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Influence of altered phospholipid composition of the membrane outer layer on red blood cell aggregation: relation to shape changes and glycocalyx structure

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Abstract. Reversible aggregation of erythrocytes was investigated after alteration of the phospholipid content in the membrane outer leaflet either by disturbance of endogenous transmembrane lipid asymmetry through changes in cellular free calcium, or by incorporation of exogenous lyso-derivatives. It was found that both calcium loading and lyso-phosphatidylcholine (LPC) addition induce a strong increase in red cell-red cell adhesive energy, whereas lyso-phosphatidylserine (LPS), added in the same amount as LPC, does not. Red cell morphological studies show differences in the shape change efficiency of LPS, LPC and calcium loading. However, it was further demonstrated that shape change is not directly responsible for the observed adhesive energy increase, since neuraminidase or trypsin treatment abolish this increase, even though the shape changes induced by alteration of phospholipid organization are not affected. The latter experiment strongly suggests that the red cell adhesive energy increase results from an alteration of the glycocalyx structure, which could be in turn a consequence of the shape change.

Key words: Erythrocytes – Aggregation – Phospholipid – Calcium – Red cell shape

I. Introduction

Alteration of phospholipid asymmetry across the red cell membrane affects adhesion and recognition of red cells by other cells (Schwartz et al. 1985; Schlegel et al. 1985; McEvoy et al. 1986; Schlegel and Williamson 1987). It has even been suggested that modifications of phospholipid asymmetry, in pathological or aged red cells, could be a signal for their clearance by splenic macrophages (McEvoy et al. 1986).

On the other hand, red blood cells also interact with one another in plasma, thereby aggregating to form reversible "rouleaux" (Fahraeus 1929; Chien and Jan 1973; Chien et al. 1984). Even though the exact mechanism of this red cell aggregation is still controversial, it involves the plasma proteins and the state of the cell membrane external surface (Mohandas and Evans 1984; Othmane et al. 1986). In particular, it has been demonstrated that pathological increases of red cell aggregation, which may play a role in the onset of vaso-occlusive syndromes, are due to plasmatic or membrane factors, according to the disease (Mohandas and Evans 1984; Friederichs et al. 1984; Othmane et al. 1989). The said membrane factors are thought to be predominantly linked to the structure of the glucidic coating of the membrane (or glycocalyx), for instance through the negative charges carried by sialic acids (Brooks 1973; Snabre and Mills 1986a). In contrast, little is known about the role of phospholipid organization in erythrocyte aggregation. An alteration in this organization might act either directly (by analogy with adhesion of red cells with other cells (Schwartz et al. 1985) or indirectly, through a modification of either the red cell shape or the lateral distribution of the glycoproteins on the red cell surface.

In this paper, we investigate the effect on erythrocyte aggregation of a disturbance of phospholipid composition in the membrane outer leaflet. This disturbance is induced either by altering the transbilayer asymmetry of endogenous phospholipids (through calcium loading of the cell cytoplasm, see Williamson et al. 1985; Chandra et al. 1987; Bitbol et al. 1987), or by incorporating exogenous lyso-phospholipids in the outer lipid layer.

II. Material and methods

1. Material

Buffers and solutions

Two buffers were used: 140 m*M* NaCl, 10 m*M* Hepes pH 7.4 (Buffer A) and 70 m*M* KCl, 70 m*M* NaCl, 1 m*M* EGTA pH 7.4 (Buffer B).

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid – EGTA, (Ethylenebis(oxyethylenenitrilo)tetraacetic acid) – DMSO, dimethyl-sulfoxide

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Dextran Solutions. Dextran T80 or T500 (Pharmacia) were dissolved in Buffer A or B, at concentrations ranging between 0.75 and 5% (w/v).

Fibrinogen Solutions. Lyophilized fibrinogen (Kabi Vitrum) was first dialysed in order to eliminate associated salts. It was then adjusted to concentrations ranging between 5 and 16% (w/v) in Buffer B.

Erythrocytes

Fresh human blood was obtained from healthy volunteers. Blood collected on EDTA was washed three times in Buffer A. The erythrocytes were then suspended at 45% hematocrit in various dextran and fibrinogen solutions.

Calcium loading

The erythrocyte suspensions (in Buffer B + fibrinogen or dextran) were incubated for 45 min at 37 °C in the presence of 20 μ*M* A23187 ionophore (from a 5 m*M* stock solution in DMSO-Ethanol 2: 1 v/v), and of various amounts of added $CaCl_2$, ranging between 0 and 1 m*M* in the suspending medium. Under these conditions, one can estimate that the intracellular free calcium concentration ranges between 0 and 20 μ*M* (see Bitbol et al. 1987 for instance).

Incorporation of exogenous lipids

Lyso-phosphatidylcholine and lyso-phosphatidylserine were prepared from egg phosphatidylcholine and from brain phosphatidylserine respectively, following Hubbel and McConnell (1971), and were dissolved in chloroform-methanol 2: 1 v/v. Desired amounts of the organic solution were dried in tubes and erythrocyte suspensions were added and gently stirred in these tubes at room temperature. Measurements bearing on these suspensions were performed within 20 min in order to avoid substantial reorientation of the incorporated lysophospholipids (Bergmann et al. 1984). The concentration in lysophospholipids are expressed in μmol/litre packed cells.

Treatment with trypsin or neuraminidase

In some experiments, the washed erythrocytes were preincubated in trypsin or neuraminidase solutions. The trypsin solution was made of 50 mg trypsin in 100 ml buffer A. The neuraminidase solution (1 U/ml) was made of 5 U neuraminidase dissolved in 5 ml of acetate buffer (50 m*M* acetate, 150 m*M* NaCl, 9 m*M* $CaCl_2$, 0.5% w/v bovine serum albumin). Treatment by trypsin involved a 10 min incubation of erythrocytes in the trypsin solution (at hematocrit 50% and at 37 °C), and then three washes in five volumes of buffer A. The neuraminidase treatment involved 90 minutes incubation of 1 ml erythrocyte pellet in 2 ml buffer A + 60 μl neuraminidase solution at 37 °C, and then three washes in five volumes of buffer A. Total sialic acid content of treated erythrocytes and of washing supernatants was determined following incubation of the samples at 80 °C for 1 h with an equal volume of H_2SO_4. The thiobarbituric acid assay (Warren 1959) was then used. This allowed us to show that about 80% and 50% of the sialic acids were removed from the cell surface by neuraminidase and trypsin treatment, respectively.

2. Methods

Light reflectometry: The critical shear stress τ_c which is necessary to complete erythrocyte disaggregation was assessed at 25 °C by means of an optical technique (Mills et al. 1980; Mills and Snabre 1983; Snabre et al. 1987; Othmane et al. 1989). Briefly this technique measures the light flux back-scattered by an erythrocyte suspension contained in the gap of a Couette flow system, and submitted to various intensities of shear stress therein. The shear stress itself is determined by performing the product of the shear rate, and of the suspension viscosity measured on a Contraves LS 30 Couette viscometer. The critical shear stress τ_c, related to contact area, was shown to reflect directly the cell surface energy (adhesive energy per unit area) without significant dependence on cell deformability or cell volume fraction in the suspension (Snabre et al. 1987). It is especially important for the present study to notice that the sensitivity of the light reflectometry technique decreases when red cell rigidity increases. Indeed, rigid red cells cannot deform themselves and thus, the contact area decreases with cell rigidity. Since it is just the variation of contact area per unit volume of the suspension which is assessed by light reflectometry, one may expect that above a certain threshold of cell rigidity, it will be impossible to detect any significant change in the reflectivity signal due to RBC aggregation. It will thus be necessary to limit observations to a range of phospholipid organization disturbance within which the cell deformability is not dramatically reduced.

Cell electrophoresis: The erythrocyte suspensions were diluted in suspending medium to a 0.01% hematocrit. Electrophoretic mobility measurements were performed at 25 °C with a device (SEFAM Company Nancy, France) taking advantage of Laser Doppler Velocimetry (Malher et al. 1983) and giving in a few seconds a mobility averaged on several thousands of cells. Viscosity measurements of the cell suspending media were performed on the same viscometer as above.

Erythrocyte morphology: Erythrocytes prepared as for the other measurements were diluted and fixed by transferring 10 μl aliquots of a 10% hematocrit suspension in 10 μl of 1% of glutaraldehyde in buffer A. The shape indexing and averaging were performed according to Daleke and Huestis (1985). Crenated cells (echinocytes) were given scores of +1 to +5, discocytes were given a score of 0 and cupped or invaginated cells were given

scores of −1 to −4: the average score of approximately 70 to 150 erythrocytes was taken as the "mean shape index" or MSI.

III. Results

1. Critical shear stress of red cell disaggregation

Influence of calcium loading: The effect of increasing the intracellular free calcium concentration, by addition of $CaCl_2$ and ionophore, was studied in the presence of dextran T80 (Fig. 1), dextran T500 (Fig. 2) or fibrinogen (Fig. 3). In all cases τ_c increases with $CaCl_2$ concentration. The results (not shown) for higher concentrations of dextran (T80 and T500) display the same trend as in Figs. 1 and 2: τ_c again increases with $[CaCl_2]$. The latter results are not plotted in Figs. 1 and 2 for reasons of clarity: whereas between 0 and 3.5% for dextran T80 or between 0 and 5% for dextran T500, τ_c increases with increasing polymer concentration, it decreases again for higher concentrations (Daleke and Huestis 1985; Snabre and Mills 1986b). Because of the progressive loss of sensitivity of the light reflectometry technique (see methods), $CaCl_2$ concentration was kept below 1 m*M*. Control experiments (not shown) demonstrated that the presence of ionophore or $CaCl_2$ alone has no effect on RBC shape or RBC aggregation in the range of concentrations used above.

Influence of lysophospholipid incorporation: The effect of lysophospholipids incorporated in the outer face of the bilayer on τ_c depends very strongly on their polar head (see Fig. 4). τ_c increases steeply with lyso-phosphatidylcholine concentration, at least above 50 μmol/litre packed cells, whereas it does not depend at all on the concentration of lyso-phosphatidylserine from 0 to 150 μmol/litre packed cells. Other experiments (not shown in Fig. 4) at higher LPS concentrations (150–250 μmol/l) show no variation in disaggregation shear stress.

Trypsin and Neuraminidase treatment: One can see from Fig. 4 that trypsin or neuraminidase treatment increase

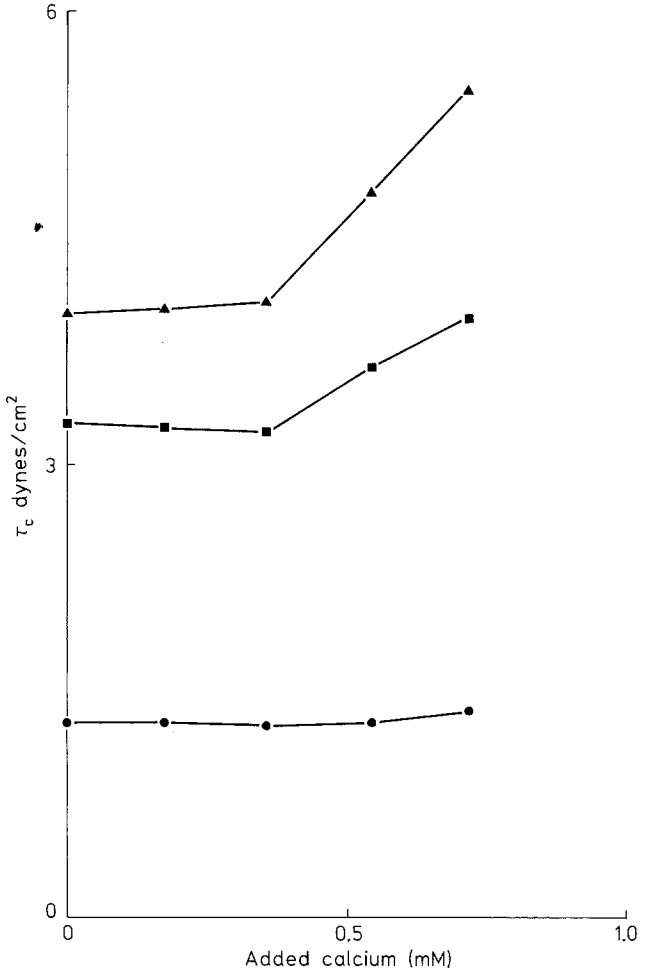

Fig. 1. Critical shear stress of red cell disaggregation versus added $CaCl_2$, in the presence of dextran T80, ionophore, and EGTA (1 m*M*). Three concentrations of dextran T80 were used: ● 1.5%; ■ 2.5%; ▲ 3.5% (w/v). The standard deviation was determined from 4 experiments. It is smaller than the size of the symbols

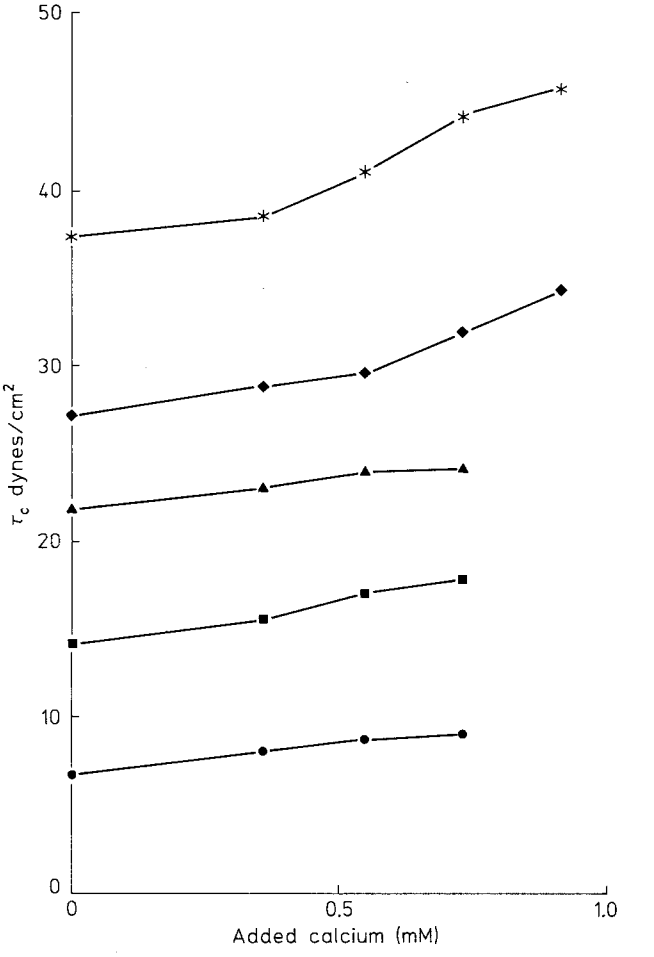

Fig. 2. Critical shear stress of red cell disaggregation versus added $CaCl_2$, in the presence of dextran T500, ionophore, and EGTA (1 m*M*). Five concentrations of dextran T500 were used: ● 0.75%; ■ 1.5%; ▲ 2.5%; ◆ 3.5%; ✳ 5% (w/v) (standard deviation, see Fig. 1)

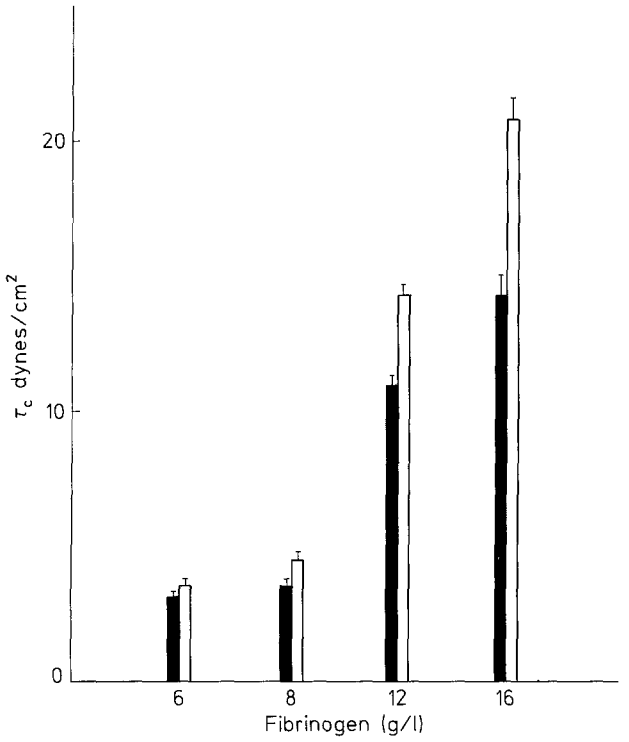

Fig. 3. Critical shear stress of red cell disaggregation versus fibrinogen concentration, without (black columns) and with (white columns) 0.55 m*M* added $CaCl_2$, in the presence of ionophore and EGTA (1 m*M*). Bars indicate the standard deviation

the value of τ_c, and abolish the lyso-phosphatidylcholine-induced increase in RBC adhesiveness. The calcium loading induced increase of RBC adhesiveness is also abolished by trypsin or neuraminidase treatment (result not shown).

2. *Morphology*

Calcium loading: Progressive alteration of red cell shape, towards crenated forms, is observed upon calcium loading (Fig. 5a).

Lyso-phospholipid incorporation: Incorporation into the outer membrane layer of both lyso-derivatives yielded red cell crenation (Fig. 5b). The striking fact here is a lower crenating efficiency of lyso-phosphatidylserine when compared to lyso-phosphatidylcholine. On the other hand, neither trypsin nor neuraminidase treatment influenced the shape change induced by lyso-phospholipids.

3. *Cell electrophoresis*

Electrophoretic mobility of erythrocytes in various suspending media shows no significant variation either when lyso-phospholipids are incorporated in the membrane outer layer or under calcium loading.

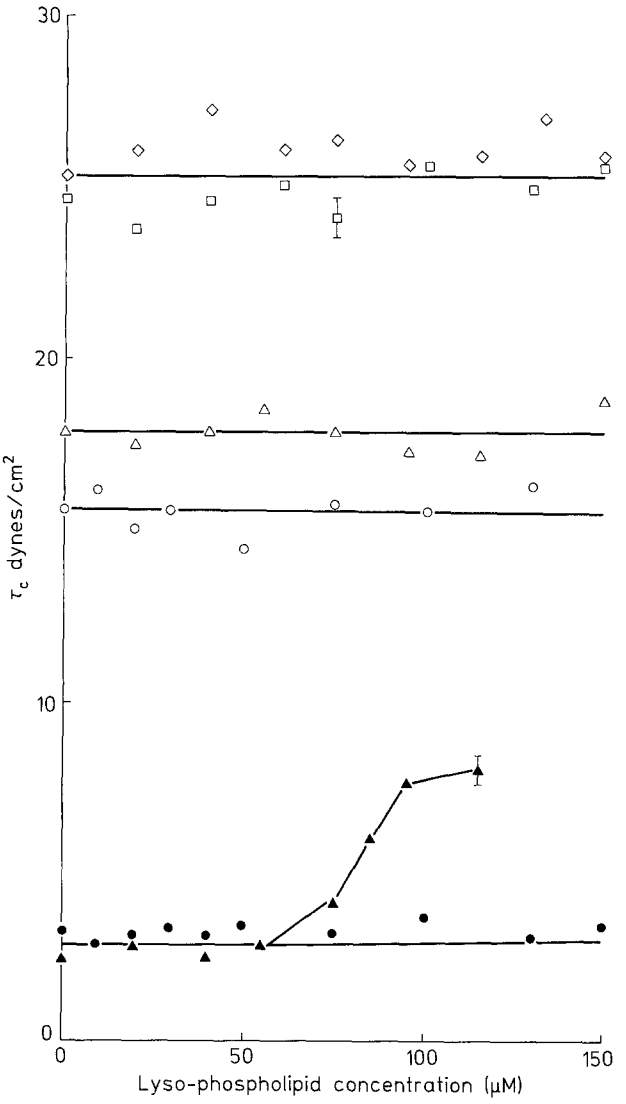

Fig. 4. Critical shear stress of red cell disaggregation versus added lysophospholipids, in the presence of dextran T80 2.5% (w/v). ● untreated cells + LPS; ▲ untreated cells + LPC; □ neuraminidase-treated cells + LPS; ◇ neuraminidase-treated cells + LPC; ○ trypsin-treated cells + LPS; △ trypsin-treated cells + LPC (standard deviation, see Fig. 1)

IV. Discussion

Calcium loading of the erythrocytes increases the critical shear stress τ_c, necessary to disaggregate the blood suspension. The presence of Ca^{2+} at the outer face of the membrane is not responsible for the increase of τ_c, since in the absence of ionophore no effect of calcium was detected. The effect of calcium is thus related to its presence inside the cell. Yet, to account for a modification of adhesive properties of the cells, the presence of free calcium within the cell must have consequences on the state of the outer face of the membrane.

One of the most striking modifications of the red cell membrane upon calcium loading is a loss of phospholipid asymmetry (Willamson et al. 1985; Chandra et al. 1987; Bitbol et al. 1987) leading to a relocation of predominantly intracellular phosphatidylserine and phosphat-

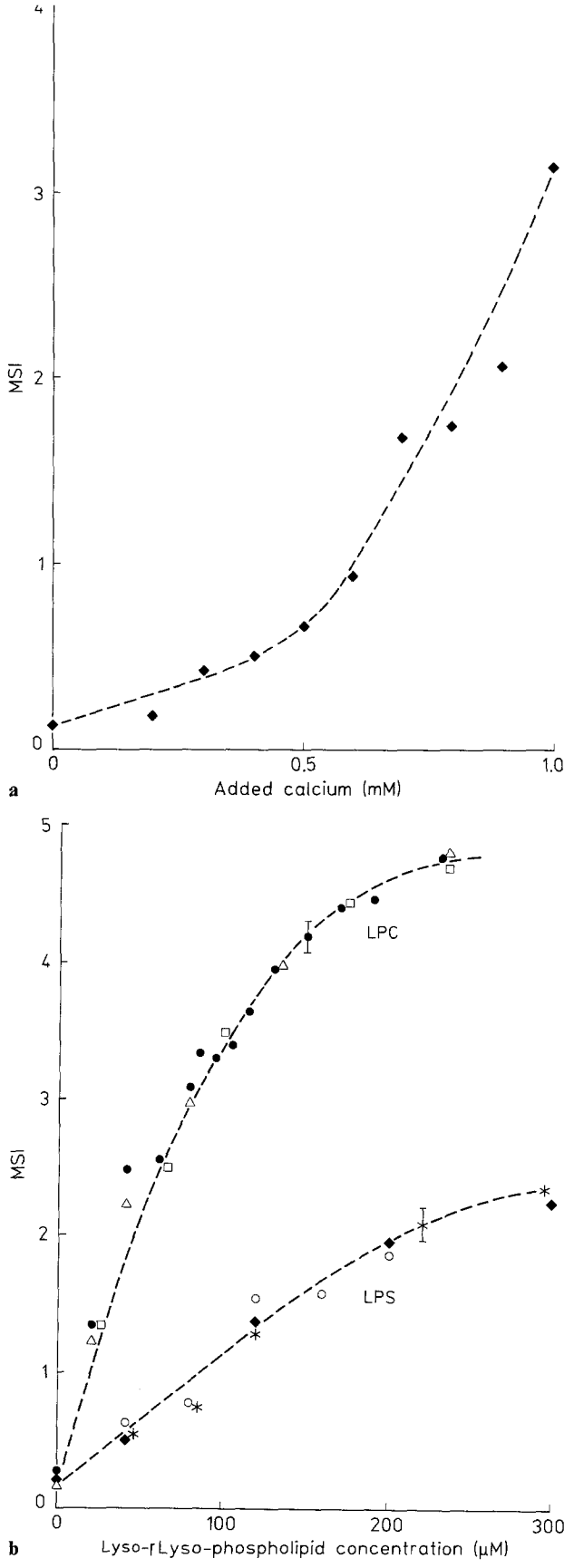

Fig. 5. **a** Mean Shape Index of the erythrocytes versus added $CaCl_2$ in the presence of ionophore and of EGTA (1 m*M*) (standard deviation, see Fig. 1). **b** Mean Shape Index of the erythrocytes versus added lyso-phospholipids. ○ untreated cells + LPS; ● untreated cells + LPC; ◆ neuraminidase-treated cells + LPS; □ neuraminidase-treated cells + LPC; ✳ trypsin-treated cells + LPS; △ trypsin-treated cells + LPC

idylethanolamine towards the outer face of the lipid bilayer. Completion of the phospholipid symmetrization was obtained after 45–60 min of incubation at 37 °C, in the presence of calcium and ionophore (Chandra et al. 1987), corresponding to the time of calcium loading of the cytoplasm. At low concentrations of intracellular free calcium up to 20 μ*M*, this relocation was related (Bitbol et al. 1987) to the inhibition of the ATP-dependent aminophospholipid translocase (Seigneuret and Devaux 1984; Zachowski et al. 1986) which, in physiological conditions, compensates inside-outside leakage by moving the aminophospholipids from the outer to the inner membrane monolayer. It could thus be tempting, at this stage of the discussion, to assume that the observed enhancement of τ_c is directly produced by changes in polar heads of the outer face of the lipid bilayer, especially through the increase in negatively charged phosphatidylserine. Such an assumption is supported by an analogy with monocyte-erythrocyte adhesion, where the adhesive process is enhanced by exogenous phosphatidylserine enrichment of the erythrocyte membrane outer face (Schwartz et al. 1985). But our results concerning the effect of incorporating lyso-phosphatidylcholine (LPS) or lyso-phosphatidylserine (LPS) on red cell aggregation are in sharp contradiction with the previous interpretation: incorporation of LPC whose polar head is predominant in the outer layer of red cell membranes (Verkleij et al. 1973) enhances aggregation, whereas incorporation of similar amounts of LPS does not. This absence of an effect of LPS cannot be due to its relocation on the inner membrane layer by the aminophospholipid translocase during the time course of the experiment (20 min) as the half-time of this phenomenon is approximately 3,5 h (Bergmann et al. 1984). Another explanation could be that LPS did not incorporate quantitatively in the membrane, as the shape change induced by LPS was always smaller than the one resulting from LPC addition, for the same amount in the incubation. This can be ruled out by assaying the amount of lyso-derivatives present in the supernatant after centrifugation of such treated cells, showing that more than 95% of the added LPS (or LPC) pelleted with the erythrocytes.

Another possibility is that aggregation enhancement is more or less directly related to red cell crenation (Meiselman 1978). Calcium loading is known to produce crenation of red cells or ghosts (Weed and Chailley 1973) (Fig. 5a displays this effect under our experimental conditions). Incorporation of amphipaths which remain predominantly at the outer face of their membrane also leads to red cell crenation, by the mechanism of bilayer couple (Sheetz and Singer 1974). The crenating effect of such an amphipath (LPC) described here has already been documented (Bierbaum et al. 1979). Figure 5b also demonstrates that LPS has a crenating effect, even though much less pronounced than LPC. As a partial insertion of LPS in the extra-cellular leaflet of the membrane cannot be put forward (see above) one might suggest that the difference between LPS and LPC crenating power could be related to the fact that the bulk of choline is large when compared to other phospholipid polar heads (Cevc and Marsh 1987).

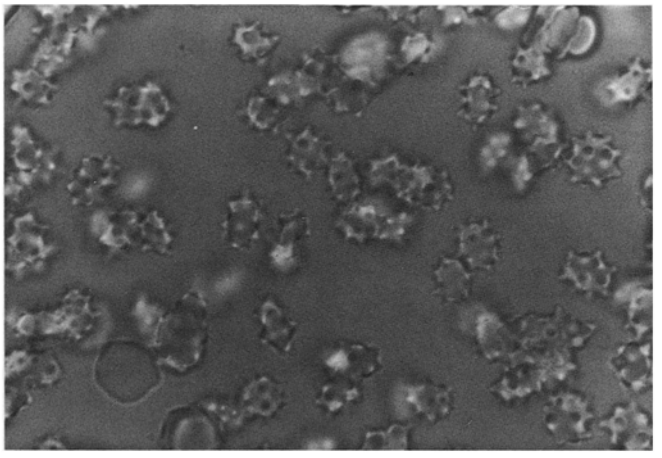

Fig. 6. Light microscopy photograph of crenated red blood cells in buffer containing 2.5% dextran and LPC (80 vmol/liter of packed cells

Data from Fig. 5 thus tend to support the possibility of a relationship between red cell shape change and aggregation, as the enhancement of τ_c occured at 50 μmol/l packed cells of LPC incorporated in the outer layer of the red cell membrane, a concentration where the mean shape index of the cells exceeds 2. However, when LPS is incorporated in the outer leaflet of the membrane bilayer instead of LPC:

(*i*) the mean shape index does not reach 2, up to 200 μ*M* of LPS;

(*ii*) τ_c does not change at all, up to 150 μ*M*.

It is thus tempting to assume that the erythrocytes must get over a certain threshold of crenation before their aggregation is enhanced. This threshold is reached at an LPC concentration of 50 μmol/l packed cells, whereas it is not reached in the range of LPS concentrations we studied. In the case of calcium loading, however, aggregation enhancement starts at concentrations of added $CaCl_2$ which correspond to a lower mean shape index of about 1.5. It is well known that with normal erythrocytes, aggregates are long cylindrical rouleaux, in which cells adhere face to face and resemble coins in a stack. The structure of RBC rouleaux directly reflects the membrane mechanics of the red cells and depends upon the cell surface adhesive energy (Chien et al. 1973, Tilley et al. 1987). However, echinocytic cells tend to form more isotropic structures (Fig. 6). Light microscopic observations of aggregated crenated cells seem to indicate that contact areas at spicules or at concave membrane surfaces are both possible. However, the critical shear stress for complete RBC disaggregation was clearly shown to characterize cell adhesiveness without significant dependence upon cell shape (Snabre et al. 1987).

Moreover, another fact precludes that the τ_c increase is a *direct* effect of crenation. The action of neuraminidase or trypsin on the erythrocytes increases the overall level of τ_c, but suppresses any effect of LPC on τ_c, although it does not alter the shape change produced by LPC.

Two non-exclusive possibilities remain open to discussion, namely:

(*i*) That red cell crenation acts *indirectly* on aggregation, for instance through alterations of the microscopic distribution of glycoproteins whose extracellular glucidic components constitute the glycocalyx. It was indeed demonstrated that crenation causes clustering of negative surface charges bound to sialyl residues (Marikovski et al. 1978; 1985). This suggests that cell adhesiveness between crenated cells could be increased owing to reduction of electrostatic repulsions at contact areas. Our results on cell electrophoresis do not necessarily disprove this structural change in the glycocalyx, since a microscopic lateral redistribution of the charges could well have no macroscopic electrophoretic consequence. As a first approximation, a lateral redistribution of glycoproteins with no change in the number of negative charges at the outer face of the cell glycocalyx has no effect on cell electrophoretic mobility) (Snabre and Mills 1986a).

(*ii*) That phospholipid distribution acts indirectly, again through the microscopic distribution of the glycocalyx. In this case, it would be necessary to understand why adding LPC dramatically alter this distribution whereas negatively charged LPS have only minor effects.

Redistribution of the glycocalyx might change the surface adhesive energy of polymer-mediated erythrocyte aggregation, according to the two recent theories on this phenomenon. From the standpoint of polymer bridging theory (Chien et al. 1984; Snabre and Mills 1985; 1986a, b), a different distribution of the glucidic residues is likely to modify the adsorption sites of the polymer and the characteristics of the local electrostatic repulsion potential between facing membranes. From the standpoint of the depleted layer theory (Gast and Leibler 1986), a lateral glycoprotein redistribution could enhance the exclusion of polymer chains from the glycocalyx, and thus modify the depletion profile near the surface membrane (Snabre et al. 1989). The increase of RBC adhesiveness induced by calcium-loading or by incorporation of exogenous lyso derivatives in the membrane would then be the consequence of a stronger polymer depletion at the cell surface. Finally, the results, showing that aggregation enhancement is no longer observed on LPC incorporation after RBC treatment with trypsin or neuraminidase confirm the role of the glycocalyx structure in polymer induced cellular interactions.

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References

Bergmann WL, Dressler V, Haest CWM, Deuticke B (1984) Reorientation rates and asymmetry of distribution of lyso-phospholipids between the inner and outer leaflet of the erythrocyte membrane. Biochim Biophys Acta 772:328–336

Bierbaum TJ, Bouma SR, Huestis WH (1979) A mechanism of erythrocyte lysis by lysophosphatidylcholine. Biochim Biophys Acta 555:102–110

Bitbol M, Fellmann P, Zachowski A, Devaux PF (1987) Ion regulation of phosphatidylserine and phosphatidylethanolamine outside-inside translocation in human erythrocyte. Biochim Biophys Acta 904:268–282

Brooks DE (1973) The effect of neutral polymers on the electrokinetic potential of cells and other charged particles. IV. Electrokinetic effect in dextran-mediated cellular interaction. J Colloid Interface Sci 43:714–726

Cevc G, Marsh D (1987) Phospholipid bilayers. Physical principles and models. Wiley, New York

Chandra R, Joshi PC, Bajpai VK, Gupta CM (1987) Membrane phospholipid organization in calcium-loaded human erythrocytes. Biochim Biophys Acta 902:253–262

Chien S, Jan KM (1973) Ultrastructure basis of the mechanisms of rouleaux formation. Microvasc Res 5:155–161

Chien S, Sung LA, Kim S, Burke AM, Usami S (1984) Quantitation of red cell aggregation under shear flow. Ann NY Acad Sci 416:190–206

Daleke DL, Huestis WH (1985) Incorporation and translocation of amino-phospholipids in human erythrocytes. Biochemistry 24:5406–5416

Fahraeus R (1929) The suspension stability of blood. Physiol Rev 9:241–258

Friederichs E, Germs J, Lakomek M, Winkler H, Tillmann W (1984) Increased erythrocyte aggregation in infectious diseases: Influence of "acute phase proteins". Clin Hemorheol 4:237–244

Gast AP, Leibler L (1986) Interactions of sterically stabilized particules suspended in a polymer solution. Macromolecules 19:686–691

Hubbell WL, McConnell HM (1971) Molecular motion in spin label phospholipids and membranes. J Am Chem Soc 93: 314–326

Malher E, Martin D, Duvivier C, Volochine B, Stoltz JF (1983) Les méthodes d'électrophorèse des cellules. In: Stoltz JF (ed) Techniques avancées en hémorhéologie, 603–623, DPIC-INPL, Nancy, France

Marikovski Y, Khodadad JK, Weinstein RS (1978) Influence of red cell shape on surface charge topography. Exp Cell Res 116: 191–197

Marikovski Y, Weinstein RS, Skutelsky E, Danon D (1985) Changes of cell shape and surface charge topography in ATP-depleted human red blood cells. Mech Ageing Dev 29:309–316

McEvoy L, Williamson P, Schlegel RA (1986) Membrane phospholipid asymmetry as a determinant of erythrocyte recognition by macrophages. Proc Natl Acad Sci 83:3311–3315

Meiselman HJ (1978) Rheologie of shape transformed human red cells. Biorheology 15:225–237

Mills P, Snabre P (1983) Agrégamétrie optique de la suspension sanguine. In: Stoltz JF (ed) Techniques avancées en hémorhéologie, 580–598, DPIC-INPL, Nancy, France

Mills P, Quemada D, Dufaux J (1980) Etude de la cinétique d'agrégation érythrocytaire dans un écoulement de Couette. Rev Phys Appl 15:1357–1366

Mohandas N, Evans EA (1984) Adherance of sickle erythrocytes to cells. Requirement for both cell membrane changes and plasma factors. Blood 64:282–287

Othmane A, Bitbol M, Snabre P, Thiam B, Mills P (1986) Etude comparative de l'agrégation érythrocytaire chez deux sujets ayant un groupe sanguin différent. Innov Tech Biol Med 7: 76–81

Othmane A, Bitbol M, Snabre P, Grimaldi A, Bosquet F (1989) Red-cell aggregation in insulin-dependent diabetics. Clin Hemorheol 9:281–295

Schlegel RA, Williamson P (1987) Membrane phospholipid organization as a determinant of blood cell-reticuloendothelial cell interactions. J Cell Physiol 132:381–384

Schlegel RA, Prendergast TW, Williamson P (1985) Membrane phospholipid asymmetry as a factor in erythrocyte-endothelial cell interactions. J Cell Physiol 123:215–218

Schwartz RS, Tanaka Y, Fidler IJ, Tsun-Yee Chu D, Lubin B, Schroit AJ (1985) Increased adherance of sickled and phosphatidylserine enriched human erythrocyte to cultured human peripheral blood monocytes. J Clin Invest 75:1965–1972

Seigneuret M, Devaux PF (1984) ATP-dependent asymmetric distribution of spin-labeled phospholipids in the erythrocyte membrane: Relation to shape changes. Proc Natl Acad Sci USA 81:3751–3755

Sheetz MP, Singer SJ (1974) Biological membranes as bilayer couples. A molecular mechanism of drug-erythrocyte interactions. Proc Natl Acad Sci USA 71:4457–4461

Snabre P, Mills P (1985) Effect of dextran polydispersity on red cell aggregation. Colloid Polym Sci 263:478–483

Snabre P, Mills P (1986a) Effect of dextran polymer on glycocalyx structure and cell electrophoretic mobility. Colloid Polym Sci 263:494–500

Snabre P, Mills P (1986b) Role des interactions électrostatiques dans les phénomènes d'agrégation érythrocytaire. In: Stoltz JF (ed) Hémorhéologie et agrégation érythrocytaire. Editions médicales internationales. Paris, pp 98–110

Snabre P, Bitbol M, Mills P (1987) Cell disaggregation behavior in shear flow. Biophys J 51:795–807

Snabre P, Mills P, Othmane A, Thiam BA (1989) Mechanisms of non specific aggregation and rheology of concentrated red blood suspension. Biorheology (in press)

Tilley D, Coakley WT, Gould RK, Hewison LA (1987) Real time observations of polylysine, dextran and polyethylene glycol induced mutual adhesion of erythrocytes held in suspension in an ultrasonic standing wave field. Eur Biophys J 14:499–507

Verkleij AJ, Zwaal RFA, Roelofsen B, Comfurius P, Kastelijn D, Van Deenen LLM (1973) The asymmetry distribution of phospholipids in the human red-cell membrane. A combined study using phospholipases and freeze-etching electron microscopy. Biochim Biophys Acta 323:178–193

Warren L (1959) The thiobarbituric acid assay of sialic acids. J Biol Chem 234:1972–1975

Weed RI, Chailley B (1973) Calcium-pH interactions in the production of shape change in erythrocytes. In: Bessis M, Weed RI, Leblond PF (eds) Red cell shape, physiology, pathology, ultrastructure. Springer, New York Berlin Heidelberg, pp 55–67

Williamson P, Algarin L, Bateman J, Choe HR, Schlegel RA (1985) Phospholipid asymmetry in human erythrocyte ghosts. J Cell Physiol 123:209–214

Zachowski A, Favre E, Cribier S, Hervé P, Devaux PF (1986) Outside-Inside translocation of aminophospholipids in the human erythrocyte membrane is mediated by a specific enzyme. Biochemistry 25:2585–2590