galactosylceramide was done according to the procedure of Kean (1966) as follows. Uniplate (Analtech Inc., Newark, DE) silica gel G 250-μm plates were dipped in the supernate of a saturated solution of sodium borate (Fisher Scientific), dried, and spotted. The plate was developed in the solvent system used for neutral glycosphingolipids, chloroform/methanol/water (60:35:8 v/v), dipped in PPO, and autoradiographed.

Enzymatic Degradation of Glycosphingolipids. The conditions for enzymatic degradation of glycosphingolipids have been previously described (Li & Li, 1977; Macher et al., 1981). The sources of the enzymes used for this work were as follows: jack bean β-galactosidase and β-hexosaminidase (Sigma); *Escherichia freundi* endo-β-galactosidase (kindly provided by Dr. Fukuda, La Jolla Cancer Research Institute) and α-galactosidase extracted from ficin (Sigma). Isolation of α-ga-

lactosidase was done following the procedure of Hakomori et al. (1971) and Li & Li (1972) to the stage of precipitating the enyzme obtained from the Bio-Gel P-60 column by adding ammonium sulfate. Enzyme assays were done at various stages of the purification following the procedure of Suzuki et al. (1970). The final enzyme product was tested with standard glycosphingolipids and showed only α-galactosidase activity.

Glycosidase assays were stopped by adding chloroform/methanol (1:1 v/v) to the reaction mixtures. Samples from exoglycosidase treatment were dried and resuspended in chloroform/methanol/water (30:60:8 v/v), chromatographed on a DEAE-Sephadex Pasteur pipet-size (0.5 × 8 cm) column to remove detergent, and analyzed by TLC autoradiography. Products obtained from endo-β-galactosidase treatment were dried and resuspended in 5 parts chloroform/methanol (2:1 v/v) and 1 part water (Folch et al., 1957). The glycosphingolipids in the lower phase were chromatographed on a Pasteur pipet-size DEAE-Sephadex column. The upper phase product (oligosaccharide) and lower phase product (neutral glycosphingolipid) were analyzed by TLC–autoradiography.

TLC Analysis of Different Types of Acetylated Trihexosylceramides and Their Enzyme Products. Joseph & Gockerman (1975) have reported a procedure (see below) which allows the separation of trihexosylceramides which contain amino sugars from those which do not. This procedure was used to separate the trihexosylceramides isolated from the cell lines. It was also used to analyze products (the trihexosylceramides that remained after treatment) obtained from exoglycosidase treatment of the trihexosylceramides from the cell lines. The following procedure was used to isolate the unhydrolyzed trihexosylceramides. The reaction mixture from the exoglycosidase treatments of the trihexosylceramides was chromatographed on TLC plates as described above, and the unhydrolyzed GL_3 bands were scraped from the plate and extracted from the silica. This material was acetylated by the procedure of Saito & Hakomori (1971) and analyzed by TLC–autoradiography using the solvent system DCE/methanol (9:1 v/v).

Results

Purification of ^{14}C-Labeled Neutral Glycosphingolipids. In this study, the neutral glycosphingolipids of three myeloid human leukemia cell lines were labeled with [^{14}C]galactose. This avoids possible errors since radioactive glycosphingolipids synthesized by the cells can be distinguished from unlabeled glycosphingolipids present in the serum of the culture medium. Purification of the neutral glycosphingolipids was done according to procedures outlined under Materials and Methods. All three cell lines incorporated [^{14}C]galactose into several neutral glycosphingolipids which were separated by TLC. An example of the pattern for HL-60 cells is shown in Figure 1, lane 1. The short-chain ^{14}C-labeled glycosphingolipids cochromatographed with standard glycosphingolipids containing one, two, three, and four sugars. Radiolabeled bands were also detected below the GL_4 standard band on the TLC autoradiogram. The major compounds in HL-60 chromatographed with standards containing two and four sugars. Each radiolabeled glycosphingolipid from the cell line occurred as a doublet which is most likely due to differences in the ceramide composition, as was previously found with glycosphingolipids from human leukocytes (Westrick et al., 1983). Similar patterns were seen with K562 and KG1 cells except that the neutral glycosphingolipid containing one sugar was predominant in these two cell lines (Figure 1, lanes 2 and 3).

Individual neutral glycosphingolipids were purified from each cell line by silicic acid column chromatography and

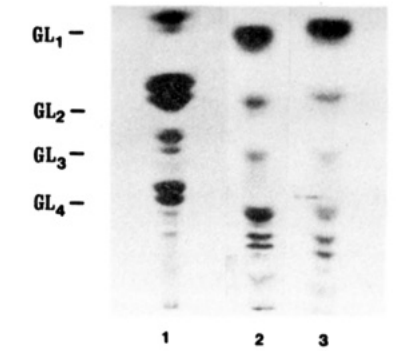

FIGURE 1: TLC autoradiograms of the total neutral glycosphingolipids from [^{14}C]galactose-labeled human myeloid leukemia cell lines; lane 1, HL-60; lane 2, KG1; lane 3, K562. Each lane is a separate chromatogram developed independent of the other two. Conditions: HPTLC silica gel 60 plate; solvent system chloroform/methanol/water (60:35:8 v/v).

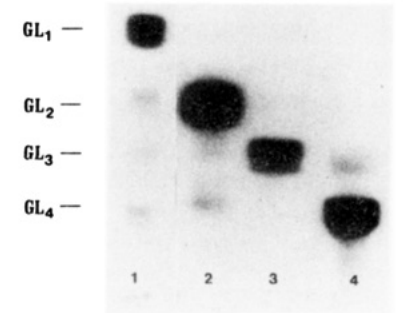

FIGURE 2: TLC autoradiogram of purified [^{14}C]galactose-labeled neutral glycosphingolipids from HL-60 cells: lane 1, monohexosylceramide; lane 2, dihexosylceramide; lane 3, trihexosylceramide; lane 4, tetrahexosylceramide. Conditions as in Figure 1.

HPLC. Figure 2 shows a TLC autoradiogram of the fractions obtained from HL-60 cells by these procedures. Lanes 1–4 contain neutral glycosphingolipids designated GL_1–GL_4, respectively. Similar fractions were purified from each cell line. The purified compounds were used for structural characterization of the mono-, di-, tri-, and tetrahexosylceramide species of the three cell lines.

Characterization of GL_1 and GL_2. The GL_1 fractions did not appear to be hydrolyzed by α-galactosidase, β-galactosidase, or β-hexosaminidase. These results indicate the presence of GlcCer and not GalCer (gala series). However, small amounts of hydrolysis would not be detected by this method. Complete or near-complete hydrolysis would result in the disappearance of the radiolabeled GL_1 band; however, a slight reduction in intensity of the band due to partial hydrolysis would not be detected. Therefore, further analyses were done with borate-dipped silica gel G TLC plates. These results indicate clearly that HL-60 cells synthesize glucosylceramide exclusively and that both K562 and KG1 cells synthesize glucosylceramide predominately, with a small amount of galactosylceramide (Figure 3).

The GL_2 fraction from each cell line was not hydrolyzed by β-hexosaminidase, or α-galactosidase, whereas they were hydrolyzed by β-galactosidase. These results indicate that lactosylceramide is the only GL_2 species of each cell line.

Characterization of Trihexosylceramides. The HPLC chromatograms of the enriched trihexosylceramide fractions from the three cell lines show distinct patterns (Figure 4). HL-60 cells yielded one GL_3 peak, whereas the other two cell lines (K562 and KG1) yielded more than one GL_3 peak. These results suggest that HL-60 cells may contain a single type of GL_3 and that the other two cell lines contain more than one

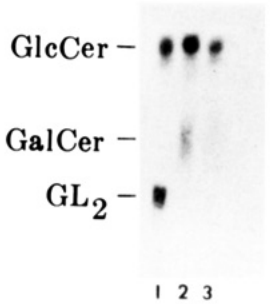

FIGURE 3: TLC autoradiogram on a borate plate of the [^{14}C]-galactose-labeled monohexosylceramides from human myeloid leukemia cell lines: lane 1, HL-60; lane 2, K562; lane 3, KG1. The monohexosylceramide fraction from the HL-60 cells used in the chromatogram also contained some dihexosylceramide. Conditions: silica gel G plate; solvent system chloroform/methanol/water (60:35:8 v/v).

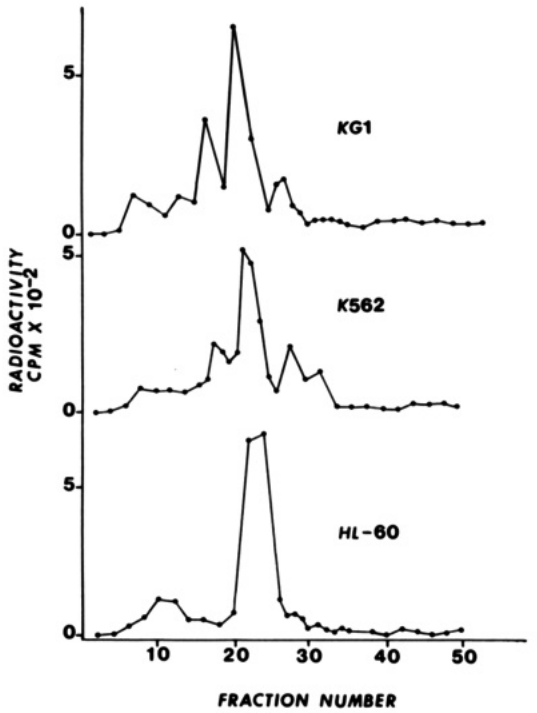

FIGURE 4: HPLC chromatograms of the [^{14}C]galactose-labeled trihexosylceramides from the human myeloid leukemia cell lines. Chromatograms of silicic acid column fractions enriched for GL_3. The peak at fractions 6–14 is due to a small quantity of LacCer. HL-60 cells yield a single GL_3 peak (fractions 18–36) whereas KG1 and K562 cells yield multiple GL_3 peaks.

type of GL_3. To confirm this, trihexosylceramides from the three cell lines were treated with exoglycosidases. Treatment of HL-60 GL_3 with β-hexosaminidase gave complete hydrolysis, whereas α-galactosidase and β-galactosidase had no effect. Trihexosylceramides from KG1 and K562 were not completely hydrolyzed by any of the exoglycosidases. However, they were partially hydrolyzed by both α-galactosidase and β-hexosaminidase as shown in Figure 5. Approximately 40% (300 cpm/800 cpm) of KG1 GL_3 was hydrolyzed by α-galactosidase (Figure 5, lane 1) and 60% (500 cpm/900 cpm) by β-hexosaminidase (Figure 5, lane 2). β-Galactosidase did not hydrolyze KG1 GL_3 (not shown). Similar results were obtained with K562 GL_3 and these three enzymes. Approximately 30% (300 cpm/1100 cpm) of the K562 GL_3 was hydrolyzed by α-galactosidase (Figure 5, lane 4) and approximately 70% (875 cpm/1290 cpm) by β-hexosaminidase (Figure 5, lane 6). K562 GL_3 was not hydrolyzed by β-galactosidase (Figure 5, lane 5).

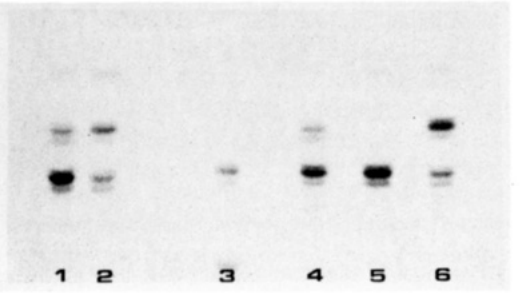

FIGURE 5: TLC autoradiogram of glycosidase-treated [^{14}C]-galactose-labeled trihexosylceramide fractions from K562 and KG1 cells: lane 1, KG1 GL_3 treated with α-galactosidase; lane 2, KG1 GL_3 treated with β-hexosaminidase; lane 3, K562 GL_3 untreated; lane 4, K562 GL_3 treated with α-galactosidase; lane 5, K562 GL_3 treated with β-galactosidase; lane 6, K562 GL_3 treated with β-hexosaminidase. Conditions as in Figure 1.

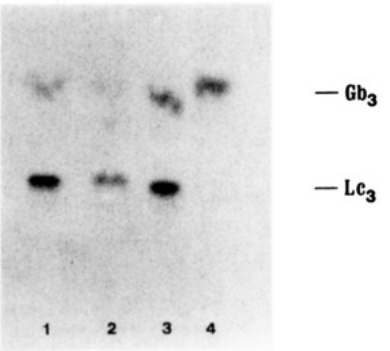

FIGURE 6: TLC autoradiogram of acetylated [^{14}C]galactose-labeled K562 GL_3 and K562 trihexosylceramide(s) remaining after treatment with glycosidase: lane 1, acetylated K562 GL_3; lane 2, acetylated K562 trihexosylceramide(s) remaining after treatment with α-galactosidase; lane 3, acetylated K562 trihexosylceramide(s) remaining after treatment with β-galactosidase; lane 4, acetylated K562 trihexosylceramide(s) remaining after treatment with β-hexosaminidase. Conditions: HPTLC silica gel 60 plate; solvent system chloroform/methanol (90:10 v/v).

These results suggest that K562 and KG1 cells synthesize at least two types of GL_3. However, since only partial hydrolysis occurred with the single enzyme treatment, there is a possibility that a GL_3 exists which is not hydrolyzed by any of the glycosidases. Therefore, a double enzyme hydrolysis of K562 GL_3 with α-galactosidase and β-hexosaminidase was done. Complete hydrolysis was observed with these two enzymes (not shown). Therefore, the trihexosylceramides that are synthesized by these cells have a nonreducing terminal residue of either α-galactose or β-*N*-acetylhexosamine. Therefore, HL-60 cells synthesize only a single type of GL_3, whereas K562 and KG1 synthesize two or more types.

To further characterize the ^{14}C-labeled trihexosylceramides, K562 GL_3 and its glycosidase products were acetylated and chromatographed on TLC to separate trihexosylceramides which contain amino sugars from those which do not. The unhydrolyzed acetylated K562 trihexosylceramides were separated into two components by TLC. One cochromatographed with standard acetylated Gb_3 (no amino sugar) (Figure 6, lane 1, upper band) and the other with standard acetylated Lc_3 (amino sugar containing) (lower band). The K562 trihexosylceramides remaining after exoglycosidase treatment were scraped from TLC plates, eluted from the silica gel, and acetylated. The K562 GL_3 remaining after α-galactosidase treatment cochromatographed with the amino sugar containing GL_3, acetylated Lc_3 (Figure 6, lane 2). The GL_3 which previously cochromatographed with Gb_3 was eliminated by this enzyme treatment. The K562 GL_3 remaining after

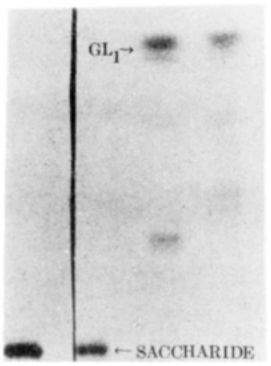

FIGURE 7: TLC autoradiogram of HL-60 GL_3 and GL_4 after treatment with endo-β-galactosidase: lane 1, saccharide product from GL_4; lane 2, saccharide product from GL_3; lane 3, glycosphingolipid product from GL_4; lane 4, glycosphingolipid product from GL_3. Conditions as in Figure 1.

β-hexosaminidase treatment cochromatographed with acetylated Gb_3 standard (Figure 6, lane 4). The GL_3 which cochromatographed with Lc_3 had been hydrolyzed to GL_2. The product of the K562 GL_3 treated with β-galactosidase was also acetylated and separated by TLC (Figure 6, lane 3). Since there was no hydrolysis, both types of GL_3 appear here as in lane 1. These results indicate the presence of both an α-Gal-terminal GL_3 and a hexosamino-terminal GL_3 in the K562 cells. These results are in agreement with those of the general exoglycosidase experiments described above.

To further elucidate the structure of the trihexosylceramides with a nonreducing terminal amino sugar from the three cell lines, each was incubated with endo-β-galactosidase, an enzyme which hydrolyzes only lacto (Galβ1→3GlcNAc) (Lc) and neolacto (Galβ1→4GlcNAc) (nLc) type glycoconjugates. Treatment of HL-60 GL_3 with endo-β-galactosidase resulted in complete hydrolysis, giving GL_1 and an oligosaccharide band on the TLC plate (Figure 7). This indicates that the only GL_3 synthesized by HL-60 cells is Lc_3. Only partial hydrolysis was observed for K562 and KG1 trihexosylceramides (not shown), suggesting that at least a portion of the trihexosylceramides from these cells is Lc_3.

Characterization of Tetraosylceramides. The HPLC chromatograms of the tetraosylceramides from the three cell lines show a single peak. TLC–autoradiographic analysis of the ^{14}C-labeled tetrahexosylceramides from each cell line gave a doublet which ran slightly below the band for standard globotetrahexosylceramide. The tetrahexosylceramides from the three cell lines were treated with exoglycosidases. Each GL_4 was completely hydrolyzed by β-galactosidase, whereas α-galactosidase and β-hexosaminidase had no effect. Hydrolysis of the GL_4 from each cell line by endo-β-galactosidase yielded an oligosaccharide and a GL_1 band only. An example for HL-60 GL_4 is shown in Figure 7. These results indicate that all three cell lines synthesize only lacto- or neolacto-type tetrahexosylceramides.

Discussion

In the parent study, we have analyzed [^{14}C]galactose-labeled neutral glycosphingolipids obtained from three myeloid leukemia cell lines. Our results show that all three cell lines synthesize GlcCer, LacCer, Lc_3Cer, and nLc_4Cer. K562 and KG1 cells synthesize two additional glycosphingolipids, GalCer and Gb_3Cer. These results are presented schematically in Figure 8. Thus, the less differentiated cells synthesize gala-, globo-, and neolacto-type neutral glycosphingolipids from [^{14}C]galactose, whereas the more differentiated promyelocytic cell line, HL-60, synthesis only neolacto neutral glycosphingolipids.

Our results with [^{14}C]galactose-labeled neutral glycosphingolipids of K562 cells can be compared with the work of others on unlabeled neutral glycosphingolipids of K562. Suzuki et al. (1981) characterized the neutral glycosphingolipids of K562 cells by TLC, carbohydrate analysis, and immunofluorescence of whole cells using polyclonal rabbit anti-glycosphingolipid antibodies. They reported the presence of GalCer, GlcCer, Lc_3Cer, and nLc_4Cer in K562 cells. In agreement, our results demonstrate that K562 cells can synthesize these glycosphingolipids. However, we also found that K562 cells can synthesize Gb_3Cer. Our results demonstrate that approximately one-third of the trihexosylceramides synthesized by K562 cells is Gb_3Cer. Kannagi et al. (1983) studied the GL_3 of unlabeled K562 cells and, using specific monoclonal antibodies directed against Gb_3Cer, showed that the trihexosylceramide of K562 cells reacted significantly with anti-Gb_3 antibody. These antibody results were supported by enzymatic degradation of the purified K562 trihexosylceramide which showed the major component to be Gb_3. They also found a small quantity of Lc_3. Finally, it should be noted that although the major glycosphingolipid of K562 cells is known to be GM_2, they do not contain the asialo form of this ganglioside (i.e., Gg_3Cer) (Suzuki et al., 1981).

Concerning HL-60 cells, Momoi & Yokota (1983) have reported on the glycosphingolipids of HL-60 cells. Their work with unlabeled and tritium-labeled glycosphingolipids of HL-60 cells analyzed by TLC and TLC–autoradiography demonstrated that the major neutral glycosphingolipids present cochromatographed with LacCer and nLc_4Cer. Compounds comigrating with monohexosylceramide and Lc_3Cer were detected as minor components. Our enzymatic degradative studies of purified glycosphingolipids from HL-60 cells substantiate Momoi and Yokota's preliminary results, demonstrating that HL-60 cells contain only neolacto neutral glycosphingolipids. Recently, Nojiri et al. (1984) have studied the changes which occur in glycosphingolipid expression fol-

Pluripotent Stem Cell →	Undifferentiated Blast Cell →	Myeloblast →	Promylocyte →→→	Granulocytes (Neutrophils)
	K562	**KG1**	**HL-60**	
	GalCer GlcCer	GalCer GlcCer	GlcCer	
	LacCer	LacCer	LacCer	
	Gb_3Cer Lc_3Cer	Gb_3Cer Lc_3Cer	Lc_3Cer	
	Lc_4Cer	Lc_4Cer	Lc_4Cer	

FIGURE 8: Diagrammatic representation of the relationship between cell differentiation, cell lines, and neutral glycosphingolipids synthesized by the cell lines.

lowing in vitro differentiation of HL-60 cells by various chemicals. Differentiation of HL-60 cells to mature myeloid cells or macrophage-like cells resulted in quantitative, but not qualitative, changes in glycosphingolipids. In general, the HL-60 cells which differentiated to mature myeloid cells were enriched for glycosphingolipids with more complex oligosaccharide chains, whereas the macrophage-like cells showed an increase in the simple ganglioside GM_3 and a concomitant drop in LacCer. Thus, it appears that quantitative rather than qualitative alterations in glycosphingolipids occur in later stages of leukocyte differentiation.

Ours is the only study of neutral glycosphingolipids from KG1 cells. These cells are similar to K562 cells in that they have gala, globo, and neolacto structures. KG1 also resembles K562 in the relative incorporation of [^{14}C]galactose into neutral glycosphingolipids, showing greater incorporation into GL_1 than GL_2 or GL_4.

Our current results support the hypothesis which was proposed on the basis of our earlier work that poorly differentiated leukocytes contain both neolacto and globo neutral glycosphingolipids, but as these cells mature they lose the ability to express one or the other of these classes of neutral glycosphingolipids depending on their cell lineage (i.e., myeloid cells express only neolacto compounds, and lymphoid cells express only globo compounds). The current results also more clearly identify the stage of differentiation at which this change occurs. Using the three cell lines as models, we suggest that this alteration in neutral glycosphingolipid synthesis and expression occurs after the myeloblast stage and before or at the promyelocyte stage. A notable difference between our current results and those obtained with freshly isolated leukocytes is the stage at which gala compounds are expressed. We had found that mature myeloid cells (neutrophils and CML) have both Ga_1Cer and Ga_2Cer. However, only the less differentiated cell lines (KG1 and K562) synthesize gala compounds, whereas the more differentiated HL-60 cells did not synthesize any gala-type compounds. Further studies are necessary to determine the relationship between human leukocyte differentiation and the synthesis of gala glycosphingolipids.

Since our initial investigations showing a relationship between leukocyte cell lineage and the expression of particular glycosphingolipids (Macher et al., 1982), a number of other studies have demonstrated the restricted expression of glycosphingolipids by certain types of normal and transformed human leukocytes. The clearest example of this is the restricted expression of lacto-*N*-fucopentaose III (Le^x, My-1,X determinant, SSEA-1) by human myeloid cells (Gooi et al., 1983; Huang et al., 1983; Urdal et al., 1983; Girardet et al., 1983; Magnani et al., 1984). This sugar sequence is carried by several species of neutral glycosphingolipids of human neutrophils and is found on glycosphingolipids of myeloid cells from leukemia patients and established myeloid leukemia cell lines. Although many types of myeloid cells have glycosphingolipids with the lacto-*N*-fucopentaose sequence, it has been shown that the immunostaining pattern with glycosphingolipids from the various cells can differ. For example, neutrophils have a greater number of reactive glycosphingolipids than HL-60 cells, and the length of the carbohydrate moiety on some of the neutrophil compounds is greater (Huang et al., 1983) than that of HL-60 cells. This may represent another variation in glycosphingolipid structure which is related to the degree of cellular differentiation.

Kniep et al. (1983) and Nudelman et al. (1983) have described unique neutral glycosphingolipid patterns in cells derived from lymphoma patients. Kniep et al. (1983) demonstrated that Hodgkins cells can be distinguished from other leukocytes on the basis of the expression of Gg_3Cer. This glycosphingolipid can be isolated from Hodgkins cells and detected on these cells by immunofluorescence with an anti-Gg_3Cer monoclonal antibody. Burkitt lymphoma cells can also be distinguished from other leukocytes on the basis of their glycosphingolipid expression. However, it is not the expression of a particular neutral glycosphingolipid that distinguishes these cells but rather the quantity and cell surface expression of Gb_3Cer. Burkitt cells contain more than 100 times as much Gb_3Cer as other lymphoma and leukemia cells which can be detected by an anti-Gb_3Cer monoclonal antibody derived from a Burkitt cell hybridoma (Lipinski et al., 1982).

Ganglioside differences have also been noted among human leukocytes, leukemia cells, and leukemia cell lines. Rosenfelder et al. (1982) and Saito et al. (1981) have shown that ganglioside TLC patterns can be useful in distinguishing different leukemia cell lines. These authors have suggested that the degree of cell differentiation can be correlated with particular TLC patterns. Our recent work has demonstrated a difference in the expression of a specific ganglioside by human leukocytes and leukemia cells (Siddiqui et al., 1984). Using an anti-GD_3 monoclonal antibody, we showed that this ganglioside was not found in well-differentiated leukocytes but was present in poorly differentiated leukocytes. Finally, Majdic et al. (1984) have prepared a monoclonal antibody (VIM-2) which has a specificity for myelomonocytic cells and binds to an antigen (M2) which has a nonreducing terminal neuraminic acid residue. M2 is found on normal myeloid cells, leukemia cells, and myeloid leukemia cell lines. We have found that M2 is a ganglioside with a poly(lactosamine) structure and therefore is another example of a leukocyte glycosphingolipid with a restricted cellular distribution (Uemura et al., 1985). In summary, it is clear that human leukocyte differentiation is accompanied by multiple changes in the synthesis and expression of glycosphingolipid antigens.

Registry No. Gb_3Cer, 71965-57-6; Lc_3Cer, 73467-80-8; Lc_4Cer, 71950-33-9; GlcCer, 85305-87-9; GalCer, 85305-88-0; LacCer, 4682-48-8.

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Apolipoprotein E: Phospholipid Binding Studies with Synthetic Peptides Containing the Putative Receptor Binding Region†

James T. Sparrow,* Doris A. Sparrow, Alan R. Culwell, and Antonio M. Gotto, Jr.
Department of Medicine, Baylor College of Medicine and The Methodist Hospital, Houston, Texas 77030
Received February 22, 1985; Revised Manuscript Received June 10, 1985

ABSTRACT: To define the lipid and receptor binding regions of apolipoprotein E (apoE), we have synthesized four peptides beginning at residue 169 and continuing through the putative receptor binding region and ending at residue 129 so as to include a proposed lipid binding domain. The peptides were synthesized by solid-phase techniques, cleaved with anhydrous HF, and purified by ion-exchange and semipreparative reversed-phase high-performance liquid chromatography (HPLC). The peptides had the correct amino acid composition and were greater than 99% pure by analytical reversed-phase HPLC. The circular dichroic spectrum of each peptide was recorded before and after mixing with dimyristoylphosphatidylcholine. With apoE(148-169), apoE(144-169), and apoE(139-169), no changes were observed in the ellipticity at 222 nm. However, with apoE(129-169), an increase in α-helicity to ~42% was observed. Density gradient ultracentrifugation of the lipid-peptide mixture permitted isolation of a complex with apoE(129-169) with a molar ratio of lipid to peptide of 125:1, which was stable to recentrifugation. The α-helicity of the peptide in the complex was estimated to be 56%. No complexes were isolated from the gradients of the shorter peptides. Therefore, we conclude that the amphipathic helix formed by residues 130-150 contains one of the lipid binding regions of apoE.

Apolipoprotein E (apoE)[1] is a constituent of human plasma chylomicrons, very low density lipoproteins, and high-density lipoproteins (Mahley, 1978) and exhibits sequence heterogeneity that is genetically determined (Utermann et al., 1980; Zannis & Breslow, 1981). The apoprotein is responsible for the interaction of the lipoproteins with cellular receptors for control of cholesterol metabolism [for review, see Mahley & Innerarity (1983)]. Due to the sequence heterogeneity, several apoE isoforms are defective in their ability to interact with receptors, which results in the lipoprotein disorder type III hyperlipoproteinemia (Mahley & Angelin, 1984).

The amino acid sequence of apoE and three common isoforms has been determined (Rall et al., 1982a,b, 1983). The

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[1] Abbreviations: apoE, apolipoprotein E isolated from human plasma very low density lipoproteins; apoE(129-169), a synthetic peptide comprising residues 129-169 of apolipoprotein E; DMPC, dimyristoylphosphatidylcholine; CD, circular dichroism; HPLC, high-performance liquid chromatography; *t*-BOC, *tert*-butyloxycarbonyl; Tris, tris(hydroxymethyl)aminomethane; TEAP, triethylammonium phosphate; AUFS, absorbance units full scale.

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