Lipid composition of plasma membranes from human leukemic lymphocytes

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Abstract The specific activity of adenosine 5′-monophosphatase and the concentrations of cholesterol, glucosylceramide, lactosylceramide, and phospholipids were compared in the whole homogenates and in plasma membrane fractions in four preparations of human leukemic lymphocytes taken over a 1-yr period from a patient with chronic lymphocytic leukemia. There was a 69.5-fold enrichment of the specific activity of adenosine 5′-monophosphatase in plasma membrane fractions. This enzyme appeared to be the best plasma membrane marker of all compounds studied. The increase in lactosylceramide concentration in the plasma membranes was 34.4-fold. It was significantly higher than that of glucosylceramide. The enrichment of glucosylceramide in the plasma membranes was similar to that of cholesterol and total phospholipids. The pattern of individual phospholipids in the plasma membrane fraction, as compared with the whole homogenate, was characterized by a decrease in phosphatidylcholine and an increase in sphingomyelin.

Supplementary key words adenosine 5′-monophosphatase · glucosylceramide · lactosylceramide · phospholipids

Changes in lipids, especially glycolipids, have been observed during the malignant transformation in several experimental systems (1, 2). Some main characteristics of neoplastic cells such as new antigenic properties or loss of contact inhibition due to modifications in cell surface may be related to these changes (3, 4) because it is assumed that glycolipids are localized primarily if not exclusively in plasma membranes.

This assumption, however, is based on studies performed in only a few tissues (5–8). In a large number of cell types this hypothesis still awaits further investigations. A method for isolation of plasma membranes from human leukemic lymphocytes was recently described by this laboratory (9), and the presence of neutral glycolipids in these cells has been previously demonstrated (10).

The purpose of this study is to compare the distribution of glycolipids and adenosine 5′-monophosphatase (5′-AMPase), a plasma membrane marker, in the whole homogenate and in plasma membranes of human leukemic lymphocytes and to report the lipid composition of these membranes.

MATERIALS AND METHODS

Isolation of leukemic lymphocytes

For subcellular fractionations, preparations of lymphocytes containing 5′-AMPase activity were taken from a single patient with CLL on four separate occasions over a 1-yr period. The cells were isolated by an IBM experimental blood-cell separator. The diagnosis was made 1 yr prior to the beginning of this study, and the patient was treated only by leukapheresis. Further purification of lymphocytes was achieved by allowing the erythrocytes to sediment overnight at 0°C in a suspending medium containing 1.5 vol of an anticoagulant solution of ACD formula A (0.8% citric acid, 2.2% sodium citrate, and 2.45% dextrose) and 1 vol of 0.9% NaCl for 15 vol of lymphocyte-enriched preparation. Lymphocytes were washed and stored as previously described (9). Final lymphocyte preparations contained, per 100 white blood cells, less than 5 polymorphonuclear cells, from 10 to 15 erythrocytes, and less than 30 platelets. Additional lymphocyte preparations from different patients were analyzed for glycolipids. In all cases, CLL was diagnosed shortly before the analysis of glycolipids, and the patients were without treatment.

Preparation of plasma membrane fraction and electron microscopic examination

Lymphocytes were homogenized in 10 vol of 1 mM $NaHCO_3$, 0.5 mM $CaCl_2$, at pH 7.5, with a Dounce homogenizer using a tight-fitting pestle. Aliquots of this homogenate were used for further chemical determinations.

Abbreviations: 5′-AMPase, adenosine 5′-monophosphatase; CMH, ceramide monohexoside (glucosylceramide); CDH, ceramide dihexoside (lactosylceramide); CLL, chronic lymphocytic leukemia.

Subcellular fractionation of human leukemic lymphocytes was achieved by a method recently described (9). For electron microscopy, samples of plasma membrane preparations were fixed in 4% glutaraldehyde, washed in a buffered phosphate medium, embedded in Epon 812, and examined in a Siemens Elmiskop I. The details of these methods have been previously reported (11).

Chemical studies

5′-AMPase was assayed in 2-ml incubation mixtures containing 2 mM 5′-AMP (Sigma Chemical Co., St. Louis, Mo.), 4 mM $MgCl_2$, 10 mM Veronal buffer (pH 7.5), and about 1 mg of protein when the whole homogenate was used as enzyme source or about 0.02 mg of protein in assays with plasma membrane fractions. The reaction was performed at 37°C for 60 min and stopped with 0.5 ml of 10% trichloroacetic acid. The liberated phosphorus was measured by the method of Fiske and SubbaRow (12). Proteins were determined by the method of Lowry et al. (13).

Total lipids were extracted at room temperature, first with 20 vol of chloroform–methanol 2:1 (v/v) for 12 to 16 hr and then with 5 vol of chloroform–methanol 1:1 (v/v) for 2 hr. The two lipid extracts were combined and were partitioned and washed following the method of Folch, Lees, and Sloane Stanley (14).

Glycolipids were isolated by thin-layer chromatography after a mild alkaline hydrolysis of the total lipid extract (15). They were characterized by cochromatography with previously characterized glycolipid standards isolated from leukemic leukocytes (10) and determined by the procedure of Hakomori, Saito, and Vogt (4).

Total cholesterol and total phospholipids were measured in the lipid extract by the methods of Sperry and Webb (16) and Bartlett (17), respectively. Individual phospholipids were separated by two-dimensional thin-layer chromatography performed on silica gel G in chloroform–methanol–7 N ammonia 60:35:5 (v/v/v) and chloroform–methanol–7 N ammonia 35:60:5 (v/v/v) (18) and were determined according to the procedure described by Broekhuyse (19).

Statistical significance of the data was determined by Student's *t* test.

RESULTS

Morphological data

Fig. 1 shows a typical final plasma membrane preparation consisting primarily of membranous structures with frequent vesicles. The preparation is still contaminated by particles of dense material that cannot be identified morphologically.

Biochemical data

Neutral glycolipids. Two neutral glycolipids were found in the whole homogenates of leukemic lymphocytes: glucosylceramide (CMH) and lactosylceramide (CDH). CMH was found in all five preparations at concentrations ranging from 1.07 to 2.20 μg/mg of protein. CDH was identified in only three cases, including the patient who provided lymphocytes for subcellular fractionation; the concentration was from 0.21 to 0.64 μg/mg of protein. Qualitatively, the neutral glycolipids were the same in various subcellular fractions, particularly the whole homogenate and the plasma membranes.

Distribution of 5′-AMPase and lipids in subcellular fractions. The distribution of protein, 5′-AMPase activity, and lipids in various subcellular fractions isolated from CLL lymphocytes is given in **Table 1.** The greatest enrichments in measured substances were found in S1, the top fraction collected on the continuous sucrose gradient performed with the 12,800 *g* microsomal pellet P2, and in S2B, the final plasma membrane fraction. The total phospholipid content was two times higher in S1 than in S2B, and total cholesterol and CMH concentrations were equally enriched in the two fractions, whereas the specific activity of 5′-AMPase and the concentration of CDH were higher in S2B. The calculated molar ratios of cholesterol to phospholipids were, respectively, 0.33 and 0.68 for S1 and S2B.

Enrichment of plasma membrane fraction in 5′-AMPase and lipids. Comparisons of the increase of 5′-AMPase specific activity and of total cholesterol, CMH, CDH, and phospholipid concentrations between the whole homogenate and the plasma membrane fraction are shown in **Table 2.**

5′-AMPase activity was enriched 69.5 ± 27.3 times. In all preparations examined, this enrichment was significantly ($P < 0.02$) higher than that of any other determined compound. The CDH concentration in the plasma membrane fraction was increased 34.4 ± 14.1 times. Its enrichment was significantly higher ($P < 0.01$) when compared with total cholesterol, CMH, and total phospholipids. Enrichment of these last three substances in the plasma membrane fraction was similar.

Distribution of individual phospholipids in the whole homogenate and in the plasma membrane fraction. As shown in **Table 3,** the phospholipids in the whole leukemic lymphocytes and in plasma membrane preparations were the same, as judged by two-dimensional thin-layer chromatography. Quantitatively, however, there was a significant ($P < 0.05$) decrease in phosphatidylcholine and an increase in sphingomyelin in plasma membrane fractions.

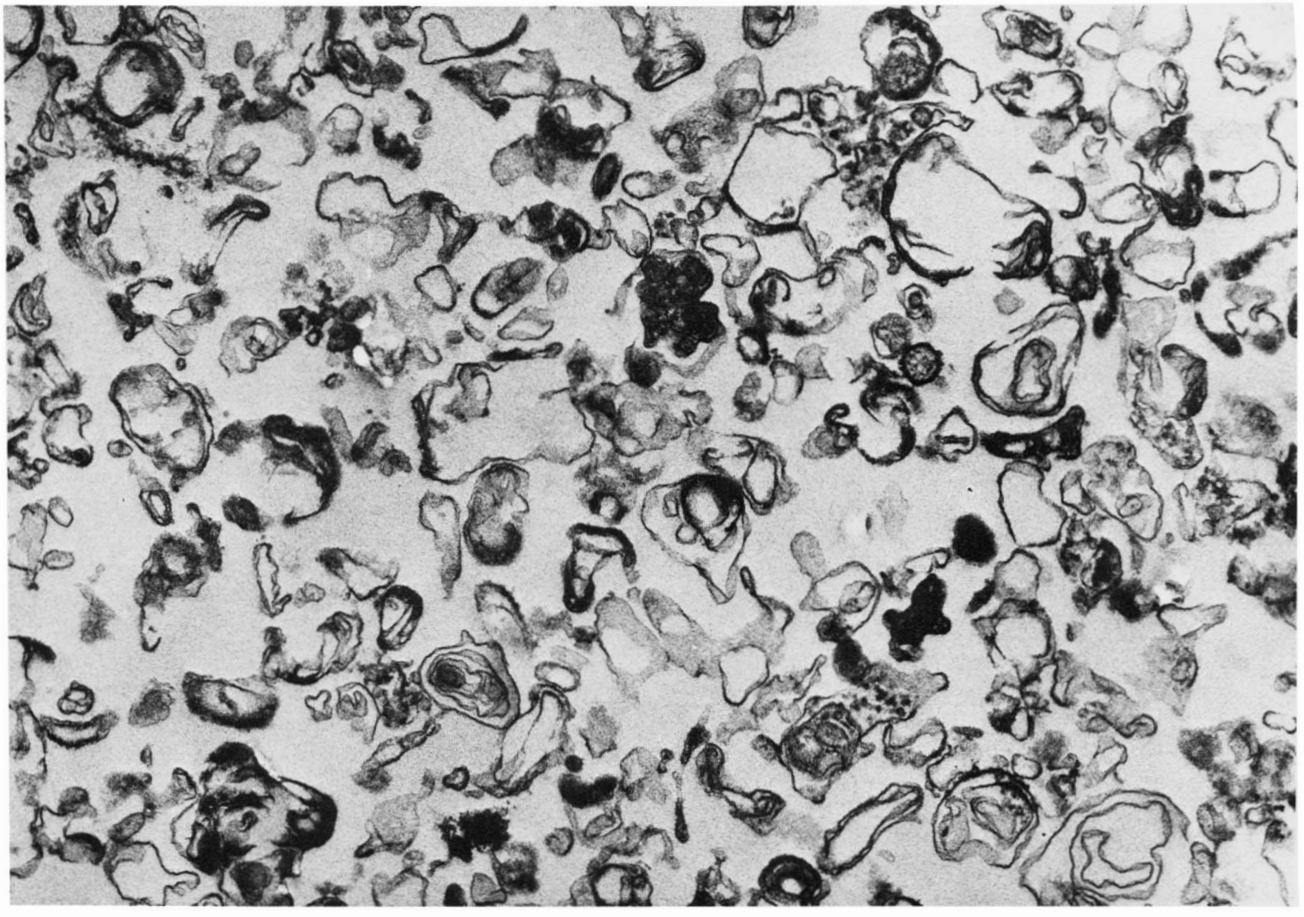

Fig. 1. Typical aspect of leukemic lymphocyte plasma membranes. ×24,000.

DISCUSSION

The mean enrichment of the plasma membrane fraction in 5′-AMPase was 69.5-fold, indicating a very satisfactory degree of purification of this subcellular fraction.

Recently, Misra, Gill, and Estes (20), using a cytochemical method, demonstrated that in rat lymphocytes 5′-AMPase was located exclusively in the plasma membranes. Although this has not yet been established for CLL lymphocytes, 5′-AMPase has been chosen as a plas-

TABLE 1. Distribution of protein, 5′-AMPase, and lipids in subcellular fractions of human leukemic lymphocytes[a]

	P1[b]		SN2		S1		S3		S4		S2B		
Compound	%	Enrich-ment	%	Enrich-ment	%	Enrich-ment	%	Enrich-ment	%	Enrich-ment	%	Enrich-ment	Recovery %
Protein	51.2		27.0		0.13		0.62		9.67		0.11		88.8
5′-AMPase	13.5	0.3	54.5	2.0	9.1	71.3	17.3	27.9	36.2	3.7	10.5	98.0	141.1
Total choles-terol	26.4	0.5	25.2	0.9	3.3	25.9	6.6	10.6	18.2	1.9	2.9	26.5	82.6
CMH	25.8	0.5	48.5	1.8	2.4	18.7	7.9	12.6	11.9	1.2	1.9	17 7	98.4
CDH	26.6	0.5	37.2	1.4	4.1	32.4	10.9	17.4	22.5	2.3	5.8	54.8	107.4
Total phos-pholipids	26.8	0.5	19.3	0.7	3.1	24.4	9.8	10.3	27.2	2.8	1.3	12.2	87.4

[a] Results of a typical experiment are expressed as a percentage of the whole homogenate (%) and as percentage enrichment compared with the whole homogenate.

[b] Symbols used for the different subcellular fractions are as follows: P1, 500 *g* pellet centrifuged for 20 min; SN2, supernate of a 20-min 12,800 *g* centrifugation; S1, S2 (not mentioned in the table), S3, and S4, fractions obtained by centrifugation of the 12,800 *g* pellet on a continuous sucrose gradient of density 1.080 to 1.150 for 2 hr; and S2B, material corresponding to fraction S2 after two successive centrifugations on a continuous sucrose gradient.

TABLE 2. 5′-AMPase specific activity and concentrations of cholesterol, CMH, CDH, and total phospholipids in whole homogenate and in plasma membranes

Compound	Whole Homogenate	Plasma Membrane Fraction	Enrichment in Plasma Membranes
5′-AMPase	2.69 ± 1.67	154.3 ± 20.6	69.5 ± 27.3
Total cholesterol	13.5 ± 3.7	208.1 ± 31.4	16.7 ± 7.0
CMH	1.30 ± 0.76	21.57 ± 8.61	15.1 ± 4.0
CDH	0.45 ± 0.18	13.70 ± 2.10	34.4 ± 14.1
Total phospholipids	86.8 ± 14.8	1116.3 ± 328.2	12.9 ± 1.9

Results for 5′-AMPase are given in units/mg of protein; all others are given in μg/mg of protein. Values are means ± SD of four experiments.

TABLE 3. Distribution of individual phospholipids in whole homogenate and in plasma membranes from human leukemic lymphocytes

	Whole Homogenate	Plasma Membrane Fraction	*P*
	%	%	
Phosphatidylcholine	45.9 ± 3.4	36.5 ± 7.6	<0.05
Lysophosphatidylcholine	1.2 ± 0.8	4.1 ± 3.7	NS
Phosphatidylethanolamine	27.4 ± 4.6	26.9 ± 4.4	NS
Phosphatidylinositol	4.3 ± 1.8	4.4 ± 3.6	NS
Phosphatidylserine	6.7 ± 2.8	7.3 ± 2.7	NS
Phosphatidic acid	3.0 ± 1.7	1.9 ± 1.8	NS
Sphingomyelin	4.1 ± 2.4	8.4 ± 2.6	<0.05
Unidentified	7.6 ± 2.8	10.3 ± 4.1	NS

Results are means ± SD of five experiments for the whole homogenate and three experiments for the plasma membrane fraction. *P* values were calculated by Student-Fisher's *t* test.

ma membrane marker in this study. Indeed, in various subcellular preparations from human normal (21, 22) and leukemic (9, 23) lymphocytes, electron microscopic examinations revealed an enrichment in plasma membranes in fractions in which the specific activity of 5′-AMPase was increased. In addition, in a microsomal fraction of CLL lymphocytes, the distributions, on a continuous sucrose gradient, of 5′-AMPase and Mg^{2+}–Na^{+}–K^{+}–ATPase, another possible plasma membrane marker in lymphocytes (24), were similar (9).

The composition of individual phospholipids in the whole lymphocytes found in our study is consistent with the results published by Gottfried (25). To the best of our knowledge, the pattern of individual phospholipids has not been previously studied in lymphocyte plasma membranes. It is characterized by a relative decrease of phosphatidylcholine and an increase of sphingomyelin compared with the whole homogenate.

Two neutral glycolipids, CMH and CDH, were found in the CLL lymphocytes. The major one, CMH, was present at concentrations similar to those reported in four other preparations of leukemic lymphocytes (10). CDH, not identified in our previous study in lymphocytes (10), was found at a concentration threefold lower than that of CMH. In addition, CDH was identified in only three patients out of five with CLL. The presence of CDH probably represents individual variations, and we must correct our previous conclusion that CLL lymphocytes contained exclusively CMH. Nevertheless, CMH still appears as the major glycolipid in mature lymphocytes and accounts for less than 1.5% of total lipids. These results are in disagreement with observations of Gottfried, who reported that CDH was the major glycolipid in cultured normal lymphocytes (26) and leukemic lymphocytes.[1] We have no explanation for this discrepancy.

The same glycolipids were found in plasma membranes as in the leukemic lymphocytes. Similar observations were made in other tissues also, such as bovine kidney (6), hamster kidney fibroblasts (7), and rat intestinal mucosa (8). In the present study, the enrichment of CDH in plasma membranes was significantly higher than in all other lipids, including CMH.

As pointed out by Renkonen et al. (7), glycolipids possess a great advantage over the enzyme markers in that they can be quantified chemically, whereas with enzymes, only activity can be measured. These authors also demonstrated that, in hamster kidney fibroblasts, gangliosides, ATPase, and surface antigens were equally good as plasma membrane markers (7). Nevertheless, in our study, CDH appeared to be a less useful plasma membrane marker than 5′-AMPase and cannot be considered as an exclusive component of the plasma membrane. Although the highest enrichment in CDH was associated with the highest enrichment in 5′-AMPase and with the highest molar ratio of cholesterol to phospholipids, comparison of enrichments in 5′-AMPase and CDH indicates that only about 50% of the total CDH was present in plasma membranes. The corresponding figure for the CMH was only 22%.

Studies with other tissues such as bovine mammary gland and rat liver (27) or NIL_2 hamster cells (28) also indicate that glycolipids are nonspecific for surface membranes. Also, in rat intestinal mucosa, invertase was a better cell surface marker than any tested glycolipid (8), and, in L cells, Weinstein et al. (29) found CDH and a monosialoganglioside in whole homogenate but not in plasma membranes. Finally, glycosphingolipids have been identified in secondary lysosomes from rat liver (30, 31), but these structures may derive from plasma membranes.

Our conclusion agrees with the opinion expressed by Critchley, Graham, and Macpherson (28), that glycolipids are located primarily but not exclusively on the cell surface. Their subcellular localization may vary from one cell type to another and from one type of glycolipid to another.

[1] Gottfried, E. L. 1971. Personal communication.

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