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DIFFERENCES IN LIPID FLUIDITY AMONG ISOLATED PLASMA MEMBRANES OF NORMAL AND LEUKEMIC LYMPHOCYTES AND MEMBRANES EXFOLIATED FROM THEIR CELL SURFACE

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Summary

The fluorescence polarization technique with 1,6-diphenyl 1,3,5-hexatriene as a probe was used to determine the lipid microviscosity, $\bar{\eta}$, of isolated plasma membranes of mouse thymus-derived ascitic leukemia (GRSL) cells and of extracellular membraneous vesicles exfoliated from these cells and occurring in the ascites fluid. For comparison, $\bar{\eta}$ was also determined in isolated plasma membranes of normal thymocytes and extracellular membranes of thymus cell supernatants.

For isolated plasma membranes of thymocytes and GRSL cells $\bar{\eta}$ values at 25°C amounted to 4.67 and 3.28 P, respectively, which were higher than the microviscosities of the corresponding intact cells, 3.24 and 1.73 P, respectively.

Microviscosities in extracellular membranes of thymocytes and GRSL cells were 5.96 and 5.83 P, respectively. The fluidity difference between these membranes and plasma membranes was most pronounced for the leukemic cells and was thereby correlated with a large difference in cholesterol/phospholipid molar ratio (1.19 for extracellular membranes and 0.37 for plasma membranes). It is proposed that extracellular membraneous vesicles are shed from the surface of GRSL cells similar to the budding process of viruses, that is by selection of the most rigid parts of the host cell membrane.

Liposomes of total lipid extracts of plasma membranes and extracellular membranes of both cell types exhibited about the same microviscosity as the corresponding intact membranes, indicating virtually no contribution of (glyco)-protein to the lipid fluidity as measured by the fluorescence polarization technique. For both cell types $\bar{\eta}$ (25°C) values of liposomes consisting of membrane phospholipids varied between 1.5 and 1.9 P, much lower than the values for

Abbreviation: DPH, 1,6-diphenyl 1,3,5-hexatriene.

total lipids, indicating a significant rigidizing effect of cholesterol in each type of membrane.

Introduction

The dynamics of cell surface membrane constituents may play a major role in processes associated with growth, recognition and differentiation of cells, metastasis of tumor cells and their escape from host immune surveillance (reviewed in ref. 1). An obvious parameter in this respect is the degree of fluidity of the plasma membrane lipids [1—3]. This quantity can be measured with the fluorescence polarization technique using 1,6-diphenyl-1,3,5-hexatriene (DPH) as a probe which is embedded in the membrane lipid core [2—13]. Such studies have indicated that leukemic cell membranes have a higher fluidity (lower microviscosity) than those of normal lymphocytes [3—6]. Although it has been argued that in intact cells the DPH monitors mainly the surface membrane [8], unequivocal proof is lacking. Therefore, in the present investigation we have determined the microviscosities of (a) purified plasma membranes of thymus-derived leukemic (GRSL) cells and normal thymocytes, in comparison with (b) the intact cells and (c) surface vesicles derived from the cells by other means, referred to as extracellular membranes (see below). Similar measurements were performed on liposomes consisting of total lipids or phospholipids extracted from purified membranes, in order to evaluate the contribution of (glyco)proteins and neutral lipids to the fluidity of the membrane lipids as measured by DPH fluorescence polarization. In addition, the effect of drugs that interact with microtubuli and microfilaments was also investigated.

In our previous studies [14—17] it was found that the GRSL ascites cells contain mammary tumour virus-induced cell surface antigens (MLr) exhibiting highly dynamic properties (shedding, antigenic modulation and capping) but produce only very little virus. However, the ascites fluid contains extracellular membraneous vesicles, which could be isolated and purified in the same manner as were plasma membranes from the cell homogenate [17], and which showed properties similar to the isolated plasma membranes (Van Blitterswijk et al., manuscript in preparation). Such vesicles, presumably derived from the cell surface by shedding, contain MLr tumour antigens, and can also be released from intact cells by washing. In the present study the lipid fluidity of these exfoliated vesicles was compared to that of the plasma membranes isolated from the GRSL cells, in order to find out whether vesicle formation may occur from selected surface-membrane domains, as is the case with enveloped viruses [10,11,18—21].

Materials and methods

Cells. GRSL2 cells from a spontaneous thymus-derived lymphoid leukemia in the GR/A mouse strain were maintained (passage 126 to 142) by weekly intraperitoneal transplantation in 2—3-months-old GR/A mice. The cells were harvested 7 days after transplantation and washed three times with 10—20 vol-

umes of Hanks solution (Oxoid, London, U.K.).

Thymocytes from 3—5-weeks-old GR/A mice were obtained by mincing thymuses in a few drops of Hanks solution with scissors and, after dilution, filtrating the single cells through four layers of prewetted surgical gauze. The cells were washed three times with Hanks solution.

Isolation of plasma membranes. Plasma membranes of GRSL cells were purified according to the procedure designated as "sucrose method", described previously [17]. Plasma membranes of thymocytes were prepared similarly, except that for homogenization of the cells by "nitrogen cavitation", equilibration of the cells in the pressure vessel [22] was performed with 800 lb/inch^2 N_2 for 20 min.

Isolation of extracellular membranes. (a) From GRSL ascites fluid. 4-fold diluted ascites fluid and/or combined cell washes were centrifuged at 4000 rev./min (1950 × *g*) for 10 min in a Sorvall RC-2B centrifuge. Supernatants were centrifuged at 30 000 rev./min (105 000 × *g*) in the 30 rotor of the Beckman L2 ultracentrifuge for 90 min. The pellets thus obtained were suspended in Medium A [17] and fractions were separated by sedimentation on a discontinuous sucrose gradient [17]. The extracellular membranes, present in the ascites fluid and those of the cell washes, equilibrated at the same density and showed similar properties (chemically, morphologically and immunologically; Van Blitterswijk et al., manuscript in preparation) as purified plasma membranes of GRSL cells.

(b) From thymuses. After mincing the thymuses and removal of cell clumps and connective tissue by filtration, the loose cells were spun down and the supernatant and combined cell washes were centrifuged at 4000 rev./min (1950 × *g*) for 10 min and subsequently at 30 000 rev./min (105 000 × *g*) for 90 min. The pellet thus obtained consisted of membraneous vesicles (also classified as extracellular, for convenience) which were purified on a sucrose gradient as described above. These membranes showed similar properties (unpublished) as plasma membranes purified from disrupted thymocytes.

Membrane lipids. Total lipids were extracted from purified membranes with chloroform/methanol (2 : 1, v/v) (Merck, Darmstadt, G.F.R.) followed by partition according to Folch et al. [23]. Phospholipid phosphorus and cholesterol were determined as described before [22] by the method of Morrison [24] and the Lieberman-Burchard reaction, respectively. Isolation of phospholipids from the total lipids was performed by thin-layer chromatography on precoated silica gel plates (Merck), prewashed with chloroform/methanol (2 : 1, v/v) using *n*-hexane/diethyl ether/acetic acid (85 : 15 : 2, (v/v) as described previously [25]. In this chromatography system the phospholipids remain at the origin.

Fluorescence labelling of cells, membranes and liposomes. The fluorescent hydrocarbon 1,6-diphenyl 1,3,5-hexatriene (DPH) (Koch-Light Laboratories Ltd., England) was used as a probe for monitoring the degree of fluidity of membrane lipids [3,5]. For labelling of cells or membranes, $2 \cdot 10^{-3}$ M DPH in tetrahydrofuran was first diluted 1000-fold with vigorously stirred phosphate-buffered saline, pH 7.5. Stirring was continued for 10 min at 25°C and a clear, stable aqueous dispersion of $2 \cdot 10^{-6}$ M DPH, practically void of fluorescence, was obtained. One volume of cell suspension ($2 \cdot 10^6$ cells/ml) or mem-

branes (75 μg protein/ml) was mixed with one volume of the DPH dispersion in phosphate-buffered saline and incubated at 37°C for 30 min with gentle shaking. Solutions of total lipids or phospholipids (200 μg) in chloroform/methanol (2 : 1, v/v) were evaporated to dryness under nitrogen and dispersed in 2 ml phosphate-buffered saline containing $8 \cdot 10^{-6}$ M DPH (prepared as described below for single cell measurements). The dispersions were then subjected to an ultrasonic irradiation for 5 min under nitrogen with a M.S.E. sonicator at maximum energy output with cooling on ice. The resulting mixture containing liposomes was kept at 37°C for 30 min. By using the more concentrated DPH dispersion and preparing the liposomes by sonication in the presence of DPH, we could avoid an otherwise sometimes too weak DPH fluorescence (insufficient probing).

For analysis of membrane fluidity on a single cell level, cells were labelled with a higher concentration of DPH. To this end 0.5 ml of $2 \cdot 10^{-3}$ M DPH in tetrahydrofuran was first injected into 50 ml of vigorously stirred phosphate-buffered saline. Stirring was continued for 10 min at 25°C, then the tetrahydrofuran was evaporated at 60°C under nitrogen. One volume of cell suspension ($2 \cdot 10^6$ cells/ml) in phosphate-buffered saline was mixed with one volume of the DPH dispersion and incubated at 37°C for 15 min, after which the cells were washed once with phosphate-buffered saline. The labelled cells were immediately used for fluorescence measurements.

Fluorescence polarization analysis. Fluorescence analyses were carried out by two fluorescence instruments. The degree of fluorescence polarization of DPH-labelled cell populations, isolated membranes, and lipid liposomes was determined at 25°C, immediately after labelling [3,5], with the Elscint Microviscosimeter, Model MV-1 (Elscint Ltd., Haifa, Israel). The degree of fluorescence polarization of individual cells in a given cell population was assayed at 25°C with the Elscint Single Cell microviscosimeter [6]. For excitation, a 365 nm band generated from a 200 W mercury arc, which was passed through a polarizer, was used. The fluorescence light was detected in two independent cross-polarized channels, equipped with polarizers, after passing a cut-off filter for wavelengths below 390 nm. Fluorescence measurements were obtained by simultaneous determination of the two channels, where $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities polarized parallel and perpendicular to the direction of polarization of the excitation beam, respectively. These values relate to the degree of fluorescence polarization, P, by the following equation:

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

where high P values represent low lipid fluidity, whereas low P values represent high lipid fluidity. The accuracy of the fluorescence polarization, P, measurements is ± 0.005.

The method employed for evaluation of microviscosities [3] as expressed in absolute units of poise, is based on the fluorescence polarization properties of a fluorescence probe as described by the Perrin equation for rotational depolarization of a non-spheral fluorophore:

$$\frac{r_0}{r} = 1 + C(r)\frac{T \cdot \tau}{\eta}$$

where r and r_0 are the measured and the limiting fluorescence anisotropies, T is the absolute temperature, τ is the excited state lifetime of DPH, and $\bar{\eta}$ is the microviscosity of the medium where the DPH molecules are embedded. $C(r)$ is a parameter which relates to the molecular shape (effective rotational volume) of the fluorophore, and has a specific value for each r value [26]. τ of DPH at 25°C was estimated indirectly from the temperature profile of the fluorescence intensity [3,7] assuming the limiting value τ_0 = 11.4 ns at 0°C, as determined by Shinitzky and Barenholz [7]. The fluorescence intensity is directly proportional to τ. It was thus found that τ(25°C) varied some 10% among the present systems. An average τ(25°C) = 10 ns was taken (in agreement with ref. 7) for the calculation of $\bar{\eta}$ values from P values.

Drug treatments of cells. To evaluate the effect of various drugs, interacting with microtubuli and microfilaments, on the fluorescence polarization of DPH in intact cells, GRSL cells, thymocytes and splenic lymphocytes (prepared from spleens of 3-months-old GR/A mice) were treated as follows: The cells were incubated as described above with DPH in drug-containing phosphate-buffered saline at 37°C for 30 min followed by 45 min at 25°C.

Colchicine (Merck) and vinblastine sulfate (Eli Lilly and Co., Indianapolis, Ind., U.S.A.) were both used in final concentrations of 10^{-4} and 10^{-6} M. Cytochalasin B (Serva, Heidelberg, G.F.R.) was used in final concentrations of 1 μg and 10 μg per ml; this drug was first dissolved in dimethylsulfoxide, of which the concentration in the final media was 0.1 and 1%, respectively.

Results

The degree of fluorescence polarization (P) at 25°C of DPH embedded in membrane lipids of intact leukemic (GRSL) cells and normal lymphocytes (thymocytes) was compared with that of the corresponding purified plasma membanes and extracellular membranes and their total lipids and phospholipids.

Intact cells

Table I lists the P values of intact thymocytes and GRSL cells, obtained in individual experiments. Data on a given cell type may vary, especially so in the case of the leukemic cells, where differences as large as 0.036 (corresponding to some 30% change in $\bar{\eta}$) may occur in individual mice from one transplantation generation to the next. In general, however, the P values of the leukemic cells are significantly lower than those of the thymocytes. Mean microviscosity values in poises with standard deviations are summarized in Table IV. In line with these observations is the finding that the cholesterol/phospholipid molar ratio, which is believed to be a main factor contributing to the microviscosity [2,4,27], is 0.37 for whole thymocytes and 0.22—0.30 for whole GRSL cells.

Fluorescence polarization measurements on individual cells (Fig. 1), though showing a rather broad range of values of a given cell type, allow the normal and leukemic cell populations to be distinguished.

Purified plasma membranes and extracellular membranes

The degrees of fluorescence polarization (P) and corresponding microviscosi-

TABLE I

DEGREE OF FLUORESCENCE POLARIZATION (P) OF DPH IN MEMBRANE LIPIDS OF INTACT NORMAL AND LEUKEMIC LYMPHOCYTES

Each value represents one individual experiment. Data of leukemic lymphocytes (GRSL) are listed in sequence of successive transplantation generations.

Cells	P (25° C)
Thymocytes	0.266 0.284 0.272 0.260
GRSL cells	0.211, 0.223, 0.203, 0.224, 0.188, 0.188, 0.214, 0.200, 0.219

ties of purified plasma membranes from thymocytes and GRSL cells are given in Table II. Values are higher than those of the corresponding intact cells, about 44 and 90% for thymocytes and GRSL cells, respectively (Table IV), one possible reason being an intracellular contribution to the P values of intact cells. However, the difference in P values between the two cell types is still significant on the level of the purified plasma membranes.

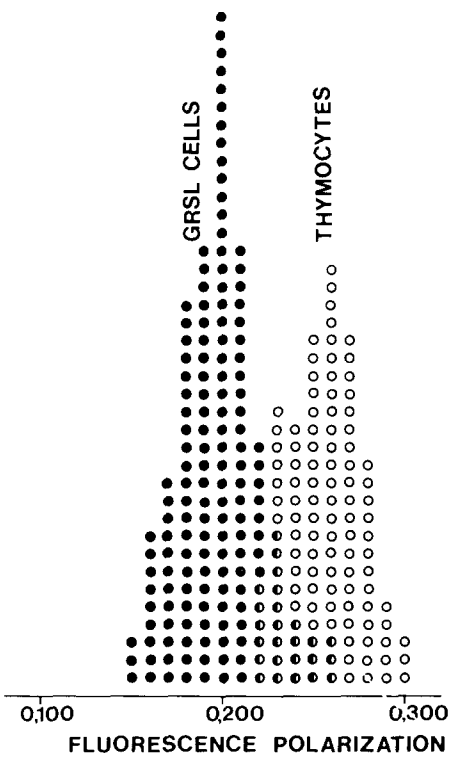

Fig. 1. Distribution of the degree of fluorescence polarization of DPH in single cells at 25°C. ○, normal lymphocytes (thymocytes); ●, leukemic lymphocytes (GRSL). Each symbol represents one single cell.

TABLE II

DEGREE OF FLUORESCENCE POLARIZATION (P) OF DPH AND MICROVISCOSITY ($\overline{\eta}$) IN PURIFIED PLASMA MEMBRANES, THEIR TOTAL LIPIDS AND PHOSPHOLIPIDS

Preparations in this and the next table which are denoted by the same experiment number, were obtained from the same set of animals.

Cells	Expt. Nr.	Plasma membranes		Total lipids		Phospholipids	
		P (25° C)	$\overline{\eta}$ (25° C)	P (25° C)	$\overline{\eta}$ (25° C)	P (25° C)	$\overline{\eta}$ (25° C)
Thymocytes	1	0.312	4.97	0.310	4.86	—	—
	2	0.307	4.70	0.309	4.80	—	—
	3	0.300	4.35	0.284	3.69	0.190	1.44
	4	—	—	0.317	5.23	0.228	2.10
GRSL cells	5	0.277	3.44	0.272	3.27	—	—
	6	0.255	2.76	0.232	2.19	0.189	1.42
	7	0.287	3.81	0.290	3.93	—	—
	8	0.258	2.84	0.226	2.06	0.189	1.42
	9	0.252	2.68	—	—	—	—
	10	0.287	3.81	0.266	3.08	0.178	1.27
	11	0.267	3.11	0.252	2.68	0.217	1.88

The so-called extracellular membranes were obtained from GRSL ascites fluid cell supernatants and from normal thymus cell supernatants, as described under Materials and Methods. The microviscosities of these membranes from both thymocytes and GRSL cells (Tables III and IV) were higher than those of plasma membranes, especially in the case of the GRSL cells. The P values of purified extracellular membranes obtained from GRSL ascites fluid supernatants were the same as those of the membraneous vesicles released from GRSL cells by washing and purified on the sucrose gradient [17].

Total lipids and phospholipids

Microviscosities of liposomes prepared from the total lipids of membranes were the same as, or sometimes only slightly lower (in some individual experi-

TABLE III

DEGREE OF FLUORESCENCE POLARIZATION (P) OF DPH AND MICROVISCOSITY ($\overline{\eta}$) IN PURIFIED EXTRACELLULAR MEMBRANES, THEIR TOTAL LIPIDS AND PHOSPHOLIPIDS

Preparations in this and the previous table which are denoted by the same experiment number, were obtained from the same set of animals.

Cells	Expt. Nr.	Extracellular membranes		Total lipids		Phospholipids	
		P (25° C)	$\overline{\eta}$ (25° C)	P (25° C)	$\overline{\eta}$ (25° C)	P (25° C)	$\overline{\eta}$ (25° C)
Thymocytes	1	0.334	6.33	0.332	6.19	—	—
	2	0.323	5.59	0.332	6.19	—	—
	3	—	—	0.297	4.22	0.193	1.48
GRSL cells	5	0.330	6.05	0.310	4.86	—	—
	6	0.332	5.52	0.328	5.92	0.215	1.84
	7	0.324	5.65	0.328	5.92	0.224	2.02
	8	0.334	6.33	—	—	—	—
	9	0.323	5.59	—	—	—	—

TABLE IV

SUMMARY OF MICROVISCOSITIES, $\bar{\eta}(25^{\circ}C)$ VALUES

Data are the mean ± S.D., expressed in poises, with numbers of different experiments in parentheses.

Cells	Intact cells		Intact membranes	Total lipids	Phospholipids
Thymocytes	3.24 ± 0.34(4)	Plasma membranes	4.67	4.65	1.77
		Extracellular membranes	5.96	5.53	1.48
GRSL cells	1.73 ± 0.23(9)	Plasma membranes	3.28 ± 0.43(10)	2.87 ± 0.70(6)	1.50 ± 0.26(4)
		Extracellular membranes	5.83 ± 0.35(5)	5.57	1.93

TABLE V

CORRELATION BETWEEN MICROVISCOSITY OF MEMBRANE LIPIDS AND CHOLESTEROL CONTENT

	$\overline{\eta}$ (25°C)	Cholesterol / Phospholipid (M/M)
GRSL extracellular membranes	5.83	1.19
Thymocyte plasma membranes	4.67	0.79
GRSL plasma membranes	3.28	0.37
Intact thymocytes	3.24	0.37
Intact GRSL cells	1.73	0.22—0.30
Membrane phospholipids	1.5—1.9 *	0

* Range in which the values for the four types of membranes fall.

ments with GRSL material) than those of the corresponding intact membranes (Tables II and III). Thus, virtually no influence of (glyco)proteins on the lipid fluidity is discernible.

The microviscosities of liposomes consisting of the phospholipids only, were much reduced in comparison to the total lipids (Tables II, III and IV). Phospholipid liposomes of the four types of membranes had roughly the same $\overline{\eta}$ values, indicating that the difference between the various intact membranes (or their total lipids) mainly arises from the relative contribution of neutral lipids, in particular cholesterol [3]. This was checked by determination of the cholesterol/phospholipid molar ratio in plasma membranes and extracellular membranes of GRSL cells, which amounted to 0.37 and 1.19, respectively. In thymocyte plasma membranes the ratio was 0.79.

Effect of drugs

The effect of colchicine, vinblastine and cytochalasin B on the lipid microviscosity of various intact lymphoid cells, was studied at the concentrations mentioned in Materials and Methods. Extra dimethylsulfoxide controls were taken for the cytochalasin B treatment. None of the treatments had a significant effect on the DPH fluorescence polarizations (P) of control cells: All P values for thymocytes fell within the range 0.275 ± 0.003. For splenocytes and GRSL cells the values were 0.267 ± 0.002 and 0.187 ± 0.002, respectively.

Discussion

In this study we determined by means of fluorescence polarization the microviscosities of isolated plasma membranes and extracellular membranes of normal and leukemic lymphocytes (thymocytes and GRSL cells, respectively) and of liposomes consisting of total lipids and phospholipids of the membranes.

The microviscosities of purified plasma membranes were higher than those of the corresponding intact cells, especially in the case of leukemic (GRSL) cells (summarized in Table IV). This may indicate that intracellular membranes also contribute to the DPH fluorescence signal, or/and that a specific tensile force which causes stretching of the plasma membrane in intact cells, increases the fluidity in its lipid core, as suggested by Aloni et al. [12], for erythrocyte

membranes. Another possibility which cannot be excluded at this moment, is that during the isolation of plasma membranes there is an enrichment of the most rigid membrane particles in the fraction designated as plasma membranes.

The higher fluidity of (leukemic) GRSL cells as compared to (normal) thymocytes is in line with previous reports [3—6]. The present experiments show that this also holds for the purified plasma membranes and liposomes consisting of their total lipids.

We found little or no difference between the lipid fluidity in intact membranes of a given type and that in liposomes made from their lipid extracts. This may indicate that the presence of (glyco)proteins in the membranes has virtually no effect on the dynamics of the lipid core as measured by the present technique and that the diverse lipid acyl chains in membranes and liposomes are equally accessible to the DPH probe. Likewise, little or no effect of intrinsic proteins has been found on the lipid fluidity of erythrocyte membranes [12] and of rhabdoviruses [10,11]. In contrast, however, a significant rigidizing effect has been described for the intrinsic protein (rhodopsin) of the bovine retinal rod outer segment disk membrane [13] and of togaviruses [10,19]. Presumably, the most important factors which determine the effect are the nature of the specific regions of the proteins which interact with the lipid bilayer and the concentration of such proteins in the membrane.

Drugs like colchicine, vinblastine and cytochalasin B, are known to destroy the microtubuli and microfilaments underlying the plasma membrane, thus influencing ligand-induced redistribution of cell surface receptors [28] and dynamics of microvilli [29]. Since these drugs may also affect several plasma membrane properties in a more direct way [30], e.g. by expansion of the surface membrane, it was of interest to search for a direct effect on the fluidity of surface membrane lipids. However, within the commonly used concentration ranges of these drugs no effect on lymphoid cells was found.

The cholesterol/phospholipid molar ratio appeared to be a major parameter in the microviscosity of membrane lipids, as measured by the fluorescence polarization technique [2,3]. This is confirmed by the positive correlation found in the present experiments between membrane microviscosity and cholesterol content (Table V). First, the microviscosity of isolated membranes and the corresponding liposomes consisting of their total lipids significantly decreased in the order extracellular membranes > thymocyte plasma membranes > GRSL plasma membranes, whereas the cholesterol/phospholipid molar ratio decreased from 1.19 to 0.37 in that order. Data for intact cells also fit in this correlation (Table V). Secondly, the microviscosity of liposomes consisting of the membrane phospholipids was markedly decreased as compared with those of membrane total lipids (Table IV). This was especially the case for extracellular membranes, which contained the highest cholesterol/phospholipid ratio. The phospholipid liposomes of the various membranes, moreover, had almost equal microviscosities, illustrating the predominant role of cholesterol in the microviscosity. The latter results also indicate that the significant differences in phospholipid composition which exist, e.g. between extracellular membranes and plasma membranes of GRSL (unpublished results), are of minor importance. It is known that in general, cholesterol imposes upon phospholipids a condi-

tion of intermediate fluidity, i.e. fluidizing gel and rigidizing liquid states of the lipid acyl chains [27,31]. Our results show that for the present lymphoid membranes, cholesterol has an overall rigidizing effect, as can be expected when the phospholipids are above their phase transition temperature.

The GRSL cells contain mammary tumor virus-induced cell surface antigens (MLr), but produce only little virus. However, the cells exfoliate (shed) membraneous vesicles, which are virtually devoid of complete virions, but contain MLr antigens. Such vesicles, which are present in the ascites fluid, can also be released in large quantities from intact cells without loss of viability (Van Blitterswijk et al., unpublished), by washing with physiological media, indicating that in vivo they are indeed released from live cells, rather than being derived from dead cells. Extracellular membranes exhibit a relatively high microviscosity and cholesterol/phospholipid molar ratio and a relatively low phosphatidylcholine/sphingomyelin ratio as compared to plasma membranes isolated from GRSL cells (Van Blitterswijk et al., manuscript in preparation), which stresses the non-random emergence of extracellular membranes. These relative lipid compositions are strikingly similar to those described by Calberg-Bacq et al. [20], and our own unpublished results, for isolated mammary tumor virus in comparison to milk fat-globule membranes. In fact, it appears to be a general observation that the lipid fluidity of viruses is much lower than the plasma membrane of the virus host cell [10,11,18–21]. Thus one might regard the selected rigid sites of the plasma membrane which are exfoliated, as analogous to virus budding sites. However, the question remains whether release of membraneous vesicles from cells in general, "spontaneously" [32] or induced [33], always concerns relatively rigid membrane domains [32]. We found that extracellular membranes of thymocytes also show a higher microviscosity (although less pronounced) than isolated thymocyte plasma membranes. The same has been found for rat thymocytes [34].

Finally, it should be noted that the increased lipid fluidity of the leukemic cells as compared to the normal cell homologues is acquired as a result of syngeneic transplantation, since almost normal microviscosity values are obtained for the primary tumours and very early transplantation generations (Hilgers et al., manuscript in preparation). It is indicated that this shift towards higher fluidity is effectuated by the immune response of the host, since by transplantation in irradiated mice the relatively high microviscosity values are retained. One possibility is that within the leukemic cell population, showing a rather broad distribution of P values for individual cells (Fig. 1), the most rigid cells are selectively killed by the immune response (immunoselection). At least part of the higher fluidity of transplanted GRSL cells, however, may be the result of the exfoliation of relatively rigid membraneous vesicles. The relevance of these possible mechanisms for the increased lipid fluidity and for the malignancy of the cells is under further investigation.

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According to our most recent results, intact and disrupted cells have similar rates and maxima of DPH uptake, as measured by the total fluorescence intensity. They show thereby the same, constant P value during the uptake of

DPH. This favours the first of the three alternatives offered in the discussion as the most important one for the lower P values in intact cells as compared with isolated plasma membranes.

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