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DIFFERENCES IN MEMBRANE LIPID COMPOSITION AND FLUIDITY OF TRANSPLANTED GRSL LYMPHOMA CELLS, DEPENDING ON THEIR SITE OF GROWTH IN THE MOUSE

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GRSL lymphoma cells were isolated from various growth sites in the host. The relative membrane lipid fluidities of these cells and of normal lymphoid cells were estimated by fluorescence polarization, using the probe diphenylhexatriene and by measuring the (free) cholesterol/phospholipid molar ratio in whole cells. The results indicate that the membrane fluidity (reciprocal of the lipid structural order) of the lymphoma cells increases in the order of their location: peripheral blood < spleen < mesenterial lymph node < ascites fluid. The membrane fluidities of normal lymphocytes from thymus, mesenterial lymph node and spleen were about the same, but higher than of peripheral blood lymphocytes, and between those of the lymphoma cells from lymph node and spleen. These results are confirmed by more extensive analysis on purified plasma membranes from the splenic and ascitic GRSL lymphoma cells and from normal splenocytes and thymocytes. The significantly higher lipid order parameter found in the GRSL plasma membrane isolated from the spleen as compared to those from the ascites cells could be fully explained by the differences measured in the major chemical determinants of the fluidity, i.e., the cholesterol/phospholipid ratio, the sphingomyelin content and the degree of saturation of the fatty acyl groups of the phospholipids. It was also found that the cholesterol/phospholipid ratio in erythrocyte membranes isolated from the peripheral blood of the tumor bearers was higher than in those from normal control mice. The observed differences in membrane fluidity between distinct subsets of tumor cells may be relevant to the sensitivity of these cells to immune attack or to drugs.

Introduction

The cell surface membrane fluidity of tumor cells has been shown to be an important parameter in determining their ability to resist or escape from immune killing, either by antibodies and complement [1,2] or by immunocompetent cells [3,4]. The degree of membrane fluidity may also be functionally linked to the sensitivity of tumor cells to chemotherapeutic drugs [5–7]. These findings suggest that therapy might be improved by artificially modulating the membrane fluidity of tumor cells in vivo, e.g., by dietary means [7–10]. Such an approach requires a clear insight into the mechanisms by which cell membrane fluidity is regulated in vivo. However, the experimental data in this respect are still fragmentary [9–15].

In this context we posed the question whether and to what extent the cell membrane fluidity of a tumor may differ according to the various

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

(micro)environments in which the cells grow. To answer this question, we studied a murine lymphoma model (GRSL) in which the tumor cells were transplanted intraperitoneally and, after outgrowth, were isolated from various locations in the body, such as the ascites fluid, the spleen and the lymph nodes.

Membrane lipid fluidity can easily be measured by fluorescence polarization, using the apolar probe 1,6-diphenyl-1,3,5-hexatriene (DPH) and may be defined as the reciprocal of the lipid structural order parameter, S_{DPH} [16]. The fluidity is determined mainly by the cholesterol- and sphingomyelin/phospholipid molar ratios and the degree of saturation in the fatty acyl chains of the phospholipids [16–18].

In this paper it is reported that these physical and chemical parameters of membrane fluidity, as measured in whole GRSL lymphoid tumor cells or in their isolated plasma membranes may differ depending on their site of growth.

Materials and Methods

Animals and cells. GRSL18 cells from a spontaneous thymus-derived lymphoma in the GR mouse strain were maintained by weekly intraperitoneal transplantation in 2-month-old male GR mice. In the present study ascites cells from tumor transplant generations 80–110 were used for the experiments. 7 days after inoculation, tumor cells were harvested from the ascites fluid, as described previously [19,20], from the enlarged spleens, mesenterial lymph nodes and the peripheral blood. Normal control white cells were isolated from similar locations in healthy mice of the same age and sex, as well as from normal thymuses from 3–5-week-old GR mice.

The various lymphoid organs were teased with scissors in Hanks' solution (Oxoid, London, U.K.) to prepare single-cell suspensions. Blood lymphocytes were isolated by metrizoate-dextran ($d =$ 1.086 g/ml), according to Bøyum [21]. Splenic lymphoid cells were separated from erythrocytes by centrifugation (20 min, $400 \times g$) at room temperature over a 17.5% metrizamide cushion.

GRSL tumor cells in their various locations in the mouse were identified histologically by Dr M.A. Van der Valk, pathologist at The Netherlands Cancer Institute, by routine techniques. Spleen and lymph nodes of GRSL-bearing animals were much enlarged (3-times or more), virtually all lymphoid cells being tumor cells. White blood cell counts in tumor bearers were not much higher (twice at the most) than normal, only a variable part of the blood cells being tumor cells. Haematocrit levels in tumor-bearers were generally decreased 40–60%.

Expression of cell surface antigens. GRSL lymphoma cells are generally characterized by the expression of mammary tumor virus-induced tumor antigens (MLr) on the cell surface [19,22]. Antiserum against the MLr tumor antigen was a rabbit anti-mammary tumor virus serum (kindly provided by Dr P. Hageman, (The Netherlands Cancer Institute), and was absorbed in vitro with milk powder from the BALB/c mouse strain and in vivo in C57BL/LiA mice. GRSL lymphoma cells furthermore exhibit a relatively high expression of a glycolipid surface antigen, detectable with a rat monoclonal antibody 30-H11 [23], which has been prepared and described by Ledbetter and Herzenberg [24].

Antigen expression on the cell surface was measured by an antibody and complement-mediated cytotoxicity test, as described before [19]. The complement source was a 5-fold diluted (with Earle's balanced salt solution, without Ca^{2+} or Mg^{2+}; Flow Laboratories) rabbit serum, selected for low cytotoxicity (less than 10%). The percentage of dead cells was counted after Trypan blue staining.

Isolation of plasma membranes. The various types of lymphoid cells were disrupted by pumping single-cell suspensions in Hanks' solution through an air-driven cell disruptor (Stansted Fluid Power Ltd., Stansted, Essex U.K.; model AO 612, disrupting valve 516), using an air pressure of 45 lb/in^2 [25]. Optimal cell disruption occured at cell densities of $5 \cdot 10^7$ cells/ml for GRSL ascites cells, $1 \cdot 10^8$ cells/ml for normal or leukemic splenocytes and $2 \cdot 10^8$ cells/ml for normal thymocytes. Under these conditions no clumps or aggregates of cellular material due to damaged nuclei were seen by phase-contrast microscopy, while almost all cells were disrupted, as judged by the uptake of Trypan blue by the remaining nuclei. Cell disruption and all subsequent steps in the isolation pro-

cedure were done at 0–4°C. The plasma membranes were purified from $(1.95–945) \cdot 10^4$ $g \cdot$ min pellets of the cell homogenates, utilizing a discontinuous sucrose gradient, as described in detail before [19]. The purity of the plasma membrane preparations was ascertained routinely by electron microscopy and by marker assays, as described previously [19].

Plasma membranes (ghosts) from the blood erythrocytes in normal and tumor-bearing mice were purified according to Hanahan and Ekholm [26].

Estimation of membrane lipid fluidity. Analysis of the degree of membrane lipid fluidity was done on the level of the intact cells and, if enough cells were available (at least $1 \cdot 10^9$), on the level of isolated plasma membranes (in the case of ascitic, thymic, splenic and red blood cells). Whole-cell analysis consisted of (a) steady-state fluorescence polarization measurements and (b) determination of the free cholesterol/phospholipid molar ratio and the ratio esterified/total cholesterol. If considered separately, these two types of analysis on whole cells have serious limitations for a proper inference of the membrane fluidity (see below). When combined, the results may allow a tentative conclusion about the relative membrane fluidity in the various lymphoid cells.

The fluorescent hydrocarbon, 1,6-diphenyl-1,3,5-hexatriene (DPH; Koch-Light Laboratories Ltd., Colnbrook, U.K.), was used as a probe for measuring the degree of lipid fluidity in whole cells and in the various plasma membrane preparations by steady-state fluorescence polarization (P_{DPH}). The measurements were performed at 25°C with an Elscint apparatus, model MV-1A (Elscint Ltd., Haifa, Israël) as described previously [19].

P_{DPH} values mainly reflect the orientational constraint of the probe, and these values, or rather the r_s values (steady-state fluorescence anisotropy) can be quantitatively converted into lipid order parameters, S_{DPH}, using a semi-empirical relationship [16,27]. Membrane lipid fluidity may be defined as the reciprocal of the lipid order parameter. In the present paper we used for calculation of S_{DPH} the equations $r_\infty = (4\ r_s/3) - 0.10$ (valid for the region $0.13 < r_s < 0.28$ or $0.18 < P_{DPH} < 0.37$) and $(S_{DPH})^2 = r_\infty / r_0$, in which r_∞ represents the limiting hindered fluorescence anisotropy, and $r_0 = 0.4$ is the theoretically maximal fluorescence anisotropy [16].

Analysis of membrane lipids. Membrane lipids were analyzed as described in detail before [20]. Briefly, lipids were extracted from the membrane preparations with chloroform/methanol (2 : 1, v/v), followed by Folch's partition. Cholesterol and cholesteryl esters were determined enzymatically, using the Merckotest cholesterol kit (Merck A.G., Darmstadt, F.R.G.). The individual phospholipids were separated by two-dimensional thin-layer chromatography on precoated activated silicagel plates (Merck), using the solvent systems chloroform/methanol/7 M ammonia (60 : 60 : 5, v/v) and chloroform/methanol/acetic acid/H_2O (50 : 30 : 8 : 4, v/v). The phospholipids were quantitated by phosphate analysis. Fatty acid analysis of the total phospholipids was done by gas-liquid chromatography after transesterification, on a wall-coated open tubular glass column, 25 m × 0.21 mm, coated with Silar 5 CP, using a temperature program.

Results

Intraperitoneally transplanted GRSL18 lymphoma cells do not only grow as an ascites tumor, as demonstrated in previous studies [19,20,28], but may also infiltrate and grow in lymphoid organs, such as the spleen and the lymph nodes. These organs become thereby significantly enlarged (gen-

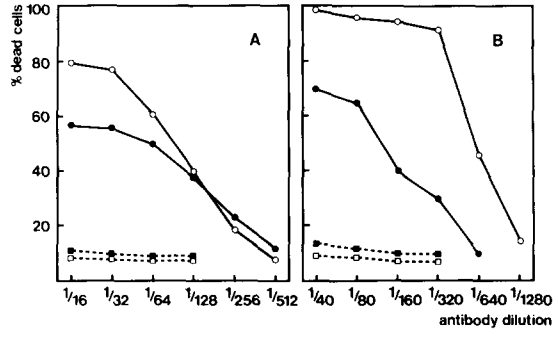

Fig. 1. Antibody and complement-dependent cytotoxicity test with anti-MLr serum (A) or monoclonal antibodies 30-H11 (B) performed on GRSL tumor cells located in the ascites (○) or the spleen (●) of tumor-bearing mice, as well as on normal splenocytes (■) and normal thymocytes (□) of healthy control mice.

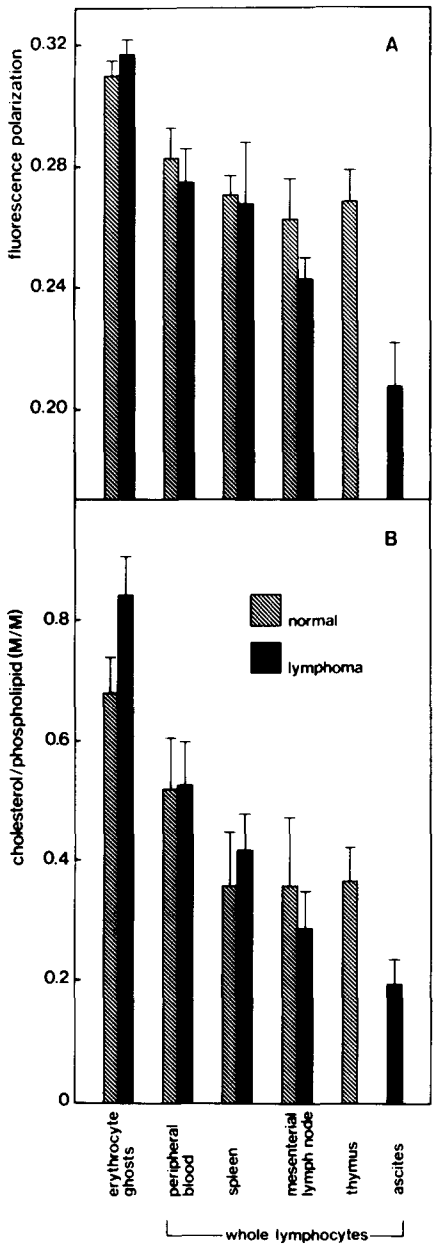

Fig. 2. Fluorescence polarization (P_{DPH}) measured at 25°C (A), and molar ratios of unesterified (free) cholesterol/phospholipid (B) in purified erythrocyte ghosts and in whole lymphocytes from different locations (indicated) in normal mice (hatched bars) and in mice bearing the GRSL lymphoma (black bars). Means ± S.D. of at least six experiments are indicated.

erally 3–4-fold). Histopathologically, most lymphoid cells (more than 75%) in the spleen and the mesenterial lymph node of tumor-bearing animals, 7 days after inoculation of the GRSL cells, could be identified as tumor cells. These cells typically express on their surface the virus-induced tumor antigen, MLr [19,22], and a glycolipid-type antigen, detectable by monoclonal antibody 30-H11 [23]. This is illustrated in Fig. 1, where typical antibody and complement-dependent cytotoxicity tests for these antigens on GRSL ascites cells, splenic GRSL cells, normal spleen cells and normal thymocytes are shown. Evidently, these antigens are present on the former two cell types and absent from the normal cells, confirming the pathological assessment of GRSL tumor cells in the ascites and the spleens of tumor-bearing animals.

Fig. 2A depicts the P_{DPH} values, measured at 25°C, in the various types of (intact) lymphoid cells from normal and GRSL-bearing mice. There is virtually no difference between the normal lymphocytes from thymus, mesenterial lymph node, peripheral blood and spleen, or between the tumor cells at the latter two locations. However, the lymphoma cells from the mesenterial lymph node showed somewhat lower and those from the ascites much lower P_{DPH} values, which indicates a higher membrane lipid fluidity.

Fig. 2B shows the free cholesterol/phospholipid molar ratios in the various types of whole lymphocytes in normal mice and tumor bearers. The graph shows some similarity to that of the P_{DPH} values (Fig. 2A) which is as expected in view of the fact that this molar ratio is the major determinant of the DPH-fluorescence polarization in membranes [15,16]. The molar ratios in the peripheral blood lymphocytes (0.53) are higher, while those in GRSL ascites cells (0.20) are lower than the other types of cells (0.30–0.42).

The data of Fig. 2A and B become much more compatible if one takes into account that the tumor cells generally contain more cytoplasmic (non-membraneous) neutral lipids (triacylglycerols and cholesteryl esters, see below) than their normal counterparts. These neutral lipids are known to depress the P_{DPH} values in intact cells [15,16] (see Discussion). The ratio of esterified/total cholesterol may be taken as a tentative measure for the amount of cytoplasmic neutral lipids in the various lymphoid cells. These ratios (not depicted in Fig. 2) amount to 11, 13, 4, 10, 5, 8, 4 and 13%

(mean values) in the lymphocytes indicated from left to right, respectively, in Fig. 2. It is clear that the relative amount of (cytoplasmic) cholesteryl esters is higher in the tumor cells than in the normal cells they were compared with. Therefore, the P_{DPH} values of the tumor cells (Fig. 2A) may overestimate the degree of lipid fluidity in the cellular membranes per se.

Taking the results of the three types of measurement described above together, it would appear that the fluidity in the plasma membrane of GRSL tumor cells increases in the following order: location in the peripheral blood < spleen < mesenterial lymph node < ascites fluid. Furthermore, the membrane fluidities of normal lymphocytes from thymus, spleen and mesenterial lymph node are about the same, but higher than of peripheral blood lymphocytes, and between those of the lymphoma cells from lymph node and spleen.

Fig. 2A also shows that the P_{DPH} values of the erythrocyte membranes isolated from tumor-bearing mice are slightly higher than those from normal mice. The difference in the free cholesterol/phospholipid molar ratio of these membranes is more significant (Fig. 2B).

A more thorough investigation of fluidity parameters was performed on purified plasma membranes of lymphoma and normal lymphoid cells isolated from the ascites fluid, spleen and thymus. Table I shows that the fluorescence polarization (P_{DPH}) and lipid order parameter (S_{DPH}) in the plasma membranes isolated from GRSL ascites cells are much lower than in those from normal thymocytes and splenocytes, the latter two showing the same values. In contrast, the plasma membranes from the lymphoma cells in the spleen exhibit somewhat higher values than in those from the normal cells. So, GRSL lymphoma cells from the spleen have much more rigid plasma membranes than those from the ascites fluid, which confirms the conclusion drawn from the whole-cell measurements, as described above.

Table I furthermore shows the cholesterol/phospholipid molar ratios and the composition of the individual phospholipids in the various plasma membranes. The cholesterol/phospholipid ratio and the sphingomyelin content, being the two major determinants of membrane structural order [16], are extremely low in the GRSL ascites cell membranes. In plasma membranes of splenic GRSL cells the ratio is similar to that in the normal cell membranes, while the sphingomyelin content (15.8%) is significantly higher, which may explain the higher order parameter, S_{DPH}, in this type of tumor cell plasma membrane.

Table II shows the fatty acid composition of the

TABLE I

LIPID STRUCTURAL ORDER, CHOLESTEROL/PHOSPHOLIPID MOLAR RATIO AND PHOSPHOLIPID COMPOSITION IN ISOLATED PLASMA MEMBRANES FROM LYMPHOMA (GRSL) AND NORMAL LYMPHOID CELLS AT DIFFERENT LOCATIONS

Values are means ± S.D. of at least three different preparations for each type of plasma membrane.

	Lymphoma cells		Normal cells	
	ascites	spleen	spleen	thymus
P_{DPH}, at 25°C	0.261 ± 0.014	0.316 ± 0.008	0.302 ± 0.005	0.303 ± 0.005
S_{DPH}, at 25°C	0.620 ± 0.030	0.731 ± 0.015	0.704 ± 0.009	0.706 ± 0.009
Cholesterol/phospholipid (molar ratio)	0.32 ± 0.08	0.70 ± 0.02	0.68 ± 0.07	0.74 ± 0.05
Phospholipid composition (%)				
Sphingomyelin	0.9 ± 0.2	15.8 ± 0.6	11.5 ± 0.8	7.6
Phosphatidylcholine	50 ± 6	50.8 ± 1.5	51.8 ± 2.0	52.0
Phosphatidylethanolamine	29 ± 4	17.1 ± 1.6	23.2 ± 0.6	19.0
Phosphatidylserine	9.8 ± 2.1	6.8 ± 1.5	6.1 ± 2.0	9.2
Phosphatidylinositol	6.5 ± 0.5	5.5 ± 0.5	5.9 ± 0.5	6.1
Lysophosphatidylcholine	3.2 ± 0.3	2.5 ± 0.2	1.2 ± 0.2	3.4
Lysophosphatidylethanolamine	2.7 ± 1.8	0.7 ± 0.3	0.9 ± 0.2	2.2

TABLE II

FATTY ACID COMPOSITION OF THE TOTAL PHOSPHOLIPIDS OF ISOLATED PLASMA MEMBRANES FROM LYMPHOMA (GRSL) AND NORMAL LYMPHOID CELLS AT DIFFERENT LOCATIONS

Data are expressed as wt% and are the means ± S.D. of three individual experiments. Fatty acids present in amounts less than 1% in any type of plasma membrane are not included in this table.

Fatty acid	Lymphoma cells		Normal cells	
	ascites	spleen	spleen	thymus
14:0	1.5±0.2	1.6±0.3	1.4±0.3	0.9±0.2
16:0	21.3±1.2	37.2±0.7	39.0±0.4	37.9±0.9
18:0	20.8±1.1	17.5±1.0	16.3±0.4	21.0±0.9
18:1	17.8±1.0	13.3±0.7	11.3±0.2	14.2±0.5
18:2	18.8±1.1	9.5±0.4	7.4±0.8	6.2±0.7
20:3	1.7±0.2	1.4±0.1	1.7±0.2	1.6±0.2
20:4	7.4±0.4	9.0±0.2	12.3±0.3	7.7±0.3
22:6	4.0±0.8	3.6±0.4	4.7±0.5	3.9±0.4
24:0	—	1.4±0.1	1.8±0.2	0.2±0.0
24:1	0.2±0.1	2.3±0.1	1.9±0.1	0.8±0.1

total phospholipids of the isolated plasma membranes from the various lymphoid cell types. There is no conspicuous difference in this composition between the normal cells and the spleen-localized tumor cells. However, the lymphoma cells in the ascites exhibit a higher degree of unsaturation, mainly due to an increased 18:1 and 18:2, and a decreased 16:0, in their plasma membrane than the other three cell types. This is also a contributing factor to the high membrane fluidity in these ascites cells.

Discussion

In the present investigation some chemical and physical parameters of membrane lipid fluidity were determined in four subpopulations of GRSL lymphoma cells which had grown at different locations after intraperitoneal transplantation of the tumor in the mouse. Since in general only small amounts of cells were available, these parameters were in the first instance confined to the fluorescence polarization (P_{DPH}) and the free cholesterol/phospholipid molar ratio of whole cells (eight cell types in total, including four types of normal control lymphocytes). Subsequently, from a larger amount of a few cell types, plasma membranes were isolated, which were subjected to a more extensive lipid analysis.

Histopathology and cytotoxicity tests with monoclonal antibodies 30-H11 (Fig. 1B) indicate that the ascites fluid contains close to a 100% GRSL tumor cells. Based on these techniques the 'leukemic' spleen may contain some 20–30% normal cells. Cytotoxicity tests with anti-MLr serum (Fig. 1A), in comparison to 30-H11 antibodies (Fig. 1B), show that some tumor cells are refractory to this complement-mediated immune killing. A likely explanation for this is that these tumor cells lack the MLr surface antigen, due to shedding and/or antigenic modulation, as previously described for this antigen [15,19,22].

P_{DPH} values measured on intact cells should be interpreted with caution, since they represent a measure of the average fluidity of all lipids being present in the cell, including endomembranes and, if present, cytoplasmic droplets of neutral lipids (triacylglycerols and cholesterylesters) [29–31]. These P_{DPH} values are generally lower than those of the corresponding isolated plasma membranes. Nonetheless, measurements on whole cells may yield some information about the relative fluidity of the plasma membrane provided that (a) the cell to nucleus volume ratio is small, (b) the measurement is used to compare cells of the same type, e.g., lymphoid cells, and most importantly, (c) the cells are devoid of intracytoplasmic lipid droplets. These lipid droplets may markedly decrease P_{DPH} values of intact cells. Of all cell types studied presently, only the GRSL ascites cells have been

clearly shown to contain substantial mounts of lipid droplets [30,32], although the increased ratio of esterified/total cholesterol in all four subsets of tumor cells would indicate that the GRSL tumor cells at other sites of growth may also contain some non-membraneous neutral lipids, at least more than the normal cells do at similar locations in healthy mice. If the latter is taken into account, the whole-cell P_{DPH} values (Fig. 2A) agree very well with the free cholesterol/phospholipid molar ratios measured in the cells (Fig. 2B). In membranes, this molar ratio represents the major determinant of the membrane lipid structural order (reciprocal of fluidity) [16]. In comparative studies, like the present one on various lymphoid cell types, the molar ratio of free cholesterol/phospholipid in whole cells may also give an indication about the relative fluidity of the plasma membrane, since free cholesterol is predominantly present in the plasma membrane [33] and overall changes in all cellular membranes can be assumed to occur parallel with the changes in the plasma membrane [34]. Considering the combined results obtained on intact cells (Figs. 2A, B), it may tentatively be concluded that the histogram in Fig. 2B represents the relative degree of membrane structural order in the various normal and tumor cells fairly well.

The above conclusion is confirmed for a restricted number of cell types by more extensive analysis of the isolated plasma membranes. Lymphoma cells from the spleen exhibit a somewhat higher membrane order parameter, while ascites cells show a much lower one than both normal spleen cells and thymocytes. These results can be fully explained by the alterations in the major chemical determinants underlying the structural order [16], i.e., the cholesterol/phospholipid ratio, the sphingomyelin content and the degree of saturation in the fatty acyl moieties of the phospholipids (see results).

The apparent difference in membrane fluidity between cells from the mesenterial lymphnode and the ascites fluid of tumor-bearing mice (Fig. 2) could possibly arise from the contribution of normal lymphocytes in the 'leukemic' lymph node. For the 'leukemic' spleen, however, which may also contain some (20–30%) normal lymphocytes, this would mean that the plasma membranes of the genuine tumor cells in this organ are even (somewhat) more rigid than depicted in Fig. 2 and Table I.

It is interesting to note that the erythrocytes in the peripheral blood of mice with GRSL lymphoma show a significantly higher membrane cholesterol/phospholipid molar ratio than in normal control mice (Fig. 2B). The erythrocyte cholesterol can readily exchange between the membrane and the ambient blood plasma. The equilibrium that is reached in vivo, and thus the cholesterol/phospholipid ratio in the erythrocyte membrane, is to a great extent determined by the composition of the plasma lipoproteins [35,36]. High-density lipoproteins (HDL) normally promote at least the major part of cholesterol efflux from peripheral cell membranes into the plasma, a process which is intimately related to the action of the plasma enzyme, lecithin-cholesterol acyltransferase, which esterifies the cholesterol in HDL, dependent on the cofactor apolipoprotein A-I [36]. Since GRSL tumor growth has been shown to be accompanied by a dramatic decrease of HDL, the major lipoprotein of the mouse [37], the increased cholesterol content in erythrocytes can be explained by a decreased in vivo esterification of plasma cholesterol by lecithin-cholesterol acyltransferase and a decreased efflux of unesterified cholesterol from the membrane into the plasma. Likewise, it has more generally been observed that where the activity of lecithin-cholesterol acyltransferase is low, either hereditarily or in certain metabolic (liver) diseases, there is an increase in erythrocyte free cholesterol [13,35,38,39].

The in vivo regulation of the membrane fluidity in the various types of lymphoid cells is not only governed by lipoprotein metabolism, as described above, but these cells, in contrast to erythrocytes, are by themselves capable of synthesizing (part of) their own cholesterol and other membrane lipids, under the control of complex regulatory mechanisms [11,14,15,40,41]. This regulation may also be dependent on the particular (micro)environment of the cells, e.g., spleen being different from lymph nodes or ascites fluid.

The existence of distinct subpopulations of cells within a tumor is regularly observed and has been recognized as a complicating factor in cancer therapy. In only a few studies have distinct subsets of

lymphoid tumor cells been found to be associated with a distinct location of their growth. Krolick et al. [42] have identified several morphological and other phenotypic differences between the splenic and peripheral blood forms of the murine B-lymphoblastic tumor BCL_1, which may possibly be related to differences in maturation of B cell differentiation. Also, in human leukemia, morphological and biochemical differences were demonstrated between prolymphocytic leukemia cells obtained from the spleen and peripheral blood of one patient [43]. As yet, such studies on lymphoid tumors have not been related to the membrane fluidity. Preliminary results in the present GRSL model have indicated that lymphoma cells in the ascites and the spleen not only show the extreme differences in membrane fluidity, as described here, but they also differ in size (splenic tumor cells are generally smaller) and in the degree of expression of various surface antigens (Van Blitterswijk, unpublished data). Whether these different expressions are determined by the different degrees of membrane fluidity according to the 'vertical displacement' hypothesis [44] or whether they represent different stages of cell maturation/differentiation, remains to be investigated. It could also be possible that the distinct subsets of tumor cells have been brought about by cell selection and/or adaptation to the particular environment.

In conclusion, different degrees of membrane fluidity, both higher and lower than normal, were found in GRSL lymphoma cells, depending on their site of growth in the mouse. A consequence of these differences could be that distinct tumor cell subpopulations show a different sensitivity to (host) immune attack and/or chemotherapeutic drugs. This should be taken into consideration whenever procedures are designed to modulate membrane fluidity in vivo for immuno- or chemotherapeutic reasons.

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