Doody, M. C., Pownall, H. J., Kao, Y. J., & Smith, L. C. (1980) *Biochemistry 19*, 108.
Duckwitz-Peterlein, G., Eilenberger, G., & Overath, P. (1977) *Biochim. Biophys. Acta 469*, 311.
Giraud, F., & Claret, M. (1979) *FEBS Lett. 103*, 186.
Gottlieb, M. H. (1980) *Biochim. Biophys. Acta 600*, 530.
Gould, R. G., LeRoy, G. V., Okita, G. T., Kabara, J. J., Keegan, P., & Bergenstal, D. M. (1955) *J. Lab. Clin. Med. 46*, 372.
Gulik-Krzywicki, T., Tardieu, A., & Luzzati, V. (1969) *Mol. Cryst. Liq. Cryst. 8*, 285.
Gurd, F. R. N. (1960) in *Lipide Chemistry* (Hanahan, D. J., Ed.) p 283, Wiley, New York.
Haberland, M. E., & Reynolds, J. A. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2313.
Hagerman, J. S., & Gould, R. G. (1951) *Proc. Soc. Exp. Biol. Med. 78*, 329.
Haran, N., & Shporer, M. (1977) *Biochim. Biophys. Acta 465*, 11.
Hauser, H. O. (1971) *Biochem. Biophys. Res. Commun. 45*, 1049.
Hauser, H. O., & Irons, L. (1972) *Hoppe-Seyler's Z. Physiol. Chem. 353*, 1579.
Holloway, P. W., & Katz, J. T. (1975) *J. Biol. Chem. 250*, 9002.
Huang, C. (1969) *Biochemistry 8*, 344.
Huang, C., & Charlton, J. P. (1972) *Biochem. Biophys. Res. Commun. 46*, 1660.
Jonas, A., & Maine, G. T. (1979) *Biochemistry 18*, 1772.
Kao, Y. J., Charlton, S. C., & Smith, L. C. (1977) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 36*, 936.
Lenard, J., & Rothman, J. E. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 391.
LeNeveu, D. M., Rand, R. P., & Parsegian, V. A. (1976) *Nature (London) 259*, 601.
Leto, T. L., Roseman, M. A., & Holloway, P. W. (1980) *Biochemistry 19*, 1911.
Litman, B. J. (1973) *Biochemistry 12*, 2545.
Martin, F. J., & MacDonald, R. C. (1976) *Biochemistry 15*, 321.
Moore, N. F., Patzer, E. J., Shaw, J. M., Thompson, T. E., & Wagner, R. R. (1978) *J. Virol. 27*, 320.
Newman, G. C., & Huang, C. (1975) *Biochemistry 14*, 3363.
Parsegian, V. A., Fuller, N., & Rand, R. P. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 2750.
Patzer, E. J., Shaw, J. M., Moore, N. F., Thompson, T. E., & Wagner, R. R. (1978) *Biochemistry 17*, 4192.
Phillips, M. C., McLean, L. R., Stoudt, G. W., & Rothblat, G. H. (1980) *Atherosclerosis (Shannon, Irel.) 36*, 409.
Poznansky, M. J., & Lange, Y. (1978) *Biochim. Biophys. Acta 506*, 256.
Quarfordt, S. H., & Hilderman, H. L. (1970) *J. Lipid Res. 11*, 528.
Roseman, M. A., & Thompson, T. E. (1980) *Biochemistry 19*, 439.
Shipley, G. G., Leslie, R. B., & Chapman, D. (1969) *Nature (London) 222*, 561.

Isolation and Structural Characterization of Human Lymphocyte Neutral Glycosphingolipids†

William M. F. Lee,* John C. Klock, and Bruce A. Macher

ABSTRACT: The neutral glycosphingolipids of human peripheral blood lymphocytes and of the lymphoid cells from a patient with B cell chronic lymphocytic leukemia were chemically analyzed. Four neutral glycosphingolipids were isolated from each of these two sources and studied by gas chromatography, methylation analysis, and electron impact–desorption mass spectrometry. The results of these studies indicate that the compounds have the following structures:

Glc1→1Cer
Gal1→4Glc1→1Cer
Gal1→4Gal1→4Glc1→1Cer
GalNAc1→3Gal1→4Gal1→4Glc1→1Cer

These compounds, belonging to the globo series, were the only neutral glycosphingolipids found in the lymphoid cells. The ceramide (Cer) moiety of all these compounds contained 4-sphingenine with $C_{16:0}$, $C_{24:0}$, and $C_{24:1}$ as the major fatty acid species. There were no structural differences in the neutral glycosphingolipids of peripheral blood lymphocytes compared to those of chronic lymphocytic leukemia cells. Peripheral blood lymphocytes contained more di- than monohexosylceramide whereas the reverse was true of the chronic lymphocytic leukemia cells. The proportion of tri- and tetrahexosylceramide was <10% for both types of cells. The results of our analyses did not support the existence of any differences in the major neutral glycosphingolipids among T, B, and chronic lymphocytic leukemia cells.

Glycosphingolipids have been isolated from most types of human blood cells, and some of these have been chemically characterized. Human erythrocytes have neutral glycosphingolipids of both the globo and lactoneo series (Hakomori & Siddiqui, 1976), whereas human platelets contain only the globo analogues (Tao et al., 1973). Recently, we have characterized the neutral glycosphinoglipids of human neutrophils and have shown that they are predominantly of the lactoneo type with smaller amounts of the gala series (Macher & Klock, 1980). Thus far, the neutral glycosphingolipids of human lymphocytes have not been completely chemically analyzed. Partial chemical analysis of lymphocyte glycosphingolipids suggests the presence of only globo-type structures (Stein & Marcus, 1977). However, data obtained by

† From the Department of Medicine, Cancer Research Institute, University of California, San Francisco, San Francisco, California 94143. *Received November 24, 1980.* This work was supported by a grant from the Leukemia Research Foundation and by Cancer Research Funds from the University of California. W.M.F.L. is the recipient of a postdoctoral fellowship from the National Institutes of Health and Giannini—Bank of America. J.K. is a scholar of The Leukemia Society of America, Inc.

0006-2960/81/0420-3810$01.25/0

using specific anti-glycosphingolipid antibodies have suggested that paragloboside[1] and asialo-GM_1 are also present on the surface of lymphocytes (Schwarting & Marcus, 1979). Furthermore, antibody studies on cells from patients with chronic lymphocytic leukemia suggest a difference in the glycosphingolipid content of these lymphocytes compared to normal lymphocytes (Schwarting & Marcus, 1979). Lack of progress in this area has been mainly due to the difficulties in obtaining sufficient quantities of normal lymphocytes for analysis. An additional problem is that lymphocytes are heterogeneous with different subpopulations having unique surface, metabolic, and functional properties. Most sources of human lymphocytes (peripheral blood, lymph nodes, and spleen) contain a mixture of these different subpopulations and any analysis of these mixtures will obscure possible glycosphingolipid differences among the individual subpopulations yielding only "average" results. Such possible differences would be important to determine because of the potential usefulness of glycosphingolipids as cell markers and as a means for the classification of lymphoid malignancies.

To approach these problems, we have chemically analyzed the neutral glycosphingolipids of pooled peripheral blood lymphocytes (PBL) and of the malignant cells from a patient with B cell chronic lymphocytic leukemia (CLL). The structural studies on these lymphoid cells are, to our knowledge, the first to be reported.

Experimental Procedures

(*A*) *Isolation of Human Lymphocytes.* Peripheral blood lymphocytes were obtained from normal donors by lymphapheresis with an IBM 2997 cell separator or an Aminco continuous flow celltrifuge (Hester et al., 1979). Normal mature lymphocytes were purified by an adaptation of the method of Böyum (1968). Forty milliliters of the lymphapheresis product was layered over 7.5 mL of Ficoll-Hypaque in a 50-mL polypropylene centrifuge tube and centrifuged at 800*g* for 20 min at room temperature. The mononuclear cell layer was resuspended in 0.9% saline containing 0.5 mM EDTA to a final concentration of $\sim 1 \times 10^8$ cells/mL. Thirty milliliters of this suspension was layered over 10 mL of 4% bovine serum albumin (BSA) and centrifuged at 800*g* for 10 min at room temperature to remove platelet contamination. The cell pellet was resuspended and the centrifugation over 4% BSA repeated. The resulting cells contained over 95% small lymphocytes by examination of the Wright-stained smear of the preparation. Monocytes constituted most of the remaining leukocytes. The ratio of the number of platelets to the number of lymphocytes was less than 1:2. We obtained a total of 15×10^{10} PBL from 12 normal donors.

B lymphocyte chronic lymphocytic leukemia cells were obtained from a patient with classic CLL. The malignant cell population consisted of normal-appearing small lymphocytes. Eighty percent of these cells were found to have μ-immunoglobulin heavy chains and κ-immunoglobulin light chains on their surface (Rose & Friedman, 1976), indicating the B lymphocyte monoclonal origin of the neoplastic cells. This patient had a peripheral white cell count of $\sim 200\,000$ cells/mm^3 of which 98% were small lymphocytes. She had previously been treated with chemotherapeutic agents including chlorambucil, cyclophosphamide, vincristine, and corticosteroids, but no treatment had been given in the 3 months prior to lymphapheresis. Lymphapheresis was performed on this patient as described for the normal donors. The major difference was that the resulting lymphocyte collection was virtually free of all contaminating cells except for erythrocytes which were eliminated by osmotic lysis (Klock & Bainton, 1976). The final cell preparation contained over 98% small lymphocytes and the ratio of the number of platelets to the number of lymphocytes was less than 1:2. We obtained a total of 150×10^{10} CLL cells from this patient for our studies.

(*B*) *Extraction and Purification of Lymphocyte Glycosphingolipids.* Immediately after purification, human lymphocytes of CLL cells were placed in 10 volumes of chloroform–methanol, 2:1, and stored at −20 °C. When we were ready to analyze the glycosphingolipids in these cells, they were extracted at room temperature with 20 volumes of each of the following solvent mixtures: chloroform–methanol, 2:1, 1:1, and 1:2 (v/v) (30 min for each extraction). After evaporation of the organic solvents in vacuo, the residue was dissolved in ~ 5 volumes of chloroform–methanol–water (30:60:8 v/v) and mixed with 0.5 g of DEAE-Sephadex A-25 (Pharmacia, Piscataway, NJ), acetate form (Ledeen et al., 1973). The sample was allowed to absorb to the column packing for 20 min and was then applied to a column (1.5 × 30 cm) of the same material. Neutral and acidic lipid fractions were eluted as described by Ando & Yu (1977). Neutral glycosphingolipids were further purified on a column of Bio-Sil A, 100–200 mesh (Bio-Rad Laboratories, Richmond, CA). The neutral lipid fraction was dissolved in 5–10 mL of chloroform–methanol (1:1 v/v), applied to a column of Bio-Sil A (2 × 30 cm), and eluted as 100-mL fractions with solvent mixtures of increasing polarity (from 100% chloroform to 100% methanol). Final purification of each neutral glycosphingolipid was by preparative thin-layer chromatography (TLC) using silica gel 60 high-performance plates (EM Laboratories Inc., Cincinnati, OH) in solvent system A (chloroform–methanol–water, 60:35:8 v/v). Glycosphingolipids were visualized by a brief exposure to iodine and eluted with chloroform–methanol–water (50:50:10 v/v) after the iodine had been sublimated, and a 0.1–1% aliquot was rechromatographed in solvent A or B (chloroform–methanol–water, 100:42:6 v/v) and visualized with orcinol reagent to demonstrate homogeneity.

(*C*) *Carbohydrate Compositional Analysis.* Me_3Si-methyl glycoside derivatives were prepared and analyzed as described by Macher et al. (1979). Known glycosphingolipid standards (human neutrophil neolactotetraosylceramide and human erythrocyte globotetraosylceramide) were derivatized and analyzed at the same time. A Bendix 2500 gas–liquid chromatograph (Ronceverte, WV) equipped with glass columns was used for the analysis. Me_3Si-methyl glycosides were separated on 3% SE-30 (Supelco Co., Bellefonte, PA) temperature programed from 140 to 240 °C at 3 °C/min.

(*D*) *Methylation Analysis.* Partially methylated alditol acetate derivatives of glycolipids were prepared as previously described (Macher et al., 1979) and analyzed with an HP-5985S combined gas–liquid mass spectrometer under the following conditions: column packing, 3% OV-225; column temperature, linear program from 150 to 250 °C at 5 °C/min; separator temperature, 275 °C; ion source temperature, 200 °C; electron impact ionization, 70 eV; electron energy, 300 μA.

(*E*) *Direct Probe Mass Spectrometry.* Glycosphingolipids (30–100 μg) were permethylated (Hakomori, 1964) and

[1] Abbreviations used: Cer, ceramide; PBL, peripheral blood lymphocytes; CLL, chronic lymphocytic leukemia; GL1, monohexosylceramide; GL2, dihexosylceramide; GL3, trihexosylceramide; GL4, tetrahexosylceramide; paragloboside, neolactotetraosylceramide; asialo-GM_1, gangliotetraosylceramide; globoside, globotetraosylceramide; HPLC, high-pressure liquid chromatography; TLC, thin-layer chromatography; EDTA, ethylenediaminetetraacetic acid; DEAE, diethylaminoethyl; GC, gas chromatography.

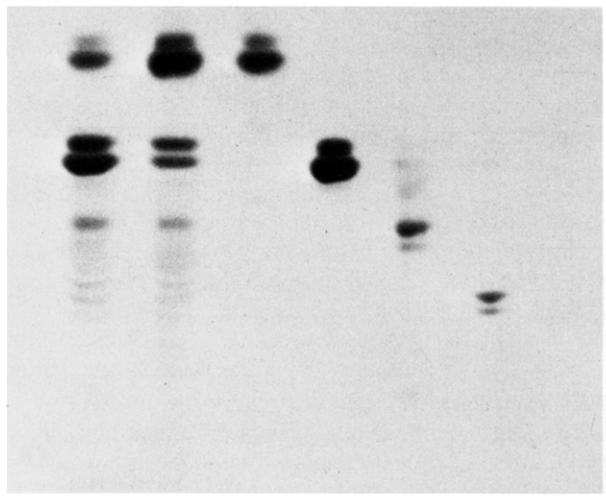

FIGURE 1: Thin-layer chromatogram of peripheral blood lymphocyte and chronic lymphocyte leukemia cell neutral glycosphingolipids. The separation is on a plate of silica gel 60 (HPTLC) in the solvent chloroform–methanol–water (60:35:8 v/v), and the glycolipids were visualized with orcinol reagent spray. PBL, neutral glycosphingolipids from peripheral blood lymphocytes; CLL, neutral glycosphingolipids from chronic lymphocyte leukemia cells; neutral glycosphingolipids from human erythroytes (glucosylceramide, lactosylceramide, globotriaosylceramide, and globotetraosylceramide, sequentially).

samples (<5 μg) were subjected to electron impact–desorption analysis with a Varian MAT CH-5 DF mass spectrometer under the following conditions: emission current, 300 μA; electron energy, 70 eV; acceleration voltage, 3 kV; ion source temperature, 160 °C; emitter wire current, programed from 0 to 35 mA.

Results

Isolation of Neutral Glycosphingolipids. The total yield of neutral glycosphingolipids from the PBL was ~1 mg/10^{10} cells. A similar yield was obtained from CLL cells. Peripheral blood lymphocyte neutral glycosphingolipids showed four major bands or doublets with TLC mobilities corresponding to those of mono-, di-, tri- and tetrahexosylceramide standards from human erythrocytes; these were labeled PBL GL1–PBL GL4 sequentially (Figure 1). CLL neutral glycosphingolipids had components which, on TLC, corresponded to those of the PBL GL1–GL4 (labeled CLL GL1–GL4). For both the PBL and the CLL cells, GL1 and GL2 accounted for over 90% of the total neutral glycosphingolipids. PBL had a GL1/GL2 ratio of 1:4 whereas CLL cells had a GL1/GL2 ratio of 2:1. PBL and CLL GL1, GL2, and GL4 chromatographed on TLC as doublets, and, in all subsequent studies, both components of the doublets were analyzed together.

Carbohydrate Composition. The carbohydrate compositions of PBL and CLL GL1–GL4 as determined by gas chromatography are given in Table I. Both sources of neutral glycosphingolipid yielded *N*-acetylgalactosamine as the only amino sugar, and this was found only in the GL4 species. The molar ratios of the monosaccharides were similar for each species of glycosphingolipid obtained from both PBL and CLL cells. Thus, both CLL and PBL GL1 contained only glucose, CLL and PBL GL2 contained approximately equimolar amounts of glucose and galactose, and CLL and PBL GL3 contained approximately twice the amount of galactose as glucose. CLL GL4 contained glucose, galactose, and *N*-acetylgalactosamine in the ratio 1.0:2.0:0.8. The PBL GL4 contained these compounds in the ratio of 1.0:2.3:0.5. Our findings were in keeping with the general experience that the recovery of *N*-acetylamino sugars on gas chromatographic analysis is less than that of neutral sugars (Chambers & Clamp, 1971).

Table I: Carbohydrate Composition of Neutral Glycosphingolipids from Human Lymphocytes

		molar ratios		
cell type	fraction	glucose	galactose	*N*-acetyl-galactosamine
PBL	GL1	1.0		
	GL2	1.0	1.2	
	GL3	1.0	2.2	
	GL4	1.0	2.3	0.5
CLL	GL1	1.0		
	GL2	1.0	1.0	
	GL3	1.0	1.8	
	GL4	1.0	2.0	0.8

Table II: Partially O-Methylated Hexoses and Hexosamines Obtained from Neutral Glycosphingolipids of B Cell CLL Cells

	molar ratio of fractions			
methylated sugar	GL1	GL2	GL3	GL4
2,3,4,6-tetra-*O*-methylglucitol	1.0[a]			
2,3,4,6-tetra-*O*-methylgalactitol		1.0	1.0	
2,3,6-tri-*O*-methylglucitol		1.0	0.9	1.0
2,3,6-tri-*O*-methylgalactitol			1.0	1.0
2,4,6-tri-*O*-methylgalactitol				0.8
3,4,6-tri-*O*-methyl-*N*-methyl-acetamidogalactitol				0.6

[a] The ratios are expressed relative to 2,3,6-tri-*O*-methylglucitol, except for fraction GL1 where 2,3,4,6-tetra-*O*-methylglucitol is used for comparison.

Mass Spectrometry. The CLL neutral glycosphingolipids were analyzed by direct probe mass spectrometry and combined GC–mass spectrometry. Results of the analysis of the partially methylated alditol acetates obtained in the hydrolysate of the permethylated intact CLL GL1–GL4 fractions are presented in Table II. The hydrolysate from CLL GL1 contained only 2,3,4,6-tetra-*O*-methylglucitol, indicating the presence of 1-linked glucose. The analysis of CLL GL2 showed equimolar amounts of 1,4-linked glucose and 1-linked galactose (2,3,6-tri-*O*-methylglucitol and 2,3,4,6-tetra-*O*-methylgalactitol). CLL GL3 had nearly equimolar amounts of 1,4-linked glucose, 1,4-linked galactose, and 1-linked galactose (2,3,6-tri-*O*-methylglucitol, 2,3,6-tri-*O*-methylgalactitol, and 2,3,4,6-tetra-*O*-methylgalactitol). CLL GL4 had nearly equimolar amounts of 1,4-linked glucose, 1,4-linked galactose, and 1,3-linked galactose (2,3,6-tri-*O*-methylglucitol, 2,3,6-tri-*O*-methylgalactitol, and 2,4,6-tri-*O*-methylgalactitol) and slightly less 1-linked *N*-acetylgalactosamine (3,4,6-tri-*O*-methyl-2-deoxy-*N*-methylacetamidogalactitol).

The direct probe mass spectrum of the permethylated derivative of CLL GL3 is shown in Figure 2, together with a simplified formula and indication of some cleavage sites. Table III summarizes the fragments seen in the spectra of all four of the permethylated CLL neutral glycosphingolipids. GL1 had *m/z* 187, 219, 292, and 530, GL2 had *m/z* 187, 219, 423, and 496, and GL3 had *m/z* 187, 219, 423, and 700 indicating that they had one, two, and three hexoses sequentially. CLL GL4 gave ions at *m/z* 228, 260, 464, and 668, providing evidence for a saccharide chain with a hexosamine at the nonreducing end followed by a disaccharide of hexose-O-hexose (ions for the tetrasaccharide were not found).

The direct probe analysis also provided information on the type of sphingosine base and the major fatty acids of each

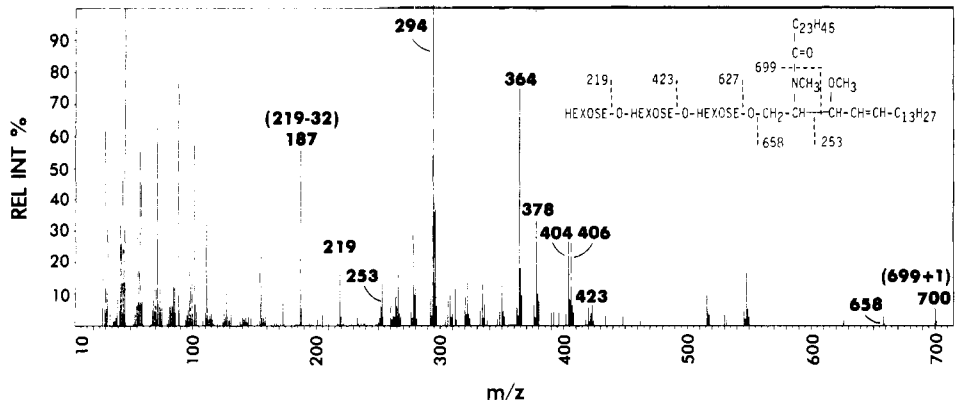

FIGURE 2: Mass spectrum of intact permethylated glycosphingolipid CLL GL3. Conditions were as described under Experimental Procedures.

neutral glycosphingolipid. All four components contained 4-sphingenine (m/z 253, 294, 312, and 364) with $C_{16:0}$, $C_{24:0}$, and $C_{24:1}$ (m/z 294, 406, and 404) as the major fatty acid species. The entire ceramide moiety gave ions at m/z 548, 658, and 660.

Discussion

Our results expand upon and clarify the information available about the neutral glycosphingolipids of human lymphoid cells. Stein & Marcus (1977) suggested that human tonsillar lymphocytes contained globoside as their major neutral tetrahexosyl glycosphingolipid as determined by gas chromatography. However, their preparations of peripheral blood lymphocytes and thymocytes contained insufficient amounts of neutral glycosphingolipids for analysis. Our data on peripheral blood lymphocytes show that these cells contain a tetrahexosyl glycosphingolipid that has the same monosaccharide composition as globoside. We were able to perform complete sequencing on the neutral glycosphingolipids containing one to four sugars from the cells of a patient with B cell chronic lymphocyte leukemia. These cells were also found to have neutral glycosphingolipids of the globo series. We propose the following structures for the neutral glycosphingolipids found in both human peripheral blood lymphocytes and our patient's CLL cells:

GL1	Glc1→1Cer
GL2	Gal1→4Glc1→1Cer
GL3	Gal1→4Gal1→4Glc1→1Cer
GL4	GalNAc1→3Gal1→4Gal1→4Glc1→1Cer

The ceramide moiety contains 18:1 sphingosine with $C_{16:0}$, $C_{24:0}$, and $C_{24:1}$ fatty acids. These results indicate that these lymphocytes or their precursors incorporate from the plasma (Vance & Sweeley, 1967) or synthesized glycosphingolipids of the globo series. Furthermore, since PBL are a mixture of T and B cells, we believe that neither of these two populations of lymphocytes has detectable neutral glycosphingolipids other than those of the globo series. To confirm this point, we attempted enzymatic digestion of PBL and CLL GL4 by using endo-β-galactosidase from *Escherichia freundii* which is known to cleave paragloboside but not globoside (Fukuda et al., 1978). We found no evidence of hydrolysis of PBL and CLL GL4 to an oligosaccharide and glucosylceramide, whereas neutrophil GL4, i.e., paragloboside (Macher & Klock, 1980), was readily cleaved (unpublished observation). These results clearly distinguish human lymphocytes from neutrophils which contain neutral glycosphingolipids predominantly of the lactoneo series

Table III: Fragments in Mass Spectra of Intact Permethylated Neutral Glycosphingolipids from B Cell CLL Cells[a]

	CLL				
m/z	GL1	GL2	GL3	GL4	assignment
187	x	x	x		terminal hexose minus OCH_3
219	x	x	x		terminal hexose
228				x	terminal hexosamine minus OCH_3
260				x	terminal hexosamine
292	x				hexose-O-CH_2-CH-NCH_3
423		x	x		hexose-O-hexose
464				x	hexosamine-O-hexose
496		x			hexose-O-hexose-O-CH_2-CH-NCH_3
530	x				hexose-O-CH_2-CH-NCH_3-$C_{16:0}$
668				x	hexosamine-O-hexose-O-hexose
700			x		hexose-O-hexose-O-hexose-O-CH_2-CH-NCH_3

[a] The spectra of all of the intact permethylated neutral glycosphingolipids analyzed had ions characteristic of ceramides (m/z 548, 658, and 660) containing $C_{18:1}$ sphingosine (m/z 312 and 364) substituted with $C_{16:0}$, $C_{24:0}$, and $C_{24:1}$ fatty acids (m/z 294, 404, and 406).

(Macher & Klock, 1980). This difference makes the neutral glycosphingolipids potentially very useful as cell surface markers to differentiate white cells of lymphoid and myeloid origin. An immediate application of these results would be to use glycosphingolipids to differentiate and classify human leukemias.

One of the objections to analyzing glycosphingolipids of lymphocytes obtained from peripheral blood, lymph nodes, or spleen is that the analysis overlooks possible differences among the subpopulations of lymphocytes that make up these organs. The existence and nature of such differences would be important to know if glycosphingolipids are to be used to differentiate among lymphocyte subpopulations and to classify the lymphoid malignancies. Unfortunately, in order to perform our chemical analyses on individual lymphocyte subpopulations, a prohibitively large amount of these cells would be required. However, two methods have been used to study lymphocyte neutral glycosphingolipids which use smaller quantities of cells. In the first method, glycosphingolipid-specific antibodies were purified by affinity chromatography, conjugated with fluorescein, and then used to label the cell surface of subpopulations of lymphoid cells (Schwarting & Marcus, 1979). In the second method, high-pressure liquid chromatography (HPLC) was used to study lymphocyte glycosphingolipids by using small quantities of cells. HPLC permits purification and identification of many glycosphingolipid species provided that reference compounds of known structures are available. The results obtained by these

methods can be compared to the results of our chemical analysis.

By use of antibodies to asialo-GM_1, 50–60% of normal B lymphocytes were labeled, <10% of normal T lymphocytes were labeled, and CLL B cells were not labeled (Schwarting & Marcus, 1979). These results suggested the presence of asialo-GM_1 on the cell surface of normal lymphocytes, perhaps with a different density or distribution of this compound on B and T lymphocytes. We did not detect asialo-GM_1 in our lymphocytes, and Schwarting (1980) did not detect it on B or T cells by HPLC. Several explanations may account for these disparities. Anti-asialo-GM_1 antibody labeling of B and T cells might be due (a) to cross-reactivity of the antibody with glycoproteins that contain the same antigenic carbohydrate determinants as the glycosphingolipid (Tonegawa & Hakomori, 1977), (b) to cross-reactivity of the antibody with more complex glycosphingolipids (containing more than four sugars) which carry asialo-GM_1 determinants, or (c) to very small quantities of asialo-GM_1 on these cells which cannot be detected by our analysis or by HPLC. This last possibility seems unlikely, since such large amounts of lymphocytes were used in the present analysis. The multitude of factors which may affect the results of anti-glycosphingolipid antibody labeling studies is highlighted by the finding that anti-globoside antibodies poorly labeled T lymphocytes and did not label CLL cells (Schwarting & Marcus, 1979), whereas both our analyses and those obtained by HPLC (Schwarting, 1980) clearly showed this compound to be present. These discrepancies might be explained (a) by a smaller amount of globoside being present on T lymphocytes and CLL cells compared to B lymphocytes or (b) by an inaccessibility of the globoside determinants present on T and CLL cell surfaces to antibody binding.

Paragloboside was identified in lymphoid cells both by antibody studies (Schwarting & Marcus, 1979) and by HPLC analysis (Schwarting, 1980). Semiquantitative information using HPLC suggested that paragloboside constituted 1–2% of lymphocyte neutral glycosphingolipids. We cannot readily account for why we did not detect paragloboside in PBL and CLL cells by using gas chromatography, mass spectrometry, or endo-β-galactosidase treatment. Perhaps our failure to find paragloboside might have been due to loss of the small amount of this compound during purification of the tetrahexosylceramide fraction. On the other hand, the small amount of paragloboside found by HPLC might have been due to the presence of contaminating cell populations. In addition, the HPLC fraction assigned as paragloboside has not been chemically analyzed and might be yet another compound which cochromatographs with paragloboside on HPLC.

Our data firmly establish the nature of the neutral glycosphingolipid molecules persent in human lymphocytes and in B cell CLL and provide a basis for the interpretation of studies using antibodies and HPLC. It points out the need for further investigations into the glycosphingolipids present in individual normal lymphocyte subpopulations and in different lymphoid malignancies. Finally, the gangliosides and the more complex glycosphingolipids (i.e., those containing more than four sugars per molecule) which exist in lymphoid cells need to be studied since these may discriminate among different normal and malignant lymphocytes.

Acknowledgments

We thank Betty Baltzer of the Michigan State University—National Institutes of Health Mass Spectrometry Facility for assistance with the mass spectrometric analyses and Jim Harris for typing this manuscript.

References

Ando, S., & Yu, R. K. (1977) *J. Biol. Chem. 252*, 6247.
Boyüm, A. (1968) *Scand. J. Clin. Lab. Invest., Suppl. No. 97 21*, 31.
Chambers, R. E., & Clamp, J. R. (1971) *Biochem. J. 125*, 1009.
Fukuda, M. N., Watanabe, K., & Hakomori, S.-I. (1978) *J. Biol. Chem. 253*, 6814.
Hakomori, S.-I. (1964) *J. Biochem. (Tokyo) 55*, 205.
Hakomori, S.-I., & Siddiqui, B. (1976) *Methods Enzymol. B 32*, 345.
Hester, J. P., Kellogg, R. M., Mulzet, A. P., Kruger, V. R., McCredie, K. B., & Freireich, E. J. (1979) *Blood 54*, 254.
Klock, J. C., & Bainton, D. F. (1976) *Blood 48*, 149.
Ledeen, R. W., Yu, R. K., & Eng, L. F. (1973) *J. Neurochem. 121*, 829.
Macher, B. A., & Klock, J. C. (1980) *J. Biol. Chem. 255*, 2092.
Macher, B. A., Pacuszka, T., Mullin, B. R., Sweeley, C. C., Brady, R. O., & Fishman, P. H. (1979) *Biochim. Biophys. Acta 558*, 35.
Rose, N. R., & Friedman, H. (1976) in *Manual of Clinical Immunology ASM*, American Society of Microbiology, Washington, DC.
Schwarting, G. A. (1980) *Biochem. J. 189*, 407.
Schwarting, G. A., & Marcus, D. M. (1979) *Clin. Immunol. Immunopathol. 14*, 121.
Stein, K. E., & Marcus, D. M. (1977) *Biochemistry 16*, 5285.
Tao, R. V. P., Sweeley, C. C., & Jamieson, G. A. (1973) *J. Lipid Res. 14*, 16.
Tonegawa, Y., & Hakomori, S.-I. (1977) *Biochem. Biophys. Res. Commun. 76*, 9.
Vance, D. E., & Sweeley, C. C. (1967) *J. Lipid Res. 8*, 621.