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FLUORESCENCE POLARIZATION STUDIES AND BIOCHEMICAL PROPERTIES OF MEMBRANES EXFOLIATED FROM THE CELL SURFACE OF RABBIT THYMOCYTES IN SITU

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Summary

In the course of our work on membrane phenomena related to the differentiation of lymphocytes in the rabbit thymus, we isolated membranous material from the extracellular compartment of this organ. With respect to their ultrastructural appearance, enzyme activity, lipid composition (cholesterol/phospholipid molar ratio, fatty acid composition of total phospholipids, phospholipid composition) and lipid fluidity, these membranes were shown to exhibit characteristics similar to those of purified plasma membranes isolated from disrupted thymocytes. Moreover, their antigenic specificity as determined in a cytotoxicity adsorption test was identical. From our experiments, we hypothesize that the extracellular membrane fragments found in the rabbit thymus are derived mainly from material shed by immature thymocytes.

Introduction

We have previously studied the composition and lipid fluidity of purified plasma membranes and other subcellular structures isolated from rabbit thymocytes [1]. A definite relationship was found between the lipid composition and the degree of fluorescence polarization measured with the probe 1,6-diphenyl-1,3,5-hexatriene (DPH). In various instances, membrane structures had also been found outside the cells under physiological conditions. This phenomenon has been particularly studied in the case of tumour cells and attributed to the shedding of plasma membrane fragments [2—9]. Van Blitterswijk et al. [6,7] have shown that the shed membrane vesicles are enriched in tumour antigens

and exhibited a lower lipid fluidity than plasma membranes isolated from the tumour cells. These authors also found extracellular membrane vesicles in cell preparations of thymocytes from normal mice [6]. They floated at the same density in a sucrose gradient as plasma membranes purified from disrupted cells. These extracellular membranes exhibited a lower lipid fluidity than the plasma membranes.

In the course of our work on membrane phenomena related to the differentiation of lymphocytes in the rabbit thymus, we also isolated extracellular material to study its lipid fluidity. From this material, membrane fragments could be obtained with the same buoyant density as thymocyte plasma membranes. They showed the same lipid fluidity as plasma membranes from immature thymocytes. This conspicuous finding in view of the results by Van Blitterswijk et al. [6] prompted us to analyse these extracellular membranes in more detail. It was found that they resembled purified plasma membranes from thymocytes in more respects: ultrastructural appearance, enzyme activities, antigenic specificity and lipid composition.

From our studies, we tentatively conclude that the extracellular membrane fragments in the rabbit thymus are mainly derived from shed material of immature lymphocytes.

Materials and Methods

Isolation of extracellular membranes. Whole thymus organs were obtained from 10-weeks-old Chinchilla rabbits (Central Institute for the Breeding of Laboratory Animals T.N.O., Zeist, The Netherlands). From these, suspensions of thymocytes were prepared as previously [1], and centrifuged at $300 \times g$ for 10 min. The $300 \times g$ supernatant was again centrifuged at $800 \times g$ for 10 min to sediment remaining cells and cell debris. After staining with eosin, phase contrast microscopy was used to verify that no cells (alive or dead) were present in the resulting supernatant. This supernatant was centrifuged at $64\,000 \times g_{av}$ for 60 min. From the pellet thus obtained, purified membrane vesicles and fragments were obtained in a 28—40% (w/v) sucrose one-step or a 24—40% (w/v) sucrose multistep gradient [1].

The cells were washed and disrupted by a cell-disrupting pump yielding nuclear, mitochondrial, endoplasmic reticulum and plasma membrane fractions [1].

Biochemical analyses. Protein, DNA, RNA, phospholipid phosphorus, cholesterol and fatty acid composition of the total phospholipid fraction were determined as described previously [1]. The composition of individual phospholipids was determined by two-dimensional thin-layer chromatography on prewashed 10×10 cm HPTLC plates (Merck, Darmstadt). The elution was performed in chloroform/methanol/25% ammonia (65 : 35 : 5, v/v) (first dimension), and acetone/chloroform/methanol/acetic acid/water (40 : 30 : 10 : 10 : 5, v/v) (second dimension). Between the runs, the plates were dried at 40°C for 20 min. The lipids were visualized by iodine vapour. The spots were marked and after evaporation of the iodine they were carefully removed by scraping with a glass blade. The phosphorus content was estimated by the

micro-method of Turner and Rouser [10]. It was observed that the silica blanks of these HPTLC plates were very low.

The enzymatic composition of the extracellular membranes was estimated by assay of 5'-nucleotidase, Mg^{2+}-ATPase, glucose-6-phosphatase and NADH-dehydrogenase as described earlier [1].

Fluorescence polarization measurements. Steady-state fluorescence polarization measurements were performed as described before [1] in an Elscint MV-1a apparatus (Elscint Ltd., Haifa, Israel) using DPH (Koch-Light Laboratories Ltd., Colnbrook Bucks) as a fluorescent probe.

Antigenic specificity. Relative amounts of thymus-specific antigens in the membranous fractions were measured by an antibody and complement mediated cytotoxicity adsorption test [11]. The antibody used was obtained by immunisation of rats with rabbit thymocytes, collection of serum and separation of the IgG fraction by Sephadex chromatography [12]. The anti-thymocyte-IgG was dissolved in RPMI 1640 (Gibco Europe, Glasgow) to a concentration twice as high as that which will kill 50% of thymocytes in a cytotoxicity test. 50 µl of this solution were added to small test tubes containing 50 µl of the sample to be tested in dilution from 2^{-1} to 128^{-1}. After shaking overnight at 0—4°C, the tubes were centrifuged to sediment antigen-antibody complexes and 20 µl samples were taken from the supernatant. To these were added 20 µl of a thymocyte suspension in RPMI 1640 ($3 \cdot 10^7$ cells/ml) and 20 µl of 1 : 4 diluted fresh guinea-pig serum as a complement source. After incubation at 37°C for 45 min in a microtiter plate, the plate was cooled on ice, 60 µl 0.2% eosin in RPMI 1640 were added and the viability was determined. From the midpoint of the sigmoid curve (percentage dead cells vs. reciprocal dilution of the sample) the specific antigenic content relative to the cell homogenate (the so-called enrichment factor) was calculated on a protein basis.

Electron microscopy. Electron micrographs of pellets of the extracellular membrane fractions were made according to the method already described [1].

Results

Isolation and characterization of extracellular membranes

The total DNA content of the extracellular fluid was found to amount to 539 µg per thymus (mean of two experiments). This can be attributed to $1.04 \cdot 10^8$ cells (calculated from Table II in Ref. 1). From this quantity, 27 µg (or 5%) was left in the supernatant after centrifugation at $800 \times g$. The rest was found in the pellet and can thus be attributed to cells and/or cell nuclei present in the original extracellular fluid. It might be stated that this amount of DNA is completely attributable to nuclei which were derived from cells disrupted during the cell isolation process. In this case, no more than approx. 10^8 cells would be disrupted, or only 2.5% of all cells from one thymus yielding approx. $4 \cdot 10^9$ thymocytes. Therefore, practically all membranous material in the extracellular fluid is already present as such in the thymus itself.

From this material, in the initial experiments, extracellular membranes were isolated and purified in a one-step sucrose gradient consisting of 28 and 40% (w/v) sucrose yielding one membrane fraction at the interphase of the two layers. As shown in Table I, the yield of purified extracellular membranes per

TABLE I

CHEMICAL ANALYSIS OF EXTRACELLULAR MEMBRANES FROM RABBIT THYMUS AND THYMOCYTE PLASMA MEMBRANES

Data are expressed as mean ±S.D. with number of preparations in parentheses. The experiments were performed with material from one thymus at a time. The cell homogenate contained 102.0 ± 11.7 (n = 10) mg protein/5 · 10^9 cells *, and the 800 × g supernatant of the extracellular fluid contained 80.7 ± 14.8 (n = 6) mg protein/5 · 10^9 cells. n.d., not determined.

	Protein recovery (%)	DNA (μg/mg protein)	RNA (μg/mg protein)
Extracellular	(n = 6)	(n = 2)	(n = 2)
800 × g supernatant	100	0.8	57
64 000 × g pellet	12.9 ± 0.9	n.d.	n.d.
64 000 × g supernatant	n.d.	0	34
Extracellular membranes	1.61 ± 0.37	0	102
Pellet from sucrose gradient	7.2 ± 1.1	14	184
Cells *	(n = 10)	(n = 4)	(n = 4)
Homogenate	100	253 ± 14	73 ± 19
64 000 × g pellet	5.5 ± 1.1	n.d.	n.d.
64 000 × g supernatant	38.9 ± 4.0	5.0 ± 1.3	148 ± 13
Plasma membranes	1.14 ± 0.17	0	50 ± 23
Pellet from sucrose gradient	2.9 ± 0.9	36 ± 22	102 ± 10

* From Ref. 1.

thymus is higher than the yield of plasma membranes isolated from all thymocytes per thymus. The extracellular membrane fragments are devoid of DNA and contain a small amount of RNA, i.e. twice as much as is found in plasma membrane vesicles from disrupted cells. This amount of RNA might be attributed to ribosomes and soluble RNA entrapped during a supposed shedding process. It is presumed than in this case, predominantly right-side-out vesicles are formed while during the mechanical disruption of cells in an isotonic medium, a fifty-fifty distribution of right-side-out and inside-out vesicles may be expected [13]. This may explain the fact that extracellular membrane vesicles contain twice as much RNA as plasma membrane vesicles obtained by mecha-

TABLE II

ENZYMIC ANALYSIS OF EXTRACELLULAR MEMBRANES FROM RABBIT THYMUS AND TMYMOCYTE PLASMA MEMBRANES

Data are expressed as the mean of duplicate (plasma membranes) or triplicate (extracellular membranes) experiments. Specific activities are expressed as μmol product liberated/mg protein per h.

	5′-Nucleotidase	Mg^{2+}-ATPase	Glucose-6-phosphatase	NADH-dehydrogenase
Extracellular membranes	1.47	13.9	0.009	5.32
Pellet from sucrose gradient	0.47	5.6	0.29	32.1
Plasma membranes *	1.16	13.0	0.003	2.67
Pellet from sucrose gradient *	0.31	3.4	0.48	12.4

* From Ref. 1.

nical disruption.

As shown in Table II, the specific activities of the plasma membrane marker enzymes 5'-nucleotidase and Mg^{2+}-ATPase were similar in the plasma membranes and extracellular membranes. NADH-dehydrogenase activity was higher in extracellular membranes than in plasma membranes. This finding, and the presence of a significant amount of glucose-6-phosphatase point to a higher contamination with intracellular components of the extracellular membranes when compared with plasma membranes which may be caused by entrapment of intracellular components during the process of shedding of the extracellular membranes. Evidence for such a sequence of events is further clearly indicated by the electron micrographs which show electron-dense material more particularly in the smaller vesicles (Fig. 1). In many instances, ribosomes are detected in the vesicles.

Table III shows the relative antigenic specificity for anti-thymocyte-IgG. The highest values were found in the plasma membranes and extracellular membranes. The presence of some antigenic activity in the other fractions, notably the sucrose gradient pellets, may be due to contamination with plasma membranes. This is consistent with the presence of plasma membrane marker enzymes (Table II).

Extracellular membranes were also purified in a 24—40% sucrose multistep gradient. The distribution of the various membrane fractions M1—M4 in the gradient was practically the same for plasma and extracellular membranes (Table IV).

Fluorescence polarization measurements

Shinitzky and co-workers (for review see Ref. 14) interpreted the steady-state fluorescence anisotropy in terms of the so-called microviscosity by applying the Perrin equation. On the basis of theoretical considerations and experimental results, this relationship has been criticised by various authors [15—17]. The steady-state fluorescence anisotropy, r_s, is a combination of a hindered rotation of the fluorescent probe by the membrane lipids and the oriental constraints imposed on the probe by the membrane, and can be repre-

TABLE III

ANTIGENIC SPECIFICITY FOR ANTI-THYMOCYTE-IgG OF EXTRACELLULAR MEMBRANES AND SUBCELLULAR FRACTIONS FROM RABBIT THYMOCYTES

Data are expressed as mean ±S.D. of four experiments. The antigenic specificity is expressed on a protein basis, relative to the cell homogenate.

	Relative antigenic specificity
Homogenate	1
Nuclei	0.4 ± 0.1
Mitochondria	0.5 ± 0.1
Plasma membranes	11.1 ± 2.3
Endoplasmic reticulum	2.3 ± 0.7
Extracellular membranes	13.4 ± 2.7
Pellet from sucrose gradient	1.8 ± 0.6

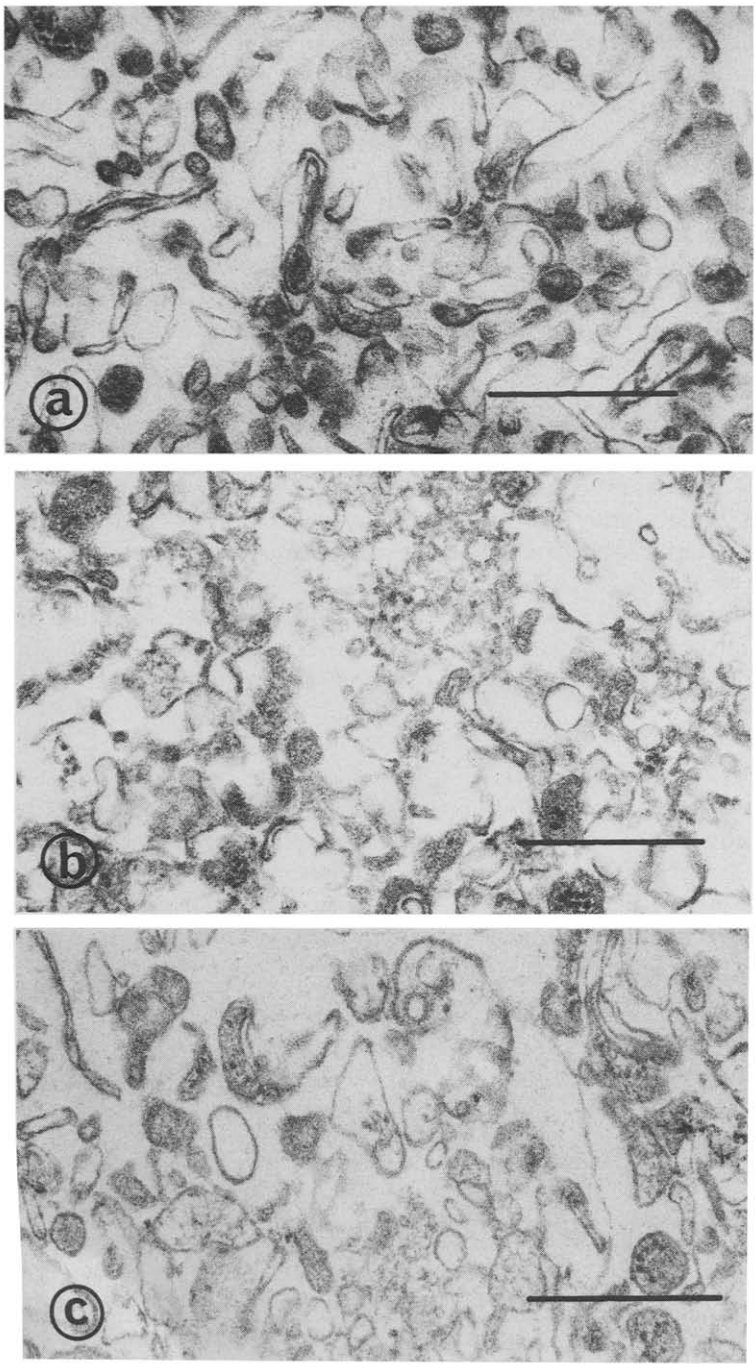

Fig. 1. Electron micrographs of extracellular membranes M2—M4 from a 24—40% sucrose multistep gradient. (a) Fraction M2; (b) fraction M3; (c) fraction M4. Bar, 0.5 μm. Magnification = 51 200×.

TABLE IV

DISTRIBUTION AND LIPID ORDER PARAMETER OF EXTRACELLULAR MEMBRANE AND PLASMA MEMBRANE FRACTIONS M1—M4 (24—40% SUCROSE MULTISTEP GRADIENT)

Data are expressed as mean ±S.D. of four experiments. The steady-state fluorescence anisotropy, r_s, is calculated from $r_s = 2P/(3 - P)$ [1]. The lipid order parameter S_{DPH} is calculated from $(S_{DPH})^2 = r_\infty/r_0$; $r_0 = 0.4$, and $r_\infty = (4/3)r_s - 0.10$ (see text). Determinations were done at 25°C.

Fractions from	Protein (% of total membranes)	P	r_s	S_{DPH}
Extracellular membranes				
M1	14.3 ± 2.7	0.304 ± 0.009	0.226 ± 0.007	0.709 ± 0.017
M2	28.3 ± 4.1	0.334 ± 0.006	0.251 ± 0.005	0.766 ± 0.010
M3	29.0 ± 3.0	0.342 ± 0.007	0.257 ± 0.006	0.779 ± 0.013
M4	28.5 ± 3.7	0.352 ± 0.002	0.266 ± 0.002	0.798 ± 0.003
Plasma membranes *				
M1	15.1 ± 1.1	0.309 ± 0.023	0.230 ± 0.019	0.719 ± 0.045
M2	20.4 ± 2.2	0.347 ± 0.005	0.262 ± 0.005	0.790 ± 0.010
M3	31.2 ± 2.2	0.352 ± 0.003	0.266 ± 0.003	0.798 ± 0.007
M4	33.3 ± 3.2	0.354 ± 0.002	0.268 ± 0.002	0.802 ± 0.004

* From Ref. 1.

sented by the relationship

$$r_s = \frac{r_0 - r_\infty}{1 + \tau/\phi} + r_\infty$$

where r_0 is the maximal fluorescence anisotropy value in the absence of any rotational motion of the fluorophore, r_∞ is the limiting anisotropy, τ is the fluorescent lifetime of the fluorophore and ϕ is the rotational correlation time. When using the probe 1,6-diphenyl-1,3,5-hexatriene (DPH), the lipid order parameter S_{DPH} can be obtained to a good approximation from the above equation and the relationship $r_\infty/r_0 = (S_{DPH})^2$ (Refs. 16, 17 and 22) van Blitterswijk et al. [22] derived empirically the relationship $r_\infty = (4/3)r_s - 0.10$ $(0.13 < r_s < 0.28)$. They also argued that the term 'lipid fluidity' is justified and may be defined as the reciprocal of the lipid structural order parameter.

The degree of fluorescence polarization, P, was determined in extracellular membrane fractions M1—M4 from a 24—40% sucrose multistep gradient. From the P value, the fluorescence anisotropy r_s and the lipid order parameter S_{DPH} were calculated. As shown in Table IV, high values were found comparable with those of plasma membranes.

Lipid composition of extracellular membranes

The fluidity of biological membranes is controlled by the lipid composition, e.g. the cholesterol/phospholipid molar ratio, the sphingomyelin content, the fatty acid composition and the polar head groups of the phospholipids (for references see Ref. 1). For this reason, we estimated the cholesterol/phospholipid molar ratio, the phospholipid composition and the fatty acid composition of the total phospholipid fraction. As shown in Table V, the cholesterol/phospholipid molar ratios of plasma membranes and extracellular membranes are

TABLE V

CHOLESTEROL/PHOSPHOLIPID MOLAR RATIO AND PHOSPHOLIPID COMPOSITION OF EXTRACELLULAR MEMBRANES AND SUBCELLULAR FRACTIONS FROM RABBIT THYMOCYTES

Data are expressed as mean ±S.D. with number of experiments in parentheses or as the mean of duplicate experiments (phospholipid composition).

	Nuclear fraction	Mitochondrial fraction	Plasma membranes		Extracellular membranes	
			Purified membranes	Pellet from sucrose gradient *	Purified membranes	Pellet from sucrose gradient
Cholesterol/phospholipid molar ratio	0.10 ± 0.02 (6) **	0.20 ± 0.02 (8) **	0.59 ± 0.04 (7) **	0.39 ± 0.04 (7) **	0.62 ± 0.07 (5)	0.33 ± 0.04 (5)
Phospholipid composition (%)						
Phosphatidylserine	12.1	8.5	12.7	9.5	11.0	9.9
Phosphatidic acid	0.5	0.7	1.1	0.7	1.1	0.7
Lysophosphatidylcholine	0.4	0.6	—	2.4	1.4	1.5
Sphingomyelin	1.5	2.4	13.5	8.8	14.5	10.1
Phosphatidylcholine	57.9	47.5	51.1	57.3	49.1	50.6
Phosphatidylethanolamine	26.3	30.6	18.9	15.2	13.9	17.3
Phosphatidylinositol	0.1	0.7	—	1.1	2.1	1.4
Diphosphatidylglycerol	1.4	8.4	0.6	1.2	—	2.4
Unknown	0.2	0.7	2.2	3.9	7.0	6.3

* Mainly endoplasmic reticulum.

** From Ref. 1.

TABLE VI

FATTY ACID COMPOSITION OF TOTAL PHOSPHOLIPIDS FROM EXTRACELLULAR MEMBRANES AND PLASMA MEMBRANES OF RABBIT THYMOCYTES

Data are expressed as mean ±S.D. with number of preparation in parentheses. Analyses were performed on two liquid phases (SP 2330 and SP 2340) for each preparation.

	Extracellular membranes (n = 6)	Plasma membranes * (n = 3)
14 : 0	1.7 ± 0.7	2.8 ± 1.7
15 : 0	1.4 ± 0.6	1.2 ± 0.2
16 · 0	51.5 ± 2.8	55.9 ± 1.9
17 : 0	1.6 ± 0.3	1.8 ± 0.1
18 : 0	18.1 ± 1.7	18.4 ± 2.2
18 : 1	12.9 ± 1.1	11.7 ± 1.6
18 : 2	3.3 ± 0.8	3.1 ± 0.7
18 : 3	—	0.3 ± 0.3
20 : 0	0.8 ± 0.2	0.1 ± 0.0
20 : 1	0.8 ± 0.1	0.3 ± 0.2
21 : 0	0.2 ± 0.2	0.4 ± 0.1
20 : 4	2.6 ± 0.4	1.7 ± 0.5
22 : 0	2.3 ± 1.0	0.6 ± 0.3
22 : 1	0.3 ± 0.3	0.1 ± 0.1
22 : 4	0.7 ± 0.4	0.4 ± 0.2
24 : 0	0.7 ± 0.1	—
Saturated	77.8 ± 1.9	80.3 ± 1.3
Monounsaturated	15.6 ± 1.1	13.9 ± 1.6
Polyunsaturated	6.6 ± 1.3	5.8 ± 0.5

* From Ref. 1.

similar and much higher than those of the sucrose pellets, mitochondria and nuclei. The phospholipid composition of plasma and extracellular membranes is very much alike, which is also true for the respective sucrose pellets. The phospholipid composition of the nuclear and mitochondrial fractions from disrupted thymocytes is further shown in Table V. As expected, the mitochondrial fraction shows the highest proportion of diphosphatidylglycerol (cardiolipin). The sphingomyelin content increases in the order: nuclei < mitochondria < sucrose pellets < purified membranes. As in the case of the cholesterol/phospholipid molar ratio, this is consistent with decreasing lipid fluidity also in that order.

We have previously shown [1] that the phospholipid fraction of purified plasma membranes from rabbit thymocytes contains a very large amount of saturated fatty acids. This is also true for purified extracellular membranes (Table VI). The fatty acid profiles are the same for plasma membranes and extracellular membranes.

Discussion

In the present study, an appreciable amount of membrane fragments (vesicles and sheets as shown by electron microscopy) was detected in the 800 × g supernatant of cell suspensions from the rabbit thymus. Analysis of these membrane fragments after purification in a sucrose gradient shows that they share with purified plasma membranes from thymocytes a considerable number of properties, such as (a) behaviour in a multistep sucrose gradient, (b) enzymatic

and (c) antigenic specificity, (d) lipid composition, and (e) lipid fluidity. Obviously, these extracellular plasma membrane fragments are released in some way from the thymocytes.

This release cannot be accounted for to a marked degree by disruption of the cells during the isolation procedure because it can be calculated from DNA estimations that only 2.5% or less of the whole cell population is liable to damage by this treatment. Shedding of plasma membrane fragments from viable cells during the isolation step is not likely because Vitetta and Uhr [18] have shown that shedding of surface Ig-plasma membrane complexes from lymphocytes hardly occurs at temperatures of 0—4°C. This is confirmed in the case of tumour cells by Koch and Smith [4]. Furthermore, in order to obtain plasma membrane vesicles in a reasonable amount in an in vitro procedure without mechanical means, treatment with low molecualr weight aldehydes or disulfide blocking agents [19] or detergents [20] is necessary. Recently, Ferber et al. [21] have developed a milder method for the induction of shedding of plasma membrane vesicles by intact calf thymocytes by application of an analogue of lysophosphatidylcholine.

In our experiments, the presence of plasma membrane fragments in the extracellular fluid may also be attributed to cell death in the thymus. We do not consider this as the main cause. First, the quantity of extracellular plasma membrane fragments obtained from one thymus is far greater than the amount of plasma membranes isolated from the total population of thymocytes. If this amount resulted from dead cells in the thymus, this would mean a massacre in this organ. Second, all other cellular structures including nuclei and mitochondria (mitochondria are only sparsely found in the extracellular compartment of the thymus by electron microscopy; Leene, W., personal communication) would be degraded at a higher rate than the plasma membranes. This is hardly conceivable. Third, the extracellular membrane vesicles contain twice as much RNA as plasma membrane vesicles obtained by mechanical disruption of viable cells; they contain more electron-dense material (amorphous and ribosomes) and show higher activities of NADH-dehydrogenase and glucose-6-phosphatase compared with purified plasma membrane fragments. This is all compatible with a preponderance of right-side-out vesicles in the case of the extracellular membranes. It is more likely that this kind of vesicle is produced during an active shedding process by viable cells than by vesiculation of dead cell membranes. Our experiments do not exclude cell death in the thymus as one possible process in the production of plasma membrane vesicles, but in view of the above we do not consider it as the main source. In our opinion this must be shedding.

The phenomenon of spontaneous membrane shedding from intact cells has been reported mainly for tumour cells [2—9]. Shedding of plasma membrane vesicles from normal cells has also previously been observed. De Broe et al. [3] detected plasma membrane fragments in duodenal fluid and urine of healthy individuals. According to the evidence available, these fragments are shed by normal viable cells. The turnover of surface Ig on spleen cells is accompanied by the shedding of Ig attached to plasma membrane fragments, as shown by Vitetta and Uhr [18]. The occurrence of these fragments in the incubation fluid was shown not to be due to cytolysis.

Van Blitterswijk et al. [6] isolated extracellular membrane fragments from cell preparations of mouse thymus. Without giving further details, they mentioned that these membrane fragments showed properties similar to plasma membranes purified from disrupted thymocytes. Their lipid fluidity, however, was lower and comparable with the lipid fluidity of extracellular membrane fragments exfoliated from thymus-derived ascitic leukemia cells and occurring in the ascites fluid.

In our previous study [1], we drew attention to the relatively high rigidity of plasma membranes from rabbit thymocytes ($P = 0.352$) when compared with mouse thymocytes ($P = 0.306$, from the experiments of Van Blitterswijk et al. [6]). Plasma membranes obtained from thymus-derived ascitic leukemia cells in mouse are even more fluid ($P = 0.269$) [6]. Membranes exfoliated from both normal lymphocytes in the thymus and leukemia cells in the ascites, however, show the same high fluorescence polarization value ($P = 0.329$) [6]. So it seems that there is some limiting value to the fluorescence anisotropy in naturally occurring membranes. We have previously found that there are differences between the lipid fluidities of plasma membranes from immature and mature thymocytes, the former showing the lower lipid fluidity [1]. We can speculate that the lipid fluidity of the plasma membranes of the immature lymphocytes is at its limit so that no preferential shedding of still more rigid membrane domains can occur.

We are currently investigating these aspects by manipulation of the plasma membrane lipid composition (and thus its lipid fluidity) of immature and mature thymocytes by incubation of the cells with unilamellar liposomes of varying composition.

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References

1 Roozemond, R.C. and Urli, D.C. (1979) Biochim. Biophys. Acta 556, 17—37
2 Nowotny, A., Grohsman, J., Abdelnoor, A., Rote, N., Cynara Yang and Waltersdorff, R. (1974) Eur. J. Immunol. 4, 73—78
3 De Broe, M.E., Wieme, R.J., Logghe, G.N. and Roels, F. (1977) Clin. Chim. Acta 81, 237—245
4 Koch, G.L.E. and Smith, M.J. (1978) Nature 273, 274—278
5 Price, M.R. and Baldwin, R.W. (1977) in Dynamic Aspects of Cell Surface Organization (Poste, G. and Nicolson, G.L., eds.), pp. 423—471, North-Holland Publ. Co., Amsterdam
6 Van Blitterswijk, W.J., Emmelot, P., Hilkmann, H.A.M., Oomen-Meulemans, E.P.M. and Inbar, M. (1977) Biochim. Biophys. Acta 467, 309—320
7 Van Blitterswijk, W.J., Emmelot, P., Hilkmann, H.A.M., Hilgers, J. and Feltkamp, C.A. (1979) Int. J. Cancer 23, 62—70
8 Raz, A., Goldman, R., Yuli, I. and Inbar, M. (1978) Cancer Immunol. Immunother. 4, 53—59
9 Petitou, M., Tuy, F., Rosenfeld, C., Mishal, Z., Paintrand, M., Jasnin, C., Mathe, G. and Inbar, M. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 2306—2310
10 Turner, J.D. and Rouser, G. (1970) Anal. Biochem. 38, 423—436

11 Van Blitterswijk., W.J., Emmelot, P., Hilgers, J., Kamlag, D., Nusse, R. and Feltkamp, C.A. (1975) Cancer Res. 35, 2743—2751
12 Roholl, P., Hogenes, T. and Leene, W. (1977) in Developmental Immunobiology (Solomon, J.B. and Horton, J.D., eds.), pp. 163—170, Elsevier/North-Holland Biomedical Press, Amsterdam
13 Walsh, F.S., Barber, B.H. and Crumpton, M.J. (1976) Biochemistry 15, 3557—3563
14 Shinitzky, M. and Barenholz, Y. (1978) Biochim. Biophys. Acta 515, 367—394
15 Hildenbrand, K. and Nicolau, C. (1979) Biochim. Biophys. Acta 553, 365—377
16 Jähnig, F. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 6361—6365
17 Heyn, M.P. (1979) FEBS Lett. 108, 359—364
18 Vitetta, E.S. and Uhr, J.W. (1972) J. Exp. Med. 136, 676—696
19 Scott, R.E. (1976) Science 194, 743—745
20 Billington, D. and Coleman, R. (1978) Biochim. Biophys. Acta 509, 33—47
21 Ferber, E., Schmidt, B. and Weltzien, H.U. (1980) Biochim. Biophys. Acta 595, 244—256
22 Van Blitterswijk, W.J., Van Hoeven, R.P. and Van der Meer, B.W. (1981) Biochim. Biophys. Acta 644, in the press