The phospholipid organisation in the membranes of McLeod and Leach phenotype erythrocytes

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The phospholipid composition, the distribution of phospholipids over the two membrane layers as well as the phosphatidylcholine-specific transfer protein-mediated exchangeability of phosphatidylcholine from the membrane, has been investigated in two types of abnormal erythrocytes – the McLeod phenotype and the Leach phenotype. The acanthocytic McLeod cells appeared to have a normal phospholipid composition and distribution, but the exchangeability of phosphatidylcholine was found to be markedly enhanced. Unlike control erythrocytes, in which 75% of all of the phosphatidylcholine can be exchanged during an 8 h incubation, the McLeod cell showed a complete exchange of this phospholipid within the same time period. This obviously indicates an enhanced transbilayer mobility of phosphatidylcholine in the membrane of McLeod cells. Erythrocytes of the Leach phenotype showed an elliptocytic shape and increased osmotic fragility, but no abnormalities were observed as to the composition and organisation of the phospholipid complement of their membranes.

Erythrocyte shape *Erythrocyte membrane* *Phospholipid distribution* *Phosphatidylcholine exchange* *Osmotic fragility*

1. INTRODUCTION

The specific composition and organisation of the lipid bilayer together with the numerous transmembrane proteins and its underlying cytoskeleton form the complex erythrocyte membrane. Evidence is accumulating that most of the individual constituents are indispensable for the maintenance of the stability, permeability and overall shape of the cell. This evidence has been obtained both from studies on erythrocytes which have been modified in vitro and from analyses carried out on naturally occurring abnormal cells [1]. Several examples of the latter group of erythrocytes have abnormal blood group antigen expression. In the Rh_{null} phenotype, the loss of the antigens of the Rhesus blood group system (as a result of the loss of two or more membrane proteins) is accompanied by changes in membrane permeability, increased osmotic fragility and abnormal cell shape and also results in a distortion of membrane phospholipid organisation [2,3,19]. This study deals with two other red cell types which have abnormal expression of blood group antigens: the McLeod phenotype and the Leach phenotype. In McLeod erythrocytes, the Kell antigens are weakly expressed, and the cells show an acanthocytic morphology, a minor increase in osmotic fragility, and a decrease in water permeability [4–7]. Cells of the Leach phenotype lack 3 minor sialoglycoproteins (β, β_1 and γ [8]), and also the Gerbich blood group antigen. A proportion of these cells are elliptocytic [8].

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2. MATERIALS AND METHODS

Erythrocytes of the McLeod phenotype from donors R.F. and R.E, erythrocytes from Leach phenotype donor P.L. and control normal erythrocytes were drawn in ACD anticoagulant. Erythrocytes were isolated by centrifugation for 10 min at 2500 × *g* and washed 3 times with 4 vols of 150 mM NaCl. Osmotic fragility was determined as described [9]. Cells were suspended in a buffer containing 10 mM Tris-HCl (pH 7.5), 10 mM $CaCl_2$, 0.25 mM $MgCl_2$ and 150 mM NaCl for phospholipase incubations. For the phosphatidylcholine exchange experiments the buffer contained 150 mM NaCl, 25 mM glucose, 1 mM EDTA, 100 IU penicillin/ml, 100 μg streptomycin/ml and 10 mM Tris-HCl, pH 7.4. Incubations with phospholipase A_2 and sphingomyelinase C from *Staphylococcus aureus* were carried out as described [10], except that in addition to the phospholipase A_2 from *Naja naja*, an equal amount (enzyme units) of bee venom phospholipase A_2 was present in the incubation mixtures.

Following the incubation, phospholipids were extracted according to Rose and Oklander [11] and analysed by thin-layer chromatography [12] and phosphate analysis [13]. Exchange experiments were carried out as in [14] using [^{14}C]phosphatidylcholine-labelled microsomes from rat liver as donor system.

3. RESULTS AND DISCUSSION

Alterations in individual membrane constituents, as is the case in the Leach and McLeod phenotypes studied here, can have far-reaching consequences on the overall properties of the cell membrane. Various parameters can be tested to illustrate this and in our studies we examined: (i) the osmotic fragility of the cells as an indication of membrane integrity; (ii) the overall shape of the erythrocytes; (iii) the phospholipid composition and distribution and (iv) the transbilayer mobility of the phosphatidylcholine.

Red cells of the McLeod phenotype show decreased osmotic water permeability [6] but their osmotic fragility is close to normal, as shown in fig.1. A clearly abnormal haemolysis pattern is shown for cells of Leach phenotype which are more fragile since they lyse at higher tonicity values than the control cells (fig.1).

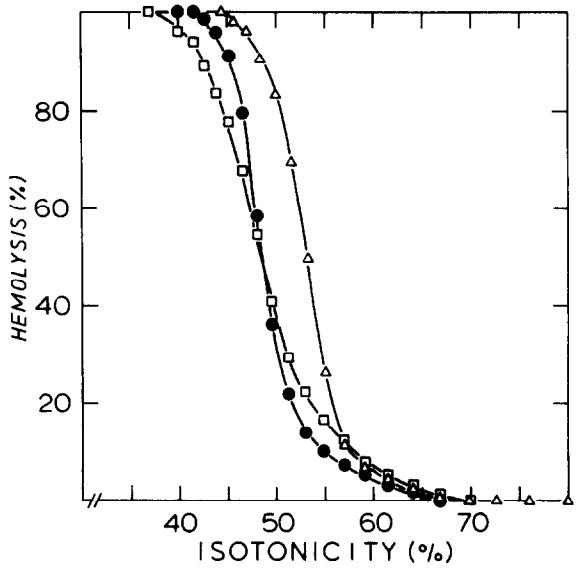

Fig.1. Osmotic fragility of control and abnormal erythrocytes. A continuous dilution procedure [9] was used to measure the osmotic fragility of normal (●), McLeod (□) and Leach (△) erythrocytes. Data are plotted as percent haemolysis vs osmolarity, expressed as a percentage of iso-osmolarity (150 mosM).

The overall shape of the two abnormal cells has been studied and the results confirm earlier observations [6–8]. About 10% of the Leach phenotype cells were elliptocytes, whereas 30% of McLeod phenotype cells showed an acanthocytic morphology. How these abnormalities in overall cell shape are correlated with defects in membrane structure is not clear. Comparison with other cell types in which integral membrane proteins are lacking may be relevant. Cells of the Leach phenotype lack 3 minor sialoglycoproteins and have abnormal shape. In contrast, abnormal cell shape has not been reported for either En(a−) erythrocytes which lack the major sialoglycoprotein α (glycophorin A) or homozygous M^k erythrocytes which lack sialoglycoproteins α and δ (glycophorin B) [15,16]. The differences could be due to the fact that the 3 minor sialoglycoproteins β, β_1 and γ are normally bound to the cytoskeleton, whereas sialoglycoproteins α and δ are not [17,18]. Thus, these 3 minor sialoglycoproteins may contribute to proper membrane-protein skeleton interaction and help to confer a discoid

shape on the cell. In an analogous manner to cells of the Leach phenotype, Rh_{null} cells lack one or more membrane proteins which are attached to the cytoskeleton and also have an abnormal shape [2,19]. How cells of the McLeod phenotype fit into this pattern remains obscure, since the precise defect in these cells is not known.

Evidence is accumulating that erythrocytes showing an abnormal shape may also exhibit an enhanced mobility of their membrane phospholipids [20,21]. Analyses of the total phospholipid content and overall phospholipid composition of cells of the McLeod and Leach phenotypes as well as their cholesterol/phospholipid ratios did not reveal any significant abnormalities when compared to normal erythrocytes (not shown). Incubations with phospholipase A_2 and/or sphingomyelinase C resulted in both cases in the hydrolysis of 80% of the sphingomyelin, 75–80% of the phosphatidylcholine, 20–30% of the phosphatidylethanolamine and none of the phosphatidylserine. These results are very similar to those found with normal red cells [22]. These observations indicate that the specific asymmetric distribution of erythrocyte phospholipids is maintained in these two abnormal cell types, despite their abnormal shape. Finally, a test was performed to estimate the transbilayer mobility of the membrane phospholipids, in particular phosphatidylcholine. This lipid, 75% of which is present in the outer layer of the erythrocyte membrane, can be exchanged for other phosphatidylcholine molecules originating from an exogenous donor system by using a specific transfer protein [14]. In fig.2, it is shown that in control cells 75% of the phosphatidylcholine is exchangeable and a similar value is found for the Leach phenotype. The clear plateau in the exchange profile shows that the residual 25% of this phospholipid present in the inner membrane layer does not become readily accessible for exchange. This implies that transbilayer mobility of the phosphatidylcholine in the Leach phenotype cells is at least as slow as that in the normal red cells [14].

A different result is obtained with the McLeod cells in which the transbilayer mobility of phosphatidylcholine is enhanced to the extent that all of this lipid is exchangeable at a relatively fast rate and in this respect the McLeod cells resemble the Rh_{null} cells [3].

Table 1 summarises the data presented here and

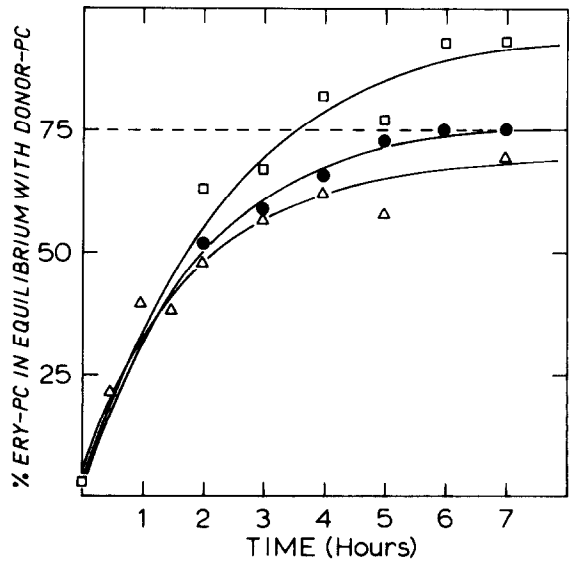

Fig.2. Exchange of erythrocyte phosphatidylcholine. Cells were incubated with phosphatidylcholine-specific exchange protein and microsomal membranes as the [^{14}C]phosphatidylcholine donor. The extent of exchange, expressed as percent of the total phosphatidylcholine present in the erythrocyte, was determined at various time points for control (●), McLeod (□) and Leach (△) cells. The horizontal broken line shows the amount (75%) of phosphatidylcholine which is present in the outer layer of the erythrocyte membrane.

those obtained previously for other phenotypes – the En(a−) phenotype and the Rh_{null} phenotype. The conclusions which may be drawn from these studies are as follows: (i) a loss of particular integral membrane proteins can result in a modification in the organisation of the lipid bilayer; (ii) in the case of En(a−) erythrocytes where such a loss is not accompanied by changes in cell morphology no abnormalities in phospholipid distribution are found; (iii) absence of particular integral membrane proteins associated with abnormal cell morphology, viz. acanthocytes. (McLeod) stomatocytes (Rh_{null}) or elliptocytes (Leach), can have a variety of effects on the phospholipid bilayer. These range from effects on both phospholipid distribution and enhanced transbilayer phospholipid movement (Rh_{null} cells), enhanced bilayer movement (McLeod) to no detectable effect (Leach).

Table 1

Some characteristics of four types of human erythrocytes having an antigenic abnormality

Cell type	Antigenic abnormality	Morphology	Osmotic fragility relative to normal cells	Biochemical membrane lesion	Organisation of the lipid bilayer	
					Phospholipid distribution	Percentage of PC exchangeable
Rh_{null}	Absence of antigens of the Rhesus blood group system	Stomatocytes	Increased	Integral protein(s) bound to membrane skeleton absent	Abnormal with respect to PE	100
McLeod	Weak expression of antigens of the Kell blood group system	Acanthocytes	Normal		Normal	100
Leach	Gerbich antigen absent	Elliptocytes	Increased	Minor sialoglycoproteins bound to membrane skeleton absent	Normal	75
En(a−)	MN antigens absent	Normal discocytes	Normal	Glycophorin A absent	Normal	n.d.

PC, phosphatidylcholine, PE, phosphatidylethanolamine; n.d., not determined; osmotic fragility is defined as the tonicity of the buffer at which 50% of the cells are haemolysed

Although detailed interpretation of these results is not possible at this stage they suggest that some of the minor membrane proteins, such as those expressing Rhesus blood group antigens, specifically interact with the bilayer phospholipids, but that abnormal cell shape is not always correlated with alterations of the lipid bilayer. Lipid bilayer destabilisation has also been observed in other cells. Erythrocytes which show defined abnormalities in the organisation of the skeletal network and/or its interaction with the membrane bilayer, i.e., spherocytes [23], sickle cells [20,21], and pyropoikilocytes (Franck et al., submitted). Thus a destabilisation of the lipid bilayer can be due to a defect in its interaction with the membrane skeleton, but the data obtained so far are not sufficient to allow a full understanding of how the absence of particular (minor) integral membrane proteins causes changes in cell shape and organisation of phospholipids in the membrane bilayer.

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