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RAPID ISOLATION AND LIPID CHARACTERIZATION OF PLASMA MEMBRANES FROM NORMAL AND MALIGNANT LYMPHOID CELLS OF MOUSE

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A rapid isolation method was developed for plasma membranes from mouse lymphoid cells such as lymph node lymphocytes, thymocytes, radiation-induced thymoma cells and L1210 cells. Lysates of these lymphoid cells were prepared by Dounce homogenization under hypotonic conditions and directly layered on sucrose step density gradients containing 2 mM $CaCl_2$ and 5 mM $MgCl_2$, and centrifuged at 52 000 × g for 1 h. Plasma membrane fractions appeared at the interface between 20 and 42% sucrose in the gradients. The procedure permitted purified membranes from cells to be obtained within 3 h, and the preparations appeared to be uniform by electron microscopy. Specific activities of $(Na^+ + K^+)$-ATPase, Mg^{2+}-ATPase and 5′-nucleotidase of the isolated plasma membranes were enriched 23- to 61-fold, 12- to 15-fold and 18- to 34-fold, respectively, in comparison with those of the corresponding cell homogenates. Cholesterol content of the malignant cell membranes was lower than that of the normal membranes and the molar ratio of cholesterol to phospholipid of the malignant cell membranes was also lower than that of the normal membranes. A decreased plasmalogen content was observed in the malignant plasma membranes, together with a higher percentage of phosphatidylethanolamine and a lower percentage of phosphatidylserine. In the normal cell membranes, thymocytes contained a higher percentage of phosphatidylcholine and a lower percentage of sphingomyelin than those of the lymph node lymphocytes. At all temperature ranges (5 to 40°C) the plasma membranes of the malignant cells had lower microviscosity than those of the normal cells.

Introduction

In order to analyze the lipids of the lymphoid cell plasma membranes it is necessary to isolate plasma membranes rapidly from the cells. Procedures of plasma membrane isolation have been developed by several investigators, which have been reviewed by Jett and Hickey-Williams [1]. One of them used nitrogen gas to disrupt cells under isotonic conditions, and is suitable for isolation of plasma membrane from cells of free type such as lymphoid cells, for example, mouse leukemia cells [2], calf and pig lymph node lymphocytes [3] and calf thymocytes [4,5]. However, a defect of this method is that it requires considerable time to isolate biologically native plasma membranes. Another method is to swell cells osmotically in hypotonic medium followed by Dounce homogenization, Marique and Hildebrand [6] applied this method to study the lipid composition of plasma membranes from human chronic lymphocytic leukemia cells. Their procedure, however, requires three ultracentrifugation steps for subsequent subcellular fractionation and took over 25 h

Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; TLC, thin-layer chromatography.

for the isolation. Therefore, we have developed a rapid isolation procedure for plasma membrane from mouse lymphoid cells.

Furthermore, because of the difficulty in obtaining a sufficient amount of the pure plasma membranes, lipid characterization of the lymphoid cell plasma membranes has not been fully investigated, except for the determination of the phospholipid and cholesterol contents [3–9]. Recently, the phospholipid composition of plasma membranes from human tonsil lymphocytes and chronic lymphocytic leukemia, from mouse lymphoma cells [10] and from calf thymocytes [11] was reported. We also have performed the analysis of phospholipid composition of several rat lymphoid cell membranes [12,13]. However, regarding lipid composition of mouse lymphoid cell plasma membrane, no data utilizing exist except for that of lymphoma cells [10]. In the present study, using the plasma membranes isolated from normal and malignant mouse lymphoid cells, we have carried out the analysis of their lipid content, phospholipid composition and microviscosity of membrane lipid region.

Materials and Methods

Cells. Normal lymph node lymphocytes were obtained from lymph nodes which were pooled from cervical, axillary, brachial and mesenteric nodes of 50 to 100 mice (average 20 g weight) of the C57BL/6 strain, and thymocytes were also obtained from thymuses of the same mice. The lymph nodes or thymuses were minced well with scissors and rubbed against metal mesh (No. 100) with a rounded glass rod to make cell suspensions in phosphate-buffered saline containing 5 mM $MgCl_2$ (pH 7.3). The thymic lymphosarcomas [14] were obtained from thymuses of 4-month-old C57BL/6 mice which received irradiation with 4 doses of X-rays (170 R) at intervals of 7 days during 1- to 2-month-old and suspended cells were prepared by the same procedure used for normal thymuses. The L1210 leukemia cells of which origin was T-cell were harvested from the abdominal cavity of the BDF_1 mice in which the cells had been maintained by intraperitoneal transplantation. To obtain sufficient quantities of plasma membranes from the X-ray-induced thymoma and L1210 cells, 10 to 20 and 20 to 30 mice, respectively, were used per experiment. To ensure complete removal of contaminating erythrocytes, each cell suspension in phosphate-buffered saline containing 5 mM $MgCl_2$ was centrifuged at $500 \times g$ for 2 min and 9 volumes of 1 mM $NaHCO_3$, 2 mM $CaCl_2$ and 5 mM $MgCl_2$ (pH 7.8) were added to the packed cells. After 25 s hypotonic treatment, the cell suspension was adjusted to isotonic osmolarity by adding 10-times concentrated phosphate-buffered saline. Then, the cells free from erythrocytes were prepared by centrifugation at $500 \times g$ for 2 min. The temperature was maintained below 4°C throughout the isolation procedures.

Isolation of plasma membranes. The following procedure was used for isolation of plasma membranes. Packed cells were suspended in 10 volumes of 1 mM $NaHCO_3$, 2 mM $CaCl_2$ and 5 mM $MgCl_2$ solution to swell the cells. Ca^{2+} and Mg^{2+} were included to prevent disruption of nuclei. After 15 to 20 min, lymphocytes and thymocytes were homogenized by a tight-fitting Dounce glass homogenizer, Kontes type B (Kontes Glass Co., Vineland, NJ), of which content was 40 ml, with 10 to 15 strokes. In X-ray-induced thymoma and L1210 cells, an optimal time of swelling of the cells in the hypotonic medium before homogenization was 5 to 10 min and numbers of stroke of pestle were 8 to 10 and 3 to 5 times, respectively, because these cells were relatively labile to hypotonic shock in comparison with normal cells.

Each homogenate was layered on sucrose layers in centrifuge tube in which the following sucrose solutions were pipetted in the following order: 7 ml of 70%, 15 ml of 42%; and 16 ml of 20% sucrose solutions. Each sucrose solution contained 2 mM $CaCl_2$ and 5 mM $MgCl_2$. The samples were centrifuged for 1 h in the Beckman L2-65B ultracentrifuge using a SW 25A-2 rotor at $52\,000 \times g$. Plasma membrane fraction was obtained as a band at the interface between 20 and 42% sucrose layers. Mitochondria, cell debris and undisrupted cells were recovered at the interface of 42 and 70% sucrose layers, and nuclei were sedimented at the bottom of tube. The hypotonic medium and 20% sucrose layer containing microsomal components were removed by aspiration and the band containing plasma membranes was carefully pipetted off. This plasma membrane fraction was diluted to isotonic sucrose concentration with 1 mM $NaHCO_3$ and centrifuged at $13\,000 \times g$ for 15 min to remove contaminating microsomes. After

centrifugation, the sediment was suspended with 1 mM $NaHCO_3$ solution to wash and centrifuged at 13 000 × *g* for 15 min. The pellet of plasma membranes was resuspended in a small volume of 1 mM $NaHCO_3$ solution and used for analysis.

Electron microscopy. Plasma membrane preparations were directly fixed with 1% osmium tetroxide in 0.05 M phosphate buffer (pH 7.4), on ice for 1 h. Samples were dehydrated in ascending concentrations of ethanol and embedded in Quetol 651. Ultrathin sections were cut and stained with uranyl acetate and lead citrate and examined in a Hitachi HU-11 transmission electron microscope.

Protein determination and enzyme assays. Protein was determined according to the method of Lowry et al. [15] with bovine serum albumin as a standard.

The activities of $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase were measured by the method of Komatsu and Fujii [16]. Briefly, 1 ml of incubation mixture for total ATPase activities consisted of 2 mM ATP, 140 mM NaCl, 14 mM KCl, 5 mM $MgCl_2$, 20 mM histidine buffer (pH 7.7) and 100 to 200 μg of enzyme source (as protein). The Mg^{2+}-ATPase activity was determined adding 0.1 mM Ouabain in the incubation mixture. The $(Na^+ + K^+)$-ATPase activity was obtained from the difference of total ATPase and Mg^{2+}-ATPase activities. Assay of 5′-nucleotidase activity was performed according to the modified method of Heppel and Hilmoe [17] as described previously [18]. Glucose-6-phosphatase activity was measured by the procedure of Nordlie and Arion [19]. Incubation was carried out at 37°C for 30 min for ATPase and for 15 min for 5′-nucleotidase and glucose-6-phosphatase. Termination of the reactions was done by addition of 5% perchloric acid solution. The liberated inorganic phosphate was determined by the Fiske-SubbaRow method [20]. The activity of NADPH-cytochrome *c* reductase was determined essentially according to the method of Tsai et al. [21], namely, by reading the rate of increase in absorbance at 550–540 nm of reduced cytochrome *c* using Hitachi model-556 double wavelength double beam spectrophotometer. NADH-cytochrome *c* reductase activity was measured by the same procedure of NADPH-cytochrome *c* reductase except for use of NADH (Sigma) instead of NADPH (Sigma). The activity of succinate dehydrogenase was estimated by the method of Green et al. [22] and the reduced cytochrome *c* was determined by the same procedure using in NADPH-cytochrome *c* reductase.

Microviscosity of membrane lipid regions. 1,6-Diphenyl-1,3,5-hexatriene (DPH) was used as a fluorescence polarization probe for monitoring the fluidity in the membrane lipid regions [23]. Labeling of the isolated plasma membranes was performed with DPH dispersion which was obtained by adding 0.1 ml of 2 mM DPH in tetrahydrofuran to 50 ml of phosphate-buffered saline containing 5 mM $MgCl_2$ and stirring vigorously. A volume of plasma membrane (100–200 μg protein/ml) suspension in phosphate-buffered saline containing 5 mM $MgCl_2$ was mixed with 2 volumes of the DPH dispersion and incubated for 30 min at 37°C. The labeled plasma membranes were then washed twice with phosphate-buffered saline containing 5 mM $MgCl_2$ and used for fluorescence measurement.

Fluorescence polarization was measured using the Elscint microviscosimeter model MV-1a (Elscint, Israel). The degree of fluorescence polarization, *P*, defined in the following equation, was directly recorded.

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

$I_{\parallel}$ and $I_{\perp}$ are the emission intensities polarized parallel and perpendicular, respectively, to the direction of polarization of the exciting light. Microviscosity, $\bar{\eta}$, was calculated from *P* value by the equation described by Shinitzky and Inbar [23] using an average value of 10 ns for τ [24].

Lipid assays. Total lipids were extracted from the whole cells and plasma membrane suspensions with 20 volumes of chloroform/methanol (2 : 1, v/v) according to the procedure of Folch et al. [25] and the extracts were used to analyze the following lipids. Analysis of cholesterol was made by thin-layer chromatography (TLC) using precoated HPTLC plates of Silica gel 60 (Merck) with petroleum ether/diethyl ether/acetic acid (60 : 40 : 1, v/v). The TLC plate was sprayed with 50% H_2SO_4 solution and charred to detect free and esterified cholesterols. Total cholesterol was determined using ferric cholride reagent by the method of Courchaine et al. [26] with free cholesterol as a standard. Phospholipid content was expressed as phospholipid-phosphorus which was determined by the method of Bartlett [27] after per-

chloric acid combustion. Each phospholipid was fractionated by two-dimensional TLC of Silica gel H (Merck) containing 2% magnesium hydroxide carbonate, with chloroform/methanol/7 M ammonia (90 : 54 : 11, v/v) and chloroform/methanol/acetic acid/water (90 : 40 : 12 : 1, v/v) [18]. After development each spot of phospholipids was detected by the reagent of Dittmer and Lester [28] and identified using authentic phospholipids. Phospholipids with free amino group or with choline were detected with ninhydrin or Dragendorff reagent [29], respectively. In order to analyze quantitatively, the developed plates were put into the I_2-vapor chamber and the visualized phospholipid spots were scraped off. After extraction from silica gel with chloroform/methanol/formic acid (1 · 1 : 2, v/v), each phospholipid was determined. The phospholipid composition was expressed as percentage of total phospholipid-phosphorus. Total fatty aldehyde was measured according to the *p*-nitrophenylhydrazine method [30] and plasmalogen (alkenyl ether linkage) by a colorimetric method using iodine reagent [31].

Results

Isolation of plasma membranes

Two steps in the procedure contributed significantly to the rapid isolation of plasma membranes. First, the homogenate was directly layered on 20% sucrose layer in the centrifuge tubes. Second, the addition of 2 mM $CaCl_2$ and 5 mM $MgCl_2$ to each sucrose solution prevented aggregation of cells and disruption of nuclei so that plasma membranes could be separated in only one ultracentrifugation step at 52 000 × *g* for 1 h.

Yields of the plasma membranes isolated from the mouse lymphoid cells, lymph node lymphocytes, thymocytes, X-ray-induced thymoma cells and L1210 leukemia cells, were 0.5 to 0.8% which are expressed in terms of percentage of membrane protein to total protein in the original whole homogenate (Table I). The yields were about the same as described in previous papers [3–5,8,12,13,32–35].

Electron microscopy

Electron micrographs of the plasma membranes isolated from lymphoid cells of mice are shown in Fig. 1. The plasma membranes from the malignant cells, X-ray-induced thymoma cells (Fig. 1C) and L1210 leukemia cells (Fig. 1D), were observed as uniform vesicles consisting of smooth membrane. The appearance was similar to plasma membranes of normal cells, lymph node lymphocytes (Fig. 1A) and thymocytes (Fig. 1B). No other subcellular organelles such as mitochondria and rough endoplasmic reticulum were observed.

Marker enzymes in plasma membranes

Table I shows the specific activities of various marker enzymes in the plasma membrane preparation (PM in Table I) in comparison with those in the homogenate of whole cells (WCH in Table I). It was apparent that the three marker enzymes for plasma membrane, $(Na^+ + K^+)$-ATPase, Mg^{2+}-ATPase and 5′-nucleotidase, were concentrated in the plasma membrane preparations. Specific activities of $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase in the plasma membranes were enriched 41- and 15-fold for lymph node lymphocytes, 47- and 15-fold for thymocytes, 61- and 13-fold for X-ray-induced thymoma cells and 23- and 12-fold for L1210 cells, respectively, and that of 5′-nucleotidase was enriched 18-, 19-, 34- and 27-fold in the respective plasma membranes in the order cited above. Specific activities of glucose-6-phosphatase and NADPH-cytochrome *c* reductase, marker enzymes for microsomes, in each plasma membrane preparation were considerably lower than those of the corresponding whole cell homogenates. Specific activity of NADH-cytochrome *c* reductase, a marker enzyme for mitochondria as well as microsomes, in the plasma membranes was about half that of the original whole cell homogenate. Specific activities of succinate dehydrogenase, another marker for mitochondria, in the plasma membranes and whole cell homogenate of L1210 cells were 1.09 and 1.90, respectively (data not cited in Table I).

Microviscosity of membrane lipid regions

Microviscosity of the isolated plasma membranes was measured at intervals of 5°C in the range of 5 to 40°C and plotted in Fig. 2, as log $\overline{\eta}$ versus $1/T$. Temperature dependence of microviscosity showed that at all temperature ranges (5 to 40°C) the plasma membranes of the malignant cells had lower microviscosity than those of the normal cells. Microviscosity of the plasma membranes from the lymph node

TABLE I

SPECIFIC ACTIVITIES OF VARIOUS MEMBRANE MARKER ENZYMES IN WHOLE CELL HOMOGENATES (WCH) AND PLASMA MEMBRANE (PM) PREPARATIONS

Specific activities of enzymes are expressed in μmol P_i released per mg protein per h at 37°C and μmol cytochrome *c* reduced per mg protein per h at 37°C for NADPH- and NADH-cytochrome *c* reductases. Values are expressed as mean ± S.D. with the number of experiments in parentheses. Yield of PM, the membrane protein percentage of the total protein in the WCH, is expressed as the mean with the number of experiments in parentheses. Each assay was carried out in duplicate.

Enzyme	Lymph node lymphocytes		Thymocytes		X-ray-induced thymoma cells		L1210 cells	
	WCH	PM	WCH	PM	WCH	PM	WCH	PM
Yield of PM (%)		0.81 (2)		0.61 (2)		0.54(3)		0 71 (2)
$(Na^+ + K^+)$-ATPase	0.038 ± 0.002 (2)	1.56 ± 0.10 (2)	0.078 ± 0.003 (2)	3.65 ± 0.35 (2)	0.12 ± 0.00 (3)	7.37 ± 0.79 (3)	0.25 ± 0.01 (2)	5 79 ± 0.41 (2)
Mg^{2+}-ATPase	2.68 ± 0.19 (2)	38.77 ± 2 63 (2)	1.49 ± 0.15 (2)	22.06 ± 2.12 (2)	1.36 ± 0.14 (3)	18.14 ± 1.35 (3)	1 42 ± 0.07 (2)	17.30 ± 1.86 (2)
5′-Nucleotidase	0.49 ± 0.01 (2)	8.82 ± 0.96 (2)	0.070 ± 0.002 (2)	1.31 ± 0.08 (3)	0.070 ± 0.008 (3)	2.38 ± 0.20 (3)	067 ± 0.03 (3)	18.40 ± 0 95 (3)
Glucose-6-phosphatase	0.38 ± 0.05 (2)	<0.01 (1)	0.55 ± 0.05 (2)	<0.01 (1)	0.53 ± 0.04 (3)	0.32 ± 0.03 (4)	0.24 ± 0.04 (2)	<0.01 (1)
NADPH-cytochrome *c* reductase	0.87 (1)	0.46 (1)	n.d. [a]	n.d. [a]	0.41 ± 0.05 (2)	0.24 ± 0.02 (2)	0.51 (1)	0.23 (1)
NADH-cytochrome *c* reductase	5.02 ± 0.92 (2)	4.99 ± 0.60 (2)	4.00 (1)	3.34 (1)	0.83 ± 0.10 (2)	0.55 ± 0.06 (2)	1.09 ± 0.16 (2)	0.19 ± 0.02 (2)

[a] n.d., not determined.

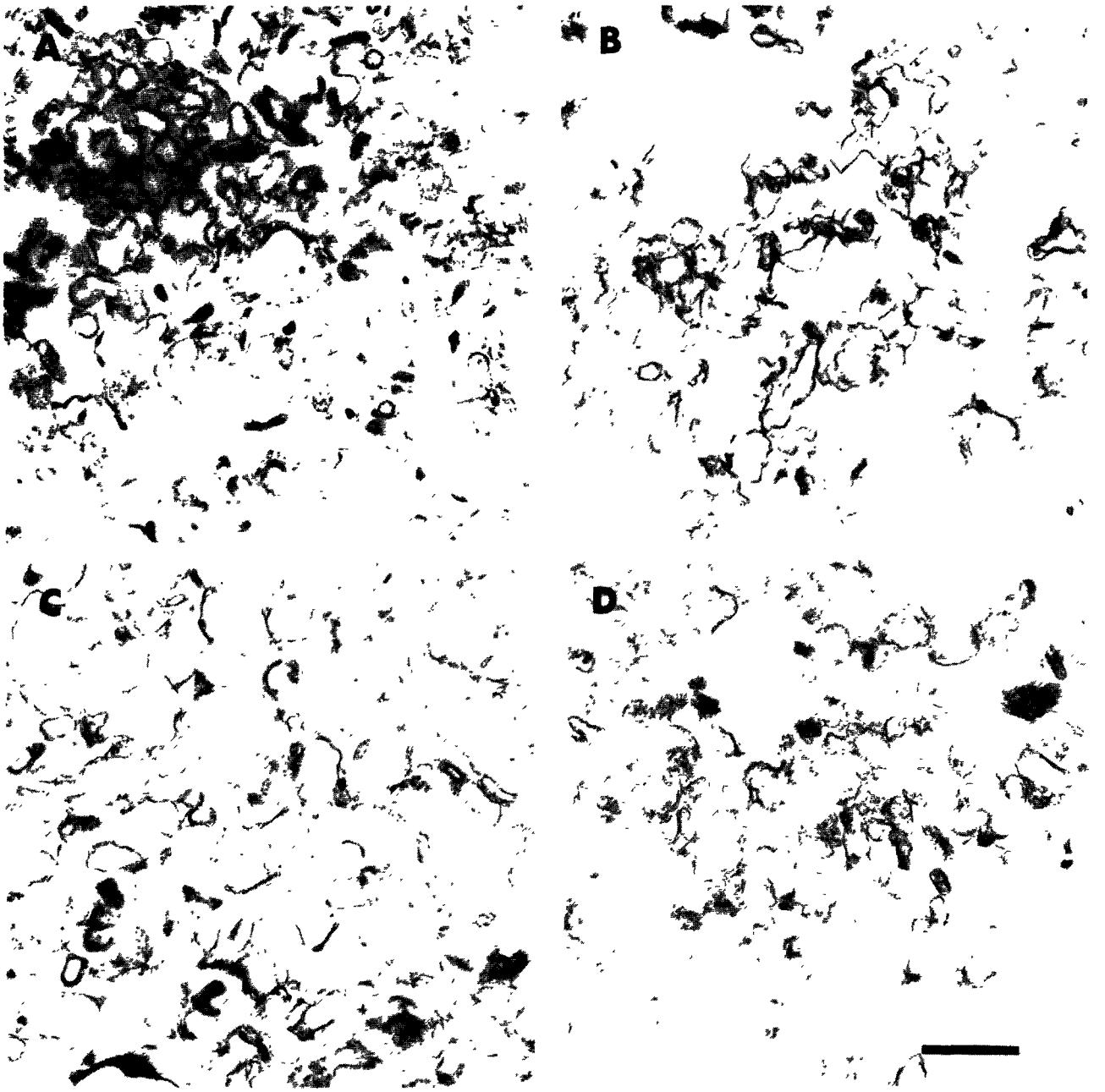

Fig. 1 Electron micrographs of the plasma membranes isolated from mouse lymphoid cells, A, lymph node lymphocytes, B, thymocytes, C, X-ray-induced thymoma cells, and D, L1210 cells. All figures are of the same magnification and the marker bar indicates 1 μm.

lymphocytes was slightly higher than that of the thymocytes.

Data points at 25°C from these plots and fluorescence polarization (P) values at the same temperature are given in Table II. Microviscosity at 25°C of the plasma membranes from two malignant cells were lower than those of two normal cells.

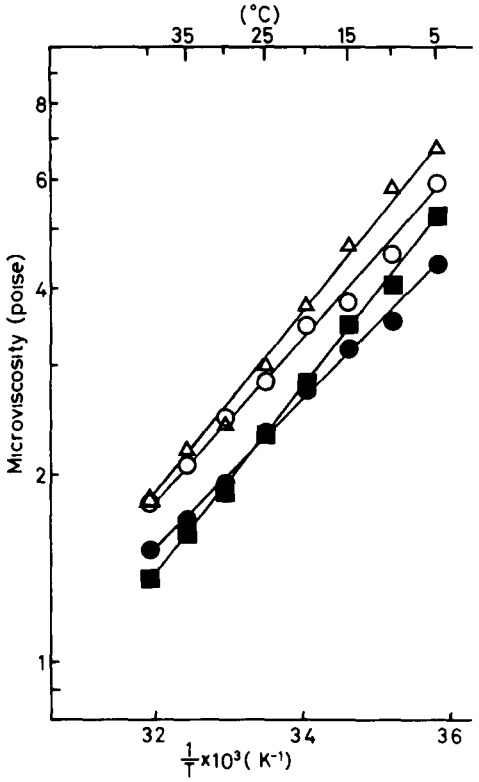

Fig. 2. Temperature dependence of microviscosity ($\bar{\eta}$) presented as log $\bar{\eta}$ versus $1/T$, obtained with DPH-labeled plasma membranes from lymph node lymphocytes (△———△), thymocytes (○———○), X-ray-induced thymoma cells (●———●) and L1210 cells (■———■). Experimental conditions used were described in Materials and Methods.

Lipid content

Table III shows the results of phospholipid and cholesterol contents of whole cells and isolated plasma membranes. The plasma membranes from the malignant cells had less lipid than the normal cell membranes. The molar ratio of cholesterol to phospholipid of the plasma membranes was higher in the normal cells (0.580 and 0.583) than in the malignant cells (0.439 and 0.546). Qualitative observation of cholesterol by TLC indicated that the plasma membranes of the mouse lymphoid cells used in this study contained little esterified cholesterol.

Table III also shows that in each plasma membrane the content of plasmalogen was equal to that of the total fatty aldehydes, and this was also observed in whole cells. It indicates that the amount of free fatty aldehyde was not significant. No difference was found in the content of plasmalogen among the plasma membranes from two normal cells and also among the plasma membranes from two malignant cells. The plasmalogen contents of the plasma membranes from the malignant cells, however, were lower than those of the normal cell membranes. The same results was observed in the case of the whole cells.

TABLE II

DEGREE OF FLUORESCENCE POLARIZATION AND MICROVISCOSITY OF DPH-LABELED PLASMA MEMBRANES

Plasma membrane preparations (100 μg protein/ml) were labeled with 2 vol. of 4 μM DPH dispersion for 30 min at 37°C. The labeled plasma membranes were washed twice with phosphate-buffered saline containing 5 mM $MgCl_2$ and used for fluorescence measurement. Degree of fluorescence polarization (P) values are range of three experiments determined at 25°C. Microviscosity ($\bar{\eta}$) was calculated from the mean of P values at 25°C of each specimen.

Plasma membrane	P	$\bar{\eta}$
Lymph node lymphocytes	0.260–0.266	2.98
Thymocytes	0.254–0.261	2.82
X-ray-induced thymoma cells	0.235–0.241	2.33
L1210 cells	0.233–0.240	2.31

Phospholipid composition

The phospholipid composition of the cells and plasma membranes (expressed as percentages of total lipid-phosphorus) is shown in Table IV. It was found that the plasma membranes generally had higher sphingomyelin and phosphatidylserine percentages and lower phosphatidylethanolamine, phosphatidylinositol and cardiolipin percentages in comparison with the corresponding whole cells. Among the plasma membranes of the normal cells, the thymocytes had a higher percentage of phosphatidylcholine and a lower percentage of sphingomyelin than the lymph node lymphocytes. However, percentages of other phospholipid classes were very similar in these two cells. The plasma membranes of the malignant cells contained higher percentages of phosphatidylethanolamine and phosphatidylinositol and a lower percentage of phosphatidylserine than the normal membranes. In addition, L1210 plasma membrane had a very high percentage of sphingomyelin and low percentage of phosphatidylcholine.

TABLE III

LIPID CONTENTS OF WHOLE CELLS AND THEIR PLASMA MEMBRANES OF NORMAL AND MALIGNANT LYMPHOID CELLS

Each lipid determination was carried out using total lipid extract of each sample. Values are expressed as mean ± S.D of two separate experiments and each assay was done in duplicate. Molar ratio of cholesterol to phospholipid-phosphorus was calculated from the means of phospholipid-phosphorus and cholesterol.

Cell	Phospholipid-phosphorus (μmol/mg protein)	Cholesterol (μmol/mg protein)	Cholesterol/phospholipid-phosphorus (molar ratio)	Fatty aldehyde/phospholipid-phosphorus (mol%)	Plasmalogen/phospholipid-phosphorus (mol%)
Whole cells					
Lymph node lymphocytes	0.132 ± 0.003	0.050 ± 0.004	0.379	9.5 ± 0.3	10.6 ± 0.2
Thymocytes	0.109 ± 0.002	0.044 ± 0 004	0.404	9.2 ± 0.2	9.9 ± 0.2
X-ray-induced thymoma cells	0.092 ± 0.003	0.021 ± 0.001	0.226	6.4 ± 0.1	6.8 ± 0.1
L1210 cells	0.112 ± 0.001	0.044 ± 0.003	0.393	6.3 ± 0.1	6.5 ± 0.1
Plasma membranes					
Lymph node lymphocytes	1.260 ± 0.065	0.731 ± 0.049	0.580	11.5 ± 0.3	12.0 ± 0.4
Thymocytes	1.670 ± 0.032	0.973 ± 0.057	0.583	11.0 ± 0.2	11.7 ± 0.2
X-ray-induced thymoma cells	1.347 ± 0.056	0.591 ± 0.023	0.439	9.0 ± 0.1	9.0 ± 0.2
L1210 cells	0.635 ± 0.061	0.347 ± 0.032	0.546	8.1 ± 0.1	8.1 ± 0 1

Discussion

Recently, Monneron and d'Alayer [36] isolated plasma membranes from calf thymus by a simplified procedure using one ultracentrifugation step. Cell disruption was carried out with hypertonic sucrose medium by Potter homogenizer and the plasma membranes were separated by ultracentrifugation for 2 h at 130 000 × *g* using discontinuous density gradient of sucrose. The levels of enrichment in the specific activities of the plasma membrane marker enzymes in their calf thymus membranes were similar to those of our plasma membranes isolated from the mouse lymphoid cells. However, they had employed only calf thymus and characterization of membrane lipid was not carried out.

Activity of 5′-nucleotidase in the plasma membranes as well as in the whole cell homogenates of the normal thymocytes was lower than that in the corresponding preparation from the normal lymph node lymphocytes. A similar result has been reported for the thymocytes and lymph node lymphocytes of pig by Allan and Crumpton [33]. Misra et al. [37] observed that the 5′-nucleotidase activity of the thymocyte plasma membranes of the ACI rat was lower than that of the splenic lymphocyte membranes of the same rat, although we found that the same enzyme activities of thymocyte membranes of the Wistar [12] and Buffalo [13] rats were much higher than that of the ACI rat [37] (in terms of specific activity). Of this enzyme in the human lymphoid cell membranes, thymocytes [38] had a low activity (specific activity 3.9), while tonsil lymphocytes [32] had a high activity (specific activity 13.5 to 14.6). These results imply that low 5′-nucleotidase activity is one of the characteristics of the thymocyte plasma membrane.

Our data on microviscosity of the plasma membranes indicate that normal mouse lymphoid cells had a higher viscosity in membrane lipid regions than malignant cells, as reported previously [24,39,40].

Van Blitterswijk et al. [4] reported that a molar ratio of cholesterol to phospholipid and phospholipid content (μmol per mg protein) of the plasma membranes from calf thymocytes were 0.61 and 1.66, respectively, which is similar to our results. Namely,

TABLE IV

PHOSPHOLIPID COMPOSITION OF WHOLE CELLS AND THEIR PLASMA MEMBRANES OF NORMAL AND MALIGNANT LYMPHOID CELLS

The phospholipid composition was determined by two-dimensional TLC using lipid extract of each sample. Values are expressed as phospholipid-phosphorus percentage (mean ± S.D.) of total phospholipid-phosphorus. Figures in parentheses are the number of experiments and each assay was carried out in duplicate.

Phospholipid	Whole cells				Plasma membranes			
	Lymph node lymphocytes (2)	Thymocytes (2)	X-ray-induced thymoma cells (2)	L1210 cells (2)	Lymph node lymphocytes (2)	Thymocytes (2)	X-ray-induced thymoma cells (3)	L1210 cells (3)
Phosphatidyl-choline	52.3 ± 2.0	52.2 ± 2.5	52.1 ± 2.8	44.9 ± 1.6	51.8 ± 2.6	55.0 ± 2 2	50.7 ± 2.3	41.2 ± 2.9
Lysophosphatidyl-choline	0.5 ± 0.2	0.7 ± 0.3	0.8 ± 0.2	1.8 ± 0.3	1.3 ± 0.2	1.0 ± 0.1	2.0 ± 0.6	1 7 ± 0.1
Sphingomyelin	5.8 ± 0.7	2.5 ± 0.3	1.0 ± 0.7	8.3 ± 1.0	7.4 ± 0.5	5.0 ± 0.4	4.2 ± 0.5	13.4 ± 2.9
Phosphatidyl-ethanolamine	23.1 ± 1.1	25.5 ± 0.9	28.9 ± 1.6	26.3 ± 1.3	21.7 ± 1.5	21.1 ± 1.1	25.5 ± 0.1	25 6 ± 1 7
Lysophosphatidyl-ethanolamine	0.5 ± 0.3	0.6 ± 0.2	0.8 ± 0.2	1.2 ± 0.4	0.8 ± 0.3	0.6 ± 0.2	1.3 ± 0.4	2.2 ± 0.3
Phosphatidyl-serine	6.2 ± 0.5	5.5 ± 0.3	3.7 ± 0.4	4.9 ± 0.3	9.2 ± 0.4	10.5 ± 0.5	7.6 ± 0.3	7.1 ± 1.0
Phosphatidyl-inositol	6.6 ± 0.3	7.5 ± 0.4	7.0 ± 0.4	7.6 ± 0.3	5.9 ± 0.3	5.6 ± 0.4	6.8 ± 0.4	6.3 ± 0.7
Phosphatidic acid	0.3 ± 0.2	0.5 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	0.9 ± 0.4	0.7 ± 0.4	0.7 ± 0.3	1.4 ± 0.8
Cardiolipin and/or phosphatidyl-glycerol	4.7 ± 0.3	5.0 ± 0.3	5.5 ± 0.4	4.7 ± 0.3	1.0 ± 0.5	0.5 ± 0.2	1.2 ± 0.7	1.1 ± 0.5

the plasma membranes isolated by our method from the normal mouse lymph node lymphocytes and thymocytes exhibited the ratio of these two lipids to be 0.580 and 0.583, respectively, and phospholipid content to be 1.26 and 1.67 μmol per mg protein, respectively, as shown in Table III. However other investigators [5,8,33,39] have reported relatively high values (0.79 to 1.3) of the molar ratio and low phospholipid content (0.50 to 0.82 μmol per mg protein) on some lymphoid cell membranes. One cause of the different results may be brought about by a loss of phospholipid by the lipid extraction procedure employed in the latter investigation, as pointed out by Johnson and Robinson [10] and Johnson [41].

The plasma membranes of the malignant cells had lower ratios of cholesterol to phospholipid than the normal plasma membranes. Similar data have been reported for pairs of mouse thymocytes-GRSL lymphoid leukemia cells [24], of rat thymocytes-RML11 thymic leukemia cells [12] and of human peripheral blood lymphocytes-leukemia cells [39, 40]. The low ratios in the malignant plasma membranes may contribute to the low lipid microviscosity of the same membranes.

Gottfried [42] reported that normal human lymphoid cells contain a high percentage of plasmalogen to phospholipid (more than 14%). We also found a considerable amount of plasmalogen in both whole cells and plasma membranes of the two normal lymphoid cells of mouse. In the malignant lymphoid cells, this content of plasmalogen decreased in both the whole cells and plasma membranes in comparison with the corresponding preparations from the normal cells.

The pattern of phospholipid composition has now been studied in the normal mouse lymphocyte plasma membranes. The lymph node lymphocyte plasma membranes had a higher percentage of sphingomyelin and a low percentage of phosphatidylcholine than those of the thymocyte membranes. The changes detected in the lymph node lymphocyte membranes may be due to difference in phospholipid composition of the plasma membranes of mature lymphoid cells in lymph nodes and immature lymphoid cells in thymuses.

Although we found recently that rat thymocyte plasma membranes were deficient in sphingomyelin in their phospholipid composition [12,13], regarding mouse lymphoid cell membranes the deficiency of sphingomyelin was not observed, but presence of a considerable amount of this phospholipid was confirmed as similar to the results of human [10,43], mouse [10] and calf [11] lymphoid cell membranes.

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