Nonidet P-40 extraction of lymphocyte plasma membrane

Characterization of the insoluble residue

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Purified preparations of lymphocyte plasma membrane were extracted exhaustively with Nonidet P-40 in Dulbecco's phosphate-buffered saline medium. The insoluble fraction, as defined by sedimentation at $10^6 g$ -min, contained about 10% of the membrane protein as well as cholesterol and phospholipid. The lipid/protein ratio, cholesterol/phospholipid ratio and sphingomyelin content were increased in the residue. Density-gradient centrifugation suggested that the lipid and protein form a common entity. As judged by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, the Nonidet P-40-insoluble fractions of the plasma membranes of human B lymphoblastoid cells and pig mesenteric lymph-node lymphocytes possessed similar qualitative polypeptide compositions but differed quantitatively. Both residues comprised major polypeptides of M_r 28 000, 33 000, 45 000 and 68 000, together with a prominent band of M_r 120 000 in the human and of M_r 200 000 in the pig. The polypeptides of M_r 28 000, 33 000, 68 000 and 120 000 were probably located exclusively in the Nonidet P-40-insoluble residue, which also possessed a 4-fold increase in 5'-nucleotidase specific activity. The results indicate that a reproducible fraction of lymphocyte plasma membrane is insoluble in non-ionic detergents and that this fraction possesses a unique polypeptide composition. By analogy with similar studies with erythrocyte ghosts, it appears likely that the polypeptides are located on the plasma membrane's cytoplasmic face.

It is apparent that many aspects of cell behaviour are regulated by the interaction of ligands with specific cell-surface receptors (Edelman, 1976; Owen & Crumpton, 1981). It is also generally accepted that the topographical arrangement of the receptors is an important feature in the regulation of cell behaviour. Various studies, particularly of lymphocytes, have demonstrated that the redistribution (i.e. 'patching' and 'capping') of receptors (antigens) induced by multivalent ligands results in a complementary intracellular re-arrangement of the cytoskeletal proteins actin, tubulin (Gabbiani *et al.*, 1977) and myosin (Schreiner *et al.*, 1977). In addition, the results of recent studies utilizing extraction with non-ionic detergents suggest that various cell-surface pro-

teins are attached to the cytoskeleton in a variety of cells, including neutrophils (Sheterline & Hopkins, 1981), platelets (Rotman *et al.*, 1982) and fibroblasts (Lehto, 1983). These observations collectively imply that cell-surface components are linked directly or indirectly to the cytoskeleton either within the membrane or at the membrane's cytoplasmic face (Bourguignon & Singer, 1977; Ben-Ze'ev *et al.*, 1979). However, the nature of this putative association as well as the mechanism by which the cell-surface topography is regulated remain obscure.

In erythrocytes, one of the major cell-surface glycoproteins, namely band 3 protein, is attached directly to the submembranous cytoskeleton (Branton *et al.*, 1981; Gratzer, 1981). This cytoskeleton, which is prepared by extracting erythrocyte ghosts with non-ionic detergents, appears to modulate the lateral mobility of band 3 protein (Sheetz *et al.*,

Abbreviations used: SDS, sodium dodecyl sulphate; Dulbecco's PBS, Dulbecco's phosphate-buffered saline medium.

1980; Smith & Palek, 1982). We have explored the possibility that a similar cytoskeleton underlies the plasma membrane of lymphocytes. An approach identical with that used by Yu *et al.* (1973) in erythrocytes was employed.

In the present paper we describe the nature of the Nonidet P-40-insoluble residues separated from the purified plasma membranes of cultured human B lymphoblastoid cells and quiescent pig mesenteric lymph-node lymphocytes. The results indicate that a highly reproducible fraction of the plasma-membrane vesicles is insoluble and that this fraction has a unique polypeptide composition. Although the polypeptide compositions of the human and pig lymphocyte membrane residues were qualitatively similar, they revealed marked quantitative differences. A preliminary report of some of this work has been published (Davies *et al.*, 1981).

Materials and methods

Reagents

[32 P]P_i (PBS.11; 10 mCi/ml), [35 S]methionine (SJ.204; 600 Ci/mmol) and [3 H]AMP (TRK.344; 19.3 Ci/mmol) were supplied by Amersham International (Amersham, Bucks., U.K.).

Complete RPMI 1640 and Eagle's culture media, and those lacking specific components, were obtained from Gibco Biocult (Paisley, Renfrewshire, Scotland, U.K.). Foetal-calf serum was from Flow Laboratories (Irvine, Ayrshire, Scotland, U.K.). Triton X-100, sodium deoxycholate, iodoacetamide and phenylmethanesulphonyl fluoride were purchased from Sigma Chemical Co. (Poole, Dorset, U.K.). SDS and Nonidet P-40 were obtained from BDH Chemicals (Poole, Dorset, U.K.), and acrylamide and bisacrylamide were from Serva (Heidelberg, Germany). Ficoll 400 was supplied by Pharmacia Fine Chemicals (Uppsala, Sweden).

Dulbecco's PBS comprised 10 mM-sodium phosphate buffer, pH 7.2, 0.17 M-NaCl, 3 mM-KCl, 1 mM-CaCl₂ and 1 mM-MgCl₂. Iodoacetamide and phenylmethanesulphonyl fluoride (final concentrations 10 mM and 1 mM respectively) were added immediately before use.

Cells and tissues

The human B lymphoblastoid cell lines Maja, MST, RPMI 1788 and BRI 8 were cultured in RPMI 1640 medium containing penicillin (100 units/ml), streptomycin (50 µg/ml) and 10% (v/v) foetal-calf serum.

Human erythrocytes were separated from fresh blood by sedimentation and were washed three times with 0.15 M-NaCl/5 mM-sodium phosphate buffer, pH 8.0. Human peripheral-blood lympho-

cytes were separated from platelet-depleted blood by layering on to an equal volume of Ficoll-Paque (Pharmacia Fine Chemicals) and centrifuging at 4 × 10⁴ g-min; the cells collecting at the interface were washed twice in phosphate-buffered saline (0.17 M-NaCl/10 mM-sodium phosphate buffer, pH 7.2).

Normal human spleen was donated by the Department of Medical Oncology, St. Bartholomew's Hospital, London E.C.1, U.K., and pig mesenteric lymph nodes were obtained from British Beef, Watford, Herts., U.K.

Radioactive labelling

Biosynthetic labelling with [35 S]methionine and [32 P]P_i was carried out with cells that had been washed twice with sterile phosphate-buffered saline or saline respectively. Cells were resuspended (5 × 10⁶/ml for phosphorylation; 2 × 10⁶/ml for methionine-labelling) in medium (Eagle's medium for phosphorylation; RPMI 1640 medium for methionine-labelling) that lacked the respective unlabelled component and that had been supplemented with 5% (v/v) dialysed foetal-calf serum. Cell suspensions were preincubated at 37°C for 1 h before the addition of [32 P]P_i (final concentration 0.1 mCi/ml) or [35 S]methionine (final concentration 20 µCi/ml). The period of labelling was 16 h for methionine and 3 h for P_i. Cells were recovered by centrifuging (2 × 10⁴ g-min) and were washed twice with phosphate-buffered saline.

Plasma-membrane preparation

Plasma membrane was separated from homogenates of pig mesenteric lymph node and of human spleen as described by Snary *et al.* (1976). Suspensions of human B lymphoblastoid cells and peripheral-blood lymphocytes in Dulbecco's PBS were disrupted and the plasma-membrane fraction was separated as described by Crumpton & Snary (1974). As judged morphologically and biochemically, the lymphocyte plasma-membrane preparations were not contaminated by significant amounts of other subcellular fractions, including endoplasmic reticulum and nuclear membrane (Allan & Crumpton, 1970; Crumpton & Snary, 1974; Snary *et al.*, 1976).

Erythrocyte ghosts were prepared by lysing whole washed erythrocytes (1 ml of packed cells to 40 ml of 5 mM-sodium phosphate buffer, pH 8.0) and were separated immediately by centrifuging at 3 × 10⁵ g-min. The upper white loose pellet was collected and resuspended in the above lysis buffer. The erythrocyte ghosts were re-sedimented at 3 × 10⁵ g-min and the washing process was repeated twice.

Purified lymphocyte and erythrocyte plasma membranes were stored at -70°C after resuspen-

sion in 8% (w/v) sucrose/10mM-Tris/HCl buffer, pH 7.4.

Extraction of plasma membrane with non-ionic detergent

The erythrocyte cytoskeleton was prepared as described by Yu *et al.* (1973) by extracting 1 ml of packed erythrocyte ghosts with 5 ml of 1% (w/v) Triton X-100 in 56mM-sodium borate buffer, pH 8.0, for 10 min at 0°C and centrifuging at $8 \times 10^5 g$ -min. The pellet was washed twice with borate buffer.

Purified lymphocyte plasma membrane (0.5 mg of protein/ml) was extracted with 0.5% (v/v) Nonidet P-40 in Dulbecco's PBS for 30 min at 0°C. The extract was centrifuged at 4°C for $10^6 g$ -min (corresponding to 20000 rev./min for 30 min in a Beckman 50.2 Ti rotor), and the resulting detergent-insoluble pellet was washed twice with Dulbecco's PBS. The residue is referred to below as the '20k pellet'.

Fractionation of 20k pellet

A stock solution of Ficoll 400 in Dulbecco's PBS was adjusted to 40% (w/v) on the basis of the refractive index (1.3998 at 20°C). Lower concentrations were prepared by appropriate dilution in Dulbecco's PBS. The 20k pellet prepared from ^{35}S -labelled BRI 8 cells (about 2 mg of protein in 450 μ l of Dulbecco's PBS; 375×10^3 c.p.m.) was fractionated on step gradients comprising 4.0 ml of 40%, 4.0 ml of 20% (refractive index 1.3638), 4.0 ml of 10% (refractive index 1.3478) and 5.5 ml of 5% (refractive index 1.3402) Ficoll 400. The gradients were centrifuged in a Beckman SW27 rotor at $6.4 \times 10^7 g$ -min at 2°C. The upper sample layer, the fractions collecting at the 5%/10%, 10%/20% and 20%/40% interfaces and the pellet were collected separately. About 75% of the radioactivity added to the gradients was recovered.

Analyses

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Samples were washed with saline by sedimentation before assay in order to remove detergent and phenylmethanesulphonyl fluoride.

Phospholipids and cholesterol extracted with chloroform/methanol (1:2, v/v) were assayed and individual phospholipids were separated by t.l.c. as described previously by Allan *et al.* (1980).

5'-Nucleotidase activity was assayed as described by Michell & Hawthorne (1965). As this procedure does not distinguish between specific 5'-nucleotidase and non-specific phosphatase activities, the enzyme was also assayed with [3H]AMP as substrate in the presence of sodium β -glycerophosphate as described by Stanley *et al.* (1980).

$Na^+ + K^+$ -dependent ATPase was measured as described by Jorgensen (1974) and glucose-6-phosphatase was assayed with glucose 6-phosphate as substrate as described by Swanson (1950). P_i was determined by the method of Fiske & SubbaRow (1925).

SDS/polyacrylamide-gel electrophoretic analysis was carried out in 7.5% (w/v) polyacrylamide slab gels in 0.1% SDS in 25mM-Tris/192mM-glycine buffer, pH 8.3, as described by Laemmli (1970). Coomassie Blue-stained gels were scanned with a Joyce-Loebl Chromoscan 3 instrument.

Results

Nonidet P-40 extraction of purified plasma membrane

Extraction of lymphocyte plasma membrane with 0.5% Nonidet P-40 in Dulbecco's PBS gave an insoluble residue (referred to as the '20k pellet'), as judged by sedimentation at $10^6 g$ -min. Centrifuging at $10^7 g$ -min gave a negligible increase in the amount of sedimented protein. Table 1 shows that the 20k pellets derived from the plasma membrane of various human B lymphoblastoid cell lines consistently comprised about 11% of the membrane protein. Extraction with 1% Triton X-100 gave a similar amount of insoluble protein. A somewhat lower proportion of the protein (about 9%) of the plasma membrane of quiescent lymphocytes (i.e. pig mesenteric lymph node, human spleen and peripheral-blood lymphocytes) was insoluble under the same conditions. In contrast, a very much larger amount of the protein of human erythrocyte ghosts (about 32%) was insoluble in 1% Triton X-100 under the extraction conditions described by Yu *et al.* (1973) and sedimentation conditions similar to those employed for lymphocyte plasma membrane ($8 \times 10^5 g$ -min compared with $10^6 g$ -min).

An important question is whether the insoluble fraction represents a limit-solubility product. This was investigated by extracting, with increasing concentrations of Nonidet P-40, samples of BRI 8-cell plasma membrane that had been labelled biosynthetically with either [^{35}S]methionine as a marker for protein or [^{32}P]P_i as a marker primarily of lipid. Fig. 1 shows that 0.5% Nonidet P-40 solubilized most of the membrane protein and lipid (78 and 81% respectively), as assessed by radioactivity. Slightly less ^{35}S and ^{32}P radioactivities were sedimented at $10^6 g$ -min after extraction with 1 and 2.5% Nonidet P-40, but no further ^{35}S - and/or ^{32}P -labelled components were solubilized by Nonidet P-40 concentrations above 2.5%. The 20k pellets obtained by extracting with 2.5% Nonidet P-40 contained 15% and 14% of the ^{35}S and ^{32}P radioactivities respectively of the plasma membrane. These results collectively indicate that a

Table 1. *Amount of lymphocyte plasma-membrane protein insoluble in non-ionic detergent*

Lymphocyte plasma-membrane preparations were extracted with non-ionic detergent in Dulbecco's PBS (0.5 mg of membrane protein/ml) for 30 min at 0°C and then centrifuged at 10⁶ g-min. Erythrocyte plasma membrane was extracted with Triton X-100 by the procedure of Yu *et al.* (1973). Where more than one, numbers of determinations are given in parentheses.

Source of plasma membrane	20k pellet (% of plasma-membrane protein)		
	0.5% Nonidet P-40	1% Nonidet P-40	1% Triton X-100
Human B lymphoblastoid cells			
Maja	11.9 ± 1.2 (5)	9.4	12.3
RPMI 1788	11.2	—	—
BRI 8	11.7 (4)	9.1 (2)	—
Human peripheral-blood lymphocytes	8.3	—	—
Human spleen lymphocytes	8.8	—	—
Pig mesenteric lymph-node lymphocytes	8.7 (2)	6.6	—
Human erythrocytes	—	—	32 (2)

defined proportion of the plasma membrane of human B lymphoblastoid cell lines is insoluble in Nonidet P-40, as judged by sedimentation at 10⁶ g-min. The insoluble residues apparently comprise lipid as well as protein.

Nature of the 20k pellet

Polypeptide composition. This was analysed by SDS/polyacrylamide-gel electrophoresis. Fig. 2 shows that the BRI 8-cell 20k pellet (track 3) had a very distinctive polypeptide composition comprising major Coomassie Blue-staining bands of *M_r* 45000 and 68000, prominent bands of *M_r* 28000, 33000 and 120000 and minor bands of *M_r* 180000, 200000 and a doublet of *M_r* 240000. Scanning the gel indicated that the 28000-, 33000-, 45000-, 68000- and 120000-*M_r* bands accounted for about 7, 10, 32, 17 and 9% respectively of the total protein, although the relative amounts of the two smaller-*M_r* bands varied somewhat for different preparations. A comparison of the composition of the pellet with those of the plasma membrane (track 5) and soluble (track 1) fractions indicated that the 68000-*M_r* and 120000-*M_r* polypeptides, and probably those of *M_r* 28000 and 33000, are located exclusively in the insoluble residue. In contrast with this exclusive distribution, the 45000-*M_r* polypeptide was distributed between the insoluble and the soluble fractions. Identical results were obtained for the corresponding fractions prepared from Maja, MST and RPMI 1788 B lymphoblastoid cells and by replacing Nonidet P-40 with 1% Triton X-100.

In contrast, the 20k pellet prepared from pig mesenteric lymph-node lymphocyte plasma membrane (Fig. 2, track 4) showed marked quantitative differences in polypeptide composition. In particular, the 45000-*M_r* and especially the 120000-*M_r* polypeptides were more poorly represented (10 and 2% of the total protein respectively), whereas the

200000-*M_r* polypeptide was much more prominent (9% of the total protein). As noted previously for the BRI 8-cell fractions, the 120000-*M_r*, 33000-*M_r* and 28000-*M_r* polypeptides (2, 9 and 8% respectively of the total protein) were probably located exclusively in the detergent-insoluble fraction. The apparent difference in distribution of the 68000-*M_r* polypeptide between the pig lymphocyte and BRI 8-cell fractions is complicated by the unique association of albumin with pig lymphocyte plasma membrane (Owen *et al.*, 1980).

Lipid composition. Analyses (Table 2) showed that 20k pellets prepared from BRI 8 cells, human spleen lymphocytes and pig lymphocytes were enriched in cholesterol and, to a lesser extent, in phospholipid. Their phospholipid compositions, furthermore, resembled those of their respective plasma membranes (Fig. 3), except that the 20k pellets contained less phosphatidylethanolamine and were enriched in sphingomyelin and, in some cases, phosphatidylcholine. These changes were enhanced when the BRI 8-cell 20k pellet was prepared with 1% instead of 0.5% Nonidet P-40.

Protein-lipid association. Transmission electron microscopy of the 20k pellets invariably revealed smooth membrane vesicles with a trilaminar structure and variable amounts of amorphous material (results not shown). The presence of 5–7 nm filaments, similar to those described by Bourguignon *et al.* (1982) for the Nonidet P-40-insoluble residue of mouse T-lymphocyte cells, was not detected.

It is important to know whether or not the protein is associated with the lipid vesicles. This was explored by sedimenting the 20k pellet, prepared from BRI 8-cell plasma membrane biosynthetically labelled with [³⁵S]methionine, through step density gradients comprising 5%, 10%, 20% and 40% (w/v) Ficoll 400; the distribution was monitored by light-scattering and radioactivity.

Fig. 1. Extraction of lymphocyte plasma membrane with Nonidet P-40

Samples of ^{32}P -labelled and ^{35}S -labelled plasma membranes from lymphoblastoid BRI 8 cells were extracted with increasing concentrations of Nonidet P-40. Insoluble residues were separated by centrifuging for $10^6 g$ -min before measurement of their radioactivities. $\circ$, ^{35}S ; $\bullet$, ^{32}P .

Although the principal features were shared by all preparations, some variation in distribution was noted for different 20k pellet preparations, especially those prepared with different concentrations of Nonidet P-40. A negligible amount of material was detected at the top of the Ficoll gradient or within the sample layer. It is therefore unlikely that the 20k pellet contains significant amounts of protein-free lipid vesicles. In each case most of the sample collected at the 10%/20% and 20%/40% interfaces, together with a small fraction at the 5%/10% step. Lipid analyses of these fractions indicated that they contained both cholesterol and phospholipid (results not shown). However, whereas the cholesterol/phospholipid ratio was similar for bands located at the same interface, the amount of lipid relative to protein decreased with increasing density, as expected. Polypeptide analyses indicated that all the fractions possessed a composition similar to that of the parent 20k pellet.

These results indicate that most of the protein of the 20k pellet is associated with lipid. The lipid-protein complexes are, however, polydisperse, differing in the amount of protein attached to lipid.

Enzyme activities. When whole cells are extracted with Nonidet P-40 the nuclei remain intact, suggesting that the nuclear membrane is not solubilized. Thus the 20k pellet could represent nuclear membrane that contaminated the plasma-membrane preparations, although the presence of cholesterol (Table 2) is contrary to this suggestion.

Fig. 2. Polypeptide compositions of lymphocyte plasma membrane and of the Nonidet P-40-insoluble and -soluble fractions

Plasma membranes of human lymphoblastoid BRI 8 cells and of pig mesenteric lymph-node lymphocytes (tracks 5 and 6 respectively) were extracted with 0.5% Nonidet P-40 as described in the Materials and methods section. Tracks 3 and 4 represent the BRI 8-cell and pig lymphocyte 20k pellets respectively; the corresponding soluble fractions are shown in tracks 1 and 2. The samples were analysed by electrophoresis on 7.5% polyacrylamide gel in the presence of SDS under reducing conditions. Track 7 represents marker proteins. The gel was stained with Coomassie Blue.

Glucose-6-phosphatase is generally accepted to be a marker of nuclear membrane, whereas 5'-nucleotidase and $\text{Na}^+ + \text{K}^+$ -dependent ATPase are plasma-membrane markers (Evans, 1978). The distributions of these enzymes between the plasma-membrane fraction and the 20k pellet are shown in Table 3. The results provide no evidence in support of the 20k pellet representing nuclear membrane. In particular, the very low glucose-6-phosphatase activity of the plasma-membrane fraction showed no preferential association with the 20k pellet. The $\text{Na}^+ + \text{K}^+$ -dependent ATPase activity of the plasma membrane was considerably decreased in the detergent-insoluble residue (no more than 3% of the plasma-membrane activity), but, interestingly, the 5'-nucleotidase activity was preferentially associated with the 20k pellet. Thus about 50% of the 5'-nucleotidase activity of the plasma membrane was recovered in the 20k pellets, leading to a 3–5-fold increase in specific activity.

Table 2. *Lipid compositions of the Nonidet P-40-insoluble fractions prepared from the plasma membrane of BRI 8 cells, human spleen lymphocytes and pig mesenteric lymph-node lymphocytes*
The results are the means of at least three determinations.

Component	Plasma membrane			20k pellet		
	Human BRI 8 cells	Human spleen lymphocytes	Pig mesenteric lymph-node lymphocytes	Human BRI 8 cells	Human spleen lymphocytes	Pig mesenteric lymph-node lymphocytes
Cholesterol ($\mu\text{mol}/\text{mg}$ of protein)	0.39	0.31	0.30	0.67	0.41	0.57
Phospholipid ($\mu\text{mol}/\text{mg}$ of protein)	0.67	0.52	1.0	0.72	0.56	1.06
Lipid (mg/mg of protein)	0.66	0.51	0.86	0.80	0.58	1.05
Cholesterol/phospholipid (molar ratio)	0.54	0.55	0.30	0.86	0.69	0.54

Fig. 3. *Phospholipid compositions of lymphocyte plasma membranes and of their respective Nonidet P-40-insoluble fractions*

(a) BRI 8-cell plasma membrane was extracted with 0.5% or 1.0% Nonidet P-40. (b) Human spleen lymphocyte plasma membrane was extracted with 0.5% Nonidet P-40. ■, Plasma membrane; □, 20k pellet prepared with 0.5% Nonidet P-40; ▨, 20k pellet prepared with 1% Nonidet P-40. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; SM, sphingomyelin. The vertical bars represent the S.D. (five different preparations were analysed).

Discussion

Extraction of purified preparations of pig and human lymphocyte plasma membrane with non-ionic detergents under conditions similar to those used to separate the erythrocyte cytoskeleton (Yu

et al., 1973) yielded an insoluble complex of lipid and protein. This complex represents a limit-solubility product, as judged by using increasing concentrations of Nonidet P-40 (Fig. 1) and from its unique polypeptide composition (Fig. 2). Although the insoluble fraction may represent, or be derived from, a contaminant of the plasma membrane, this appears unlikely, because it was enriched in two plasma-membrane markers, cholesterol and 5'-nucleotidase (Tables 2 and 3) (Evans, 1978). Fractionation of the insoluble residue on Ficoll gradients indicated that it comprised a spectrum of particles that contained the same principal polypeptides but that differed in their protein/lipid ratio; significant amounts of free protein or lipid were not detected at the top of the gradients. The intimate association of lipid and protein that these results implies may explain the detergent-insolubility of the lipid vesicles, namely that insolubility is mediated by the associated proteins. This explanation is consistent with the stabilization of the lipid bilayer by the erythrocyte cytoskeleton (Branton *et al.*, 1981). The presence of lipid (Table 2 and Fig. 3) was unexpected in view of the high ratio of Nonidet P-40 to plasma membrane used for extraction. It is important to note that the Triton X-100-insoluble residue of erythrocyte ghosts also contained lipid and that sphingomyelin was similarly preferentially represented (Yu *et al.*, 1973). However, the erythrocyte residue contained less lipid relative to protein than did its lymphocyte counterpart (about 500 $\mu\text{g}/\text{mg}$ compared with 700 $\mu\text{g}/\text{mg}$ of protein respectively). The preferential association of sphingomyelin with the residue suggests that the phospholipids located in the outer leaflet of the bilayer were preferentially retained during detergent extraction, although other interpretations based on the concept of the clustering of specific lipids into domains within the membrane are also possible (Haest, 1982).

The detergent-insoluble protein represented

Table 3. *Enzyme activities of 20k pellets compared with those of the plasma-membrane fractions*

5'-Nucleotidase activity was assayed by using the two procedures described in the Materials and methods section; one of these procedures incorporates β -glycerophosphate as an inhibitor of non-specific phosphatases. No significant difference was detected in the values obtained, and the results shown represent the average values. Plasma membranes and 20k pellets were prepared from the human B lymphoblastoid cell lines BRI 8 and Maja and from pig mesenteric lymph-node lymphocytes. Enzyme recovered in 20k pellets was calculated on the basis that the BRI 8 (Maja)-cell and pig lymphocyte 20k pellets represent 12 and 9% respectively of the total plasma-membrane protein (Table 1).

	Cell type	Specific activity (μ mol of P_i released/min per mg of protein)		Enzyme recovered in 20k pellet (%)
		Plasma membrane	20k pellet	
Glucose-6-phosphatase	Maja cells	0.03	0.04	16
5'-Nucleotidase	BRI 8 cells	82	233	34
	Maja cells	11	38	41
	pig lymphocytes	91	460	61
$Na^+ + K^+$ -dependent ATPase	BRI 8 cells	321	69	2
	pig lymphocytes	319	55	2

about 10% of the lymphocyte plasma-membrane protein compared with about 35% in the case of erythrocyte ghosts. Although the polypeptide composition showed marked quantitative differences depending on the source of lymphocytes, the qualitative compositions were similar with polypeptides of M_r 45000 and 68000 as major components. The 45000- M_r polypeptide is most probably actin, which is a major component of lymphocyte plasma membrane (Barber & Crumpton, 1976).

The present results resemble in general those of a previous study (Mescher *et al.*, 1981) in which purified plasma membrane of mouse P815 (a mastocytoma) and thymoma EL4 cells were extracted with Nonidet P-40. The most notable differences concern the larger amount of membrane protein that was insoluble (about 30%) and the less-discrete polypeptide composition of the residue. However, it appears likely that the major polypeptides of M_r 70000, 69000, 42000, 38000 and 36000 are identical with those of M_r 68000, 45000, 33000 and 28000 described in the present study. The above differences may be related to the more rigorous conditions used to sediment the insoluble fraction (4.5×10^6 g-min compared with 10^6 g-min) and the consequent capture of more dispersed particles. In contrast, these collective results show little resemblance to those reported for the Nonidet P-40-insoluble residue of the plasma membrane of mouse T lymphoma BW 5147 cells (Bourguignon *et al.*, 1982). In the latter study the lack of lipid vesicles is notable, as also is the presence of 5–7 nm filament bundles containing actin, myosin and a spectrin-related protein.

By analogy to the studies on the erythrocyte cytoskeleton (Branton *et al.*, 1981; Gratzner, 1981), the

Nonidet P-40-insoluble complex derived from lymphocyte plasma membrane may represent a sub-membranous cytoskeleton. Elucidation of the functional significance of this complex depends on a more detailed characterization of its component polypeptides. The accompanying paper describes the purification and properties of the major 68000- M_r polypeptide of the complex (Owens & Crumpton, 1984).

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