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A Set of Surface Proteins Common to the Circulating Human Platelet and Lymphocyte

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The surface proteins of the circulating human platelet and lymphocyte were labelled by using the lactoperoxidase iodination method. Polyacrylamide-gel electrophoresis showed that four corresponding labelled proteins are found on the surface of each cell type. The most intensely labelled protein contains little or no carbohydrate, but the remaining labelled proteins are all glycoproteins. The major labelled band from each cell was isolated and comparative peptide 'maps' showed that the two proteins are closely similar. The surface proteins of the lymphocyte and platelet are distinct from those on the erythrocyte, the remaining major type of circulating cell.

The plasma membrane of the human platelet has been isolated (Barber & Jamieson, 1970), and the proteins and glycoproteins of this membrane have been studied (Barber & Jamieson, 1971; Nachman & Ferris, 1972; Phillips, 1972). The plasma membrane of pig mesenteric lymph-node lymphocytes (Allan & Crumpton, 1971), calf lymph-node lymphocytes and calf and rabbit thymocytes (Schmitt-Ullrich *et al.*, 1974) have also been isolated and characterized.

The cell surface proteins of the platelet have been studied by using the lactoperoxidase method (Phillips, 1972). Similar studies have been done on the lymphocyte (e.g. Vitetta *et al.*, 1971), but these have been mainly concerned with the surface immunoglobulins of this cell, although Schmitt-Ullrich *et al.* (1974) have studied isolated labelled plasma-membrane fractions. In both cases labelling is restricted to the surface components of the intact cell (Phillips, 1972; Vitetta *et al.*, 1971).

In the present paper we compare the surface proteins labelled by iodination of circulating human platelets and lymphocytes. Our results suggest that, although they have specialized and functionally distinct plasma membranes, the two cells carry a common set of proteins at their surfaces.

Methods

Cells were prepared from fresh normal human blood containing heparin (20i.u./ml). The blood was diluted with phosphate-buffered saline (Dulbecco 'A'; Oxoid Ltd., London E.C.4, U.K.) containing 0.03M-adenosine to prevent aggregation of platelets (Zimmerman & Müller-Eberhard, 1973). Lymphocytes and platelets were separated on a Ficoll and Triosil mixture (Harris & Ukaejiofo, 1970). The platelets were obtained from the initial centrifugation of separated cells at 70–80*g*, the lymphocytes being sedimented and the platelets being left in the supernatant. The platelets were washed five times in phosphate-buffered saline containing 0.03M-adenosine, by centrifugation at 1500*g* for 15min. The lymphocytes were washed five times in phosphate-buffered saline by sedimentation at 70–80*g*. The final cell suspensions were counted on a Neubauer haemocytometer.

Iodination with lactoperoxidase was done on 1×10^8–5×10^8 platelets or 0.5×10^7–4×10^7 lymphocytes. The reaction was done in a total of 0.6ml of iso-osmotic phosphate buffer, pH7.4 (0.1M-Na_2HPO_4 titrated to pH7.4 with 0.15M-NaH_2PO_4), containing the sedimented cells, 3μM-KI, 0.0001% butylated hydroxytoluene, 1mCi of $Na^{125}I$ (carrier-free; The Radiochemical Centre, Amersham, Bucks., U.K.), 0.5mg of lactoperoxidase, 7mM-glucose and 0.01 unit of glucose oxidase (Hubbard & Cohn, 1972). The mixture was incubated at 26°C for 1h and the labelled cells were washed four times with 0.15M-NaCl.

Sodium dodecyl sulphate–polyacrylamide-gel electrophoresis, isolation of labelled protein and preparation of peptide 'maps' was done as described by Boxer *et al.* (1974). Molecular weights were determined by co-electrophoresis with the sample of a radioactive marker protein mixture containing ovotransferrin, bovine serum albumin, glutamate dehydrogenase, lactate dehydrogenase, penicillinase and haemoglobin. The marker protein mixture was labelled with $Na^{125}I$ by the chloramine-T method (Klinman & Taylor, 1969).

Results and discussion

Intact platelets and lymphocytes were ^{125}I-labelled by using lactoperoxidase. The proteins were separated on sodium dodecyl sulphate–polyacrylamide gels and the gels were stained for protein and radioautographed (Plate 1, *a–d*). The pattern of total cell

protein and of surface-labelled proteins obtained from the two cell types is similar. Four corresponding labelled bands are found in each preparation and these have apparent molecular weights of 135000, 120000, 110000 and 92000 [bands I, IIa, IIb and III respectively, based on the nomenclature of Phillips (1972) for the platelet proteins]. The same result has been obtained from four independent sets of preparations. Cell counts showed that a typical lymphocyte preparation contained 3% platelets and 1% erythrocytes and a corresponding platelet preparation contained 0.2% lymphocytes and no erythrocytes, so the common pattern of labelling is not due to cross-contamination of the cells. A small number of minor labelled bands are also found uniquely associated with each preparation, and an appreciable amount of radioactivity remains at the origin.

Phillips (1972) obtained a comparable pattern of surface-labelled proteins from human platelets, although he did not detect band IIb. His data, obtained by counting gel slices, do not completely resolve bands IIa and III. It is probable that we are able to distinguish component IIb by virtue of the higher resolution of direct radioautography. He also reported that bands I, IIa and III correspond to the three bands made visible on gels with a carbohydrate stain, and suggested that these are glycoproteins. Radioautography of gels of labelled platelets stained with periodic acid–Schiff base shows that the three carbohydrate-containing bands clearly correspond to bands I, IIa and IIb (Plate 1, *e* and *f*). The major labelled protein (III) probably contains little or no carbohydrate.

The pattern of surface labelling we obtain from circulating human lymphocytes can be compared with that obtained by Schmitt-Ullrich *et al.* (1974) on microsomal fractions (containing plasma membranes) from rabbit thymus lymphocytes. The four labelled bands described here probably correspond to their four high-molecular-weight labelled proteins, although we obtain lower apparent molecular weights for the two most slowly migrating bands. This may result from species differences or alternatively, as these bands are glycoproteins and higher polyacrylamide gel concentrations were used in this study, the lower apparent molecular weights which we obtain may result from the anomalous mobility effects which are known to occur with glycoproteins on sodium dodecyl sulphate – polyacrylamide-gel electrophoresis (Bretscher, 1971). Schmitt-Ullrich *et al.* (1974) also note a lower-molecular-weight labelled band (their band 5) which we do not observe. This may result from species differences or degradation during the plasma-membrane preparation. Phillips (1972) reported a major labelled carbohydrate-containing product of the same molecular weight (60000) after trypsin treatment of iodinated platelets. During our own preliminary experiments we noted that preparations of intact lymphocytes and platelets tended to degrade very readily, yielding lower-molecular-weight labelled products.

To assess the extent of similarity between the corresponding labelled proteins of the platelet and lymphocyte, the most intensely labelled component (band III) from each cell type was isolated from the gels. After thermolysin digestion the labelled peptides were separated on peptide 'maps' (Plate 2). The labelled peptide patterns are very similar, showing that the same protein or very closely related proteins are present on both cell surfaces. The close correspondence in the electrophoretic mobility and distribution of label in bands I, IIa and IIb suggest that the corresponding proteins are also likely to be closely similar in both cells. Comparative peptide 'maps' have not been done on these proteins.

Vitetta *et al.* (1971) estimate that only 2–4% of the total labelled material on the lymphocyte surface is immunoglobulin. It is therefore difficult to identify the expected surface immunoglobulin chains on gel electrophoresis of unfractionated material. Schmitt-Ullrich *et al.* (1974) noted a similar difficulty.

Some of the properties of the surface proteins of the blood platelet are known and it seems reasonable to extrapolate the characteristics of the corresponding lymphocyte surface proteins from them. Bands I and IIa contain sialic acid (Nachman & Ferris, 1972). Proteins I, IIa, IIb and III are all susceptible to the action of trypsin on the intact cell and they probably represent in part the fuzzy coat seen at the membrane surface (Phillips, 1972). Schmitt-Ullrich *et al.* (1974) have shown that bands I, IIa and IIb of the lymphocyte are glycoproteins.

Although blood platelets are a fairly homogeneous cell population, the lymphocyte preparations (taken from normal peripheral blood) contain 70–80% T lymphocytes, 20–30% B lymphocytes and up to 1% polymorphonuclear leucocytes. It is not clear whether the proteins discussed above are found on one or both lymphocyte types. T and B lymphocytes differ in their surface morphology (Polliack *et al.*, 1973) and content of surface immunoglobulins (Rabellino *et al.*, 1971) and receptor sites (Jondal & Klein, 1973). The surface proteins of the erythrocyte, the remaining major circulating blood cell type, appear to be different from the comparable proteins of the lymphocyte and platelet and yield labelled peptide 'maps' which are quite distinct from those shown here (Boxer *et al.*, 1974).

The platelet and the lymphocyte have different specialized functions. The proteins considered here clearly cannot be directly responsible for the interactions which are unique to haemostasis and unique to the immune response.

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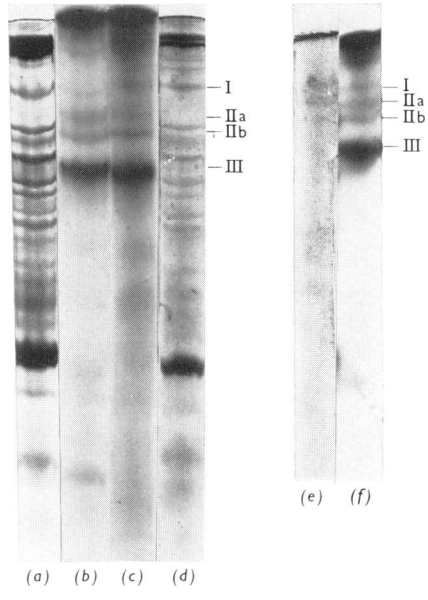
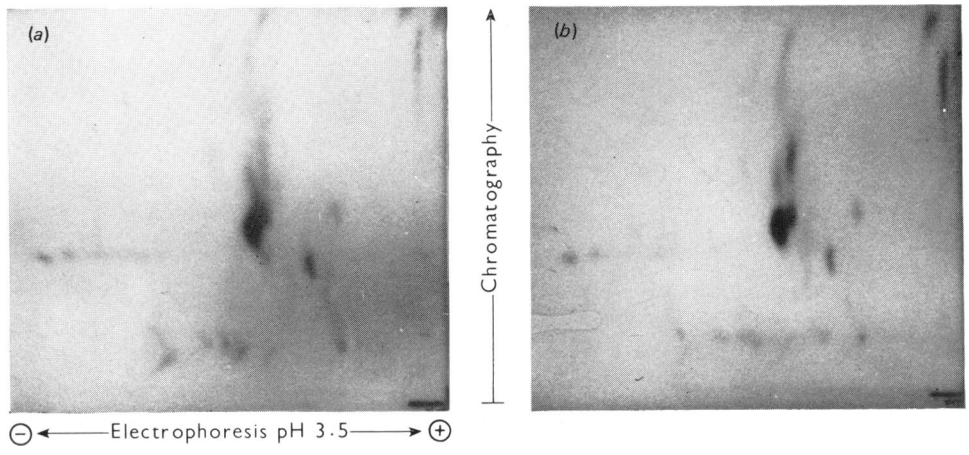


EXPLANATION OF PLATE 1

Sodium dodecyl sulphate–polyacrylamide-gel electrophoresis of labelled platelets and lymphocytes

Slices (1.1cm diam.) of 8% (w/v) polyacrylamide gel were stained for protein with Coomassie Blue, and then dried down for radioautography. Labelled whole platelets: (*a*) protein; (*b*) radioautograph. Labelled whole lymphocytes: (*c*) radioautograph; (*d*) protein. (*e*) and (*f*) Sodium dodecyl sulphate–polyacrylamide-gel electrophoresis of labelled platelets. A slice (1.1cm diam.) of 8% (w/v) polyacrylamide gel was stained for carbohydrate (Zacharias *et al.*, 1969) and then dried down for radioautography. (*e*) Carbohydrate stain; (*f*) radioautograph.



EXPLANATION OF PLATE 2

Radioautographs of peptide 'maps' obtained after thermolysin digestion of protein III from labelled lymphocytes and platelets

(*a*) Protein III from labelled lymphocytes; (*b*) protein III from labelled platelets.

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