Chemical characterization of neutral glycolipids in the human myeloid leukemias[1]

John C. Klock, Jay L. D'Angona, and Bruce A. Macher

Cancer Research Institute and the Department of Medicine,[2] and Department of Pharmaceutical Chemistry, School of Pharmacy,[3] University of California, San Francisco, CA 94143

Abstract Human neutrophils and lymphocytes have been shown to have different classes of neutral glycolipids. We have investigated alterations of glycolipids in the human myeloid leukemias to see how their neutral glycolipids differ from those of normal neutrophils. The chemical structures of the neutral glycolipids from large numbers of homogeneously purified leukemia cells were determined using column and thin-layer chromatography, gas–liquid chromatography (GLC), GLC–mass spectrometry, and direct probe mass spectrometry. Our results showed that cells from patients with acute myelogenous leukemia (AML) had less than half the amount of neutral glycolipid per cell than did cells from patients with chronic myelogenous leukemia (CML). Chromatographic mapping of the neutral glycolipids from these cells showed that AML cells had less of the polar, long-chain neutral glycolipids than did CML cells. The studies confirmed that over 99% of the neutral glycolipids were contained in a population of compounds with 1, 2, 3, and 4 sugar-containing neutral glycolipids whose structures are: Glc 1 → 1 ceramide; Gal 1 → 1 ceramide; Gal 1 → 4 Glc 1 → 1 ceramide; Gal 1 → 4 Gal 1 → 1 ceramide; GlcNAc 1 → 3 Gal 1 → 4 Glc 1 → 1 ceramide; and Gal 1 → 4 GlcNAc 1 → 3 Gal 1 → 4 Glc 1 → 1 ceramide. Lactosyl ceramide was the major glycolipid in both AML and CML cells. The studies show that human myeloid leukemia cells have the same neutral glycolipids as normal neutrophils. The alterations in neutral glycolipid distribution in leukemia suggest that they might be useful as "differentiation markers", with the morphologically more "mature" leukemias having more complex glycolipids. We were unable to detect novel or "malignancy-associated" neutral glycolipids in any of the leukemias we studied.—**Klock, J. C., J. L. D'Angona, and B. A. Macher.** Chemical characterization of neutral glycolipids in the human myeloid leukemias. *J. Lipid Res.* 1981. **22:** 1079–1083.

Supplementary key words acute myeloblastic leukemia · chronic myelocytic leukemia

A number of surface changes have been noted in cells that have undergone malignant transformation including alterations in glycolipid composition (1, 2). Several studies have shown that transformation leads to a simplification of glycolipids in the cell. This simplification is thought to be due to the diminished activity of the cell's glycosyltransferases (2). Studies in human cancers have demonstrated that these cells have altered blood group antigens and in some cases the appearance of novel glycolipid structures has been described (3–5).

We and others have investigated the structure of glycolipids in populations of purified human leukocytes, and have shown that human neutrophils and lymphocytes have characteristic families of glycolipids that can distinguish them from each other as well as from other blood cell types (6–8). Recently, we evaluated the alterations in glycolipids in the human leukemias. Our purpose was to determine if the glycolipid alterations in leukemia cells are similar to those seen in other malignant cells, to determine if the glycolipids of leukemia cells could be useful as tissue markers, and to determine if "unique" or new glycolipids would be found. We were able to find only one study of glycolipids in purified human leukemic cells (9) and no complete structural study of glycolipid alterations in the human leukemias is known to us.

Our present work demonstrates that all cells of myeloid origin (neutrophils and chronic and acute myeloid leukemia cells) share similar types of neutral glycolipids (gala- and lactoneo-types) and that the more "differentiated" leukemic cells have increased amounts of the more complex types.

MATERIALS AND METHODS

Cells

Cells from patients with cytochemically and morphologically proven acute myeloid leukemia (AML) and chronic myeloid leukemias (CML) were har-

Abbreviations: AML, acute myeloid leukemia; CML, chronic myeloid leukemia; TLC, thin-layer chromatography.

[1] Part of this material has been presented at the Society for Complex Carbohydrates, Boston, MA, September 26, 1980 (Macher, B.A., and J.C. Klock. Glycolipids of human lymphocytic, myelocytic, and hairy cell leukemias. (Abstract)).

[2] J. C. Klock and B. A. Macher.

[3] J. L. D'Angona.

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vested by continuous flow centrifugation leukapheresis and purified to homogeneity as previously described (6). Three patients with AML and two patients with CML were studied. The number of purified cells obtained per patient ranged from 2.5×10^{11} to 1×10^{12}.

Extraction and separation of glycolipids

This was done by repeated organic solvent extraction, base hydrolysis, and DEAE-Sephadex and silicic acid chromatography (6). Profile mapping and final purification of individual neutral glycolipid fractions were accomplished by applying 1–2 g of neutral glycolipid mixture to a 2 × 100 cm silicic acid column (Biosil A, 200–400 mesh, BioRad Labs, Richmond, CA) prepared with chloroform. Individual fractions were eluted using a linear chloroform–methanol gradient (100% chloroform → 100% methanol) and collected with a fraction collector. All fractions selected for analysis migrated as doublet bands on thin-layer chromatograms (6). Final preparation of individual glycolipids was by preparative thin-layer chromatography. Compounds used for gas–liquid chromatography and mass spectrometry appeared as doublets when run on thin-layer chromatography using several solvent systems. Orcinol spray was used to detect glycolipids.

Analytical methods

Quantitation of glycolipids was carried out as previously described using densitometric analysis of the thin-layer chromatograms which had been sprayed with orcinol (6). Carbohydrate compositional analysis was by gas–liquid chromatography of the trimethylsilyl methylglycoside derivatives (10). Methylation analysis to determine sugar linkage positions was done on the partially methylated alditol acetate derivatives of the glycolipids as previously described (6, 10). Mass spectrometric analysis of permethylated intact glycolipids was performed on a Kratos MS 25 double focusing instrument. The resolving power was set to 800 (10% valley) and a mass range of 28–743 was calibrated externally using high boiling perfluorokerosene. The ion source temperature was adjusted to 250°C and the electron energy was set at 70 eV. Samples were admitted using a heatable direct insertion probe over a temperature range of 100–450°C. The trap current was set at 500 μA and the accelerating voltage was 1.7 kV.

RESULTS

AML cells contained less than half the amount of neutral glycolipid per cell than did CML cells (4 mg/10^{10} cells vs 10 mg/10^{10} cells, respectively). As can be seen in **Fig. 1,** AML and CML cells have GL2 as the major neutral glycolipid species; however, AML cells have more GL1 than CML cells. To assess the long-chain neutral glycolipids, column chromatography of the neutral glycolipids was performed. As shown in **Fig. 2,** AML cells had much less of the more polar, long-chain neutral glycolipids than did CML cells. The quantitative percentages of neutral glycolipids of the different cell types are shown in **Table 1.** Carbohydrate compositional analysis of the individual bands containing one to four sugars (called GL1,2,3, and 4) is shown in **Table 2.** These data show that the GL1–4 species contain only three types of sugars: glucose, galactose, and *N*-acetylglucosamine. The GL1 and GL2 of each cell type also contained slightly higher ratios of galactose:glucose. This latter finding is similar to our findings in normal neutrophils (6), and suggests the presence of gala-type neutral glycolipids.

Results of analysis of the partially methylated alditol acetates in the hydrolysate of the permethylated intact glycolipid fractions are shown in **Table 3.** The results from AML and CML fractions GL1–4 were identical. The hydrolysate from GL1 contained 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylglucitol and 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylgalactitol in relative amounts of 5:1, indicating the presence of 1-linked glucose and 1-linked galactose. Equal amounts of 1-linked galactose and 1,4-linked glucose were found in the hydrolysate of the GL2 along with a small amount of 1,4-linked galactose. The GL3 fraction contained two tri-O-methyl-substituted hexoses (1,4,5-tri-O-acetyl-2,3,6-tri-O-methylglucitol and 1,3,5-tri-O-acetyl-2,4,6-tri-O-methylgalactitol) and a tri-O-methyl-substituted hexosamine (1,5-di-O-acetyl-3,4,6-tri-O-methyl-2-deoxy-2-N-methyl-acetamidoglucitol) in approximately equal amounts. The GL4 fraction contained a

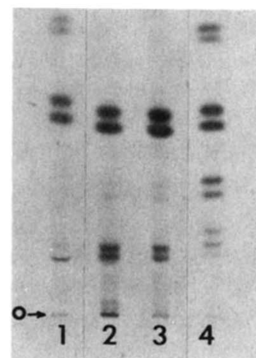

Fig. 1. Thin-layer chromatogram of neutral glycolipids from leukemic and normal cells. Lane 1, AML; lane 2, CML; lane 3, normal neutrophils; lane 4, GL1–4 from human red cells. Merck HPTLC plate, solvent chloroform–methanol–water 100:42:6 (v/v/v). Glycolipids were visualized with orcinol spray. O = origin. GL 1 doublets are at the top of the chromatogram.

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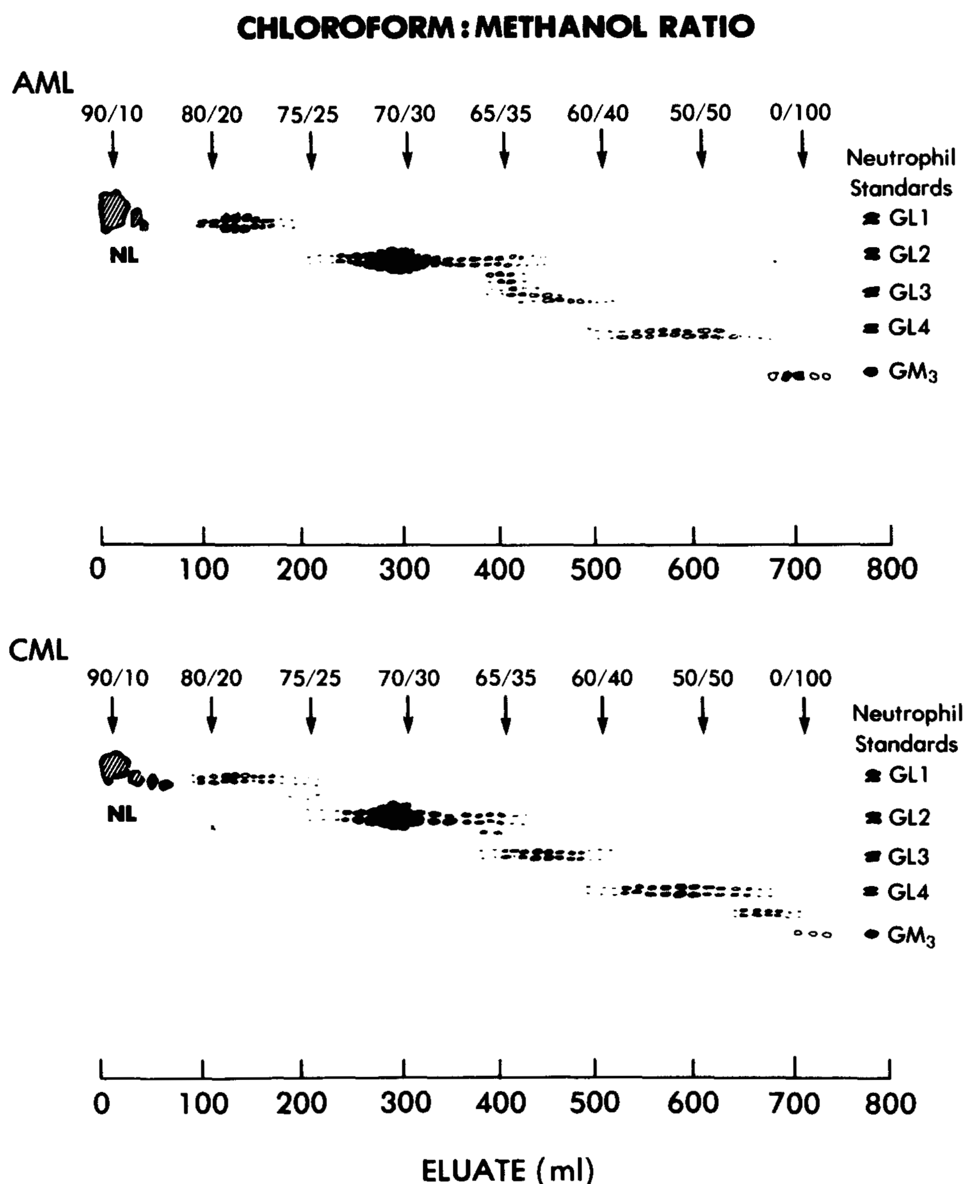

Fig. 2. Schematic representation of thin-layer chromatograms of various neutral glycolipid fractions eluted from silicic acid column. Open circles for the AML, GL3, and GL4 indicate light-staining spots on the chromatogram. NL = neutral lipid.

1,4-linked glucose, a 1,3-linked galactose, a 1,4-linked N-acetylglucosamine, and a 1-linked galactose in approximately equal amounts.

Direct probe analysis of the methylated derivative of GL1 showed m/z 187, 219, 548, and 660, indicating a monohexosyl ceramide with C18:1 long chain base plus C16:0 and C24:0 fatty acids. The methylated derivative of GL2 showed m/z 187, 219, 423, 548, and 660, indicating a dihexosylceramide with 18:1 sphingosine and C16:O and C24:O fatty acids. The permethylated GL3 fraction gave m/z 228, 260, 432, 464, 636, 660, and 668, which indicates a trihexosylceramide with a terminal amino sugar. The mass spectrum of the trihexosylceramide was identical to the one published by us for neutrophil GL3 (6). The spectrum of the methylated derivative of GL4 is shown in **Fig. 3** together with an abbreviated structural formula and indications of some fragments. The m/z 182 indicates this compound has a lactoneo-type of structure (11, 12).

DISCUSSION

The data in this report confirm and extend previous observations on neutral glycolipids in human myeloid leukemia (9). We have confirmed that glucosyl and

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TABLE 1. Quantitative percentages of neutral glycolipids in human myeloid cells[a]

	GL1	GL2	GL3	GL4	Long Chain
PMN[b]	5[c]	65	10	20	1
CML	10	65	10	15	1
AML	20	70	3	5	0

[a] Densitometric analysis is described in Methods. Values for PMN are from Ref. 6.
[b] PMN = neutrophils; CML = chronic myelogenous leukemia; AML = acute myelogenous leukemia.
[c] Percent of the total orcinol-positive material on the TLC plate.

lactosyl ceramide are present in these cells and that lactosyl ceramide is the major neutral glycolipid in AML and CML cells. We have shown that, in addition to glucosyl and lactosyl ceramide, AML and CML cells have galactosyl- and digalactosyl-ceramides and tri- and tetra-glycosyl ceramides. Long-chain neutral glycolipids are present as well (see Fig. 2) and we are presently in the process of analyzing these. Our analyses have allowed us to conclude that AML and CML cells have at least six neutral glycolipids whose structures are: Glc 1 → 1 Cer; Gal 1 → 1 Cer; Gal 1 → 4 Glc 1 → 1 Cer; Gal 1 → 4 Gal 1 → 1 Cer; GlcNAc 1 → 3 Gal 1 → 4 Glc 1 → 1 Cer; Gal 1 → 4 GlcNAc 1 → 3 Gal 1 → 4 Glc 1 → 1 Cer.

Although enzyme studies were not done to determine carbohydrate anomerity, the m/z 182 found in the mass spectrum of the GL3 and GL4 indicates a lactoneo-type structure with all β-linked sugars. In addition, treatment of the GL3 and GL4 from these cells with endo-β-galactosidase resulted in complete degradation of the compounds to GL1 and oligosaccharide (data not shown). This indicates that the GL3 and GL4 are of the neolacto family (13) and argues against the presence of any globo-type GL3 and GL4 being present in these cells. We believe that these compounds are similar to the six neutral glycolipids we found in normal neutrophils (6, 14). Our findings suggest that the distribution and type of neutral glycolipids in human leukemias may prove to be useful as biochemical markers for these cells, distinguishing them from lymphoid cells which appear to have only globo-type neutral gycolipids (7, 8).

The primary alteration in neutral glycolipids in AML is a change in distribution. More GL1 and GL2 and less of the long-chain neutral glycolipids are present than are seen in mature neutrophils. This suggests that the distribution or degree of complexity of neutral glycolipids in human myeloid leukemias may be useful as "differentiation" markers, with the more well-differentiated cells showing a more complex neutral glycolipid pattern. Even by using very large numbers of cells (10^{11}–10^{12} cells) for our studies, to date we have not been able to identify "novel" or "malignancy-associated" neutral glycolipids in human leukemias as have been described in transformed tumor cells in culture (15) and in human gastrointestinal cancer (3–5). It is possible that in the latter cases one cannot exclude, as the source of the new glycolipid, cells which are often cancer-associated (inflammatory cells, vascular proliferative cells, or fibroblastic cells). Further studies on the more complex glycosphingolipids of human leukemia cells might demonstrate a cancer-related alteration in cell surface carbohydrates. We believe that if one is to answer questions about the biologic importance of glycolipid alterations in neoplasia and the usefulness of glycolipids as clinical markers, much more information on primary structure in purified populations of cells is needed. Thus far, the usefulness of neutral glycolipids as leukocyte-specific markers in man and as differentiation-related markers appears enencouraging.

TABLE 2. Carbohydrate compositional analysis of neutral glycolipid fractions from CML cells[a]

Fraction	Molar Ratios		
	Glucose	Galactose	*N*-acetylglucosamine
GL1	1.0	0.2	0
GL2	1.0	1.3	0
GL3	1.0	1.0	0.7
GL4	1.0	1.7	0.6

[a] Results for AML cells were similar.

TABLE 3. Partially O-methylated hexoses and hexosamines obtained from neutral glycolipids of CML cells[a]

	Molar Ratios of Fractions			
	GL1	GL2	GL3	GL4
2,3,4,6-tetra-O-methylglucitol	1.0			
2,3,4,6-tetra-O-methylgalactitol	0.2	1.0		1.0
2,3,6-tetra-O-methylglucitol		1.0	1.0	1.0
2,3,6-tetra-O-methylgalactitol		0.1		
2,4,6-tetra-O-methylgalactitol			1.0	1.0
3,4,6-tetra-O-methyl-N-methylacetamidoglucitol			0.8	
3,6-di-O-methyl-N-methylacetamidoglucitol				0.8

[a] Results from AML cells were similar.

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Note added in proof: The acute leukemia cells analyzed in this study had only neolacto-types of glycolipids; however, we have recently found a patient with acute myelomonocytic leukemia who appears to have both globo- and neolacto-

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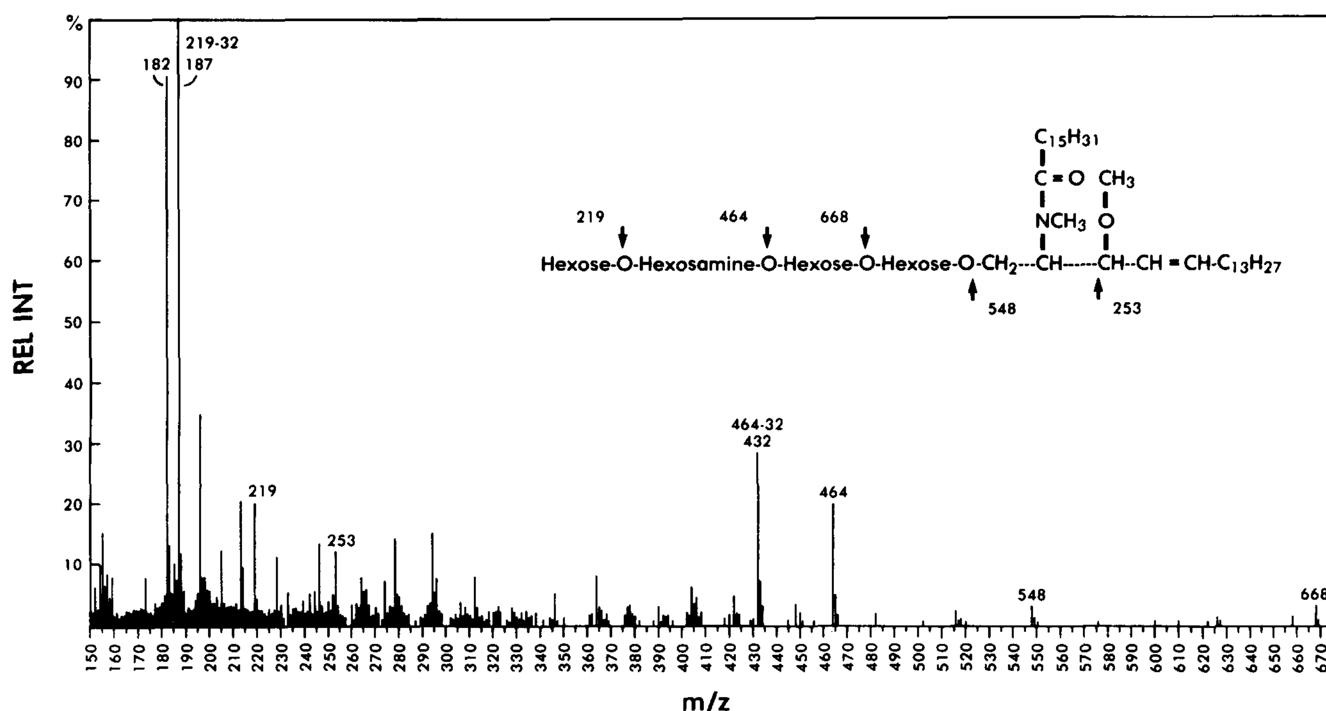

Fig. 3. Mass spectrum of the intact permethylated GL4 glycolipid from CML cells. Conditions were as described under Materials and Methods. m/z below 150 are not shown; only the major peaks of interest are demonstrated.

types of neutral glycolipids. It appears that the existence of both types is not uncommon in acute leukemia (Lee, W. M. F., M. A. Westrick, J. C. Klock, and B. A. Macher. 1981. Isolation and characterization of glycosphingolipids in human acute leukemias. A unique glycosphingolipid pattern among human leukocytes. Submitted for publication).

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