BBA 75929

ISOLATION AND COMPOSITION OF HUMAN THYMOCYTE PLASMA MEMBRANE

DAVID ALLAN AND M. J. CRUMPTON
National Institute for Medical Research, Mill Hill, London NW7 1AA (Great Britain)
(Received December 27th, 1971)

SUMMARY

Plasma membrane was isolated from human thymus cells using an identical procedure with that described previously for the plasma membrane of pig lymphocytes. Its chemical and enzymic compositions, apart from a lower and more variable 5'-nucleotidase activity, were similar to those of the pig lymphocyte plasma membrane. Polyacrylamide gel electrophoresis of the human thymocyte membrane in sodium dodecyl sulphate gave a large number of protein bands about seven of which stained also for carbohydrate. A comparison of the protein and carbohydrate patterns with those of pig thymocyte and lymphocyte plasma membranes revealed a number of differences in glycoprotein composition.

INTRODUCTION

The therapeutic potential of antilymphocytic serum in man is limited by the availability of potent immunosuppressive and nontoxic antisera. Evidence has been presented that the immunosuppressive antibodies are directed primarily against the surface of lymphoid cells[1-3] and that antithymocyte sera are superior as immunosuppressive agents[4-6]. It seems likely on the basis of this evidence that potent and nontoxic antisera would be produced by using human thymocyte plasma membrane as the antigen. The need for a purified and well-characterised membrane preparation is, however, emphasised by the suggestion that reticulum and capillary basement membranes in a whole thymus homogenate may provoke the formation of nephrotoxic antibodies[7]. The present paper reports the isolation and composition of human thymocyte plasma membrane. A comparison of its properties with those of pig thymocyte and lymphocyte plasma membranes indicated that the membranes differed in their glycoprotein compositions and that the thymocyte membranes possessed lower 5'-nucleotidase activities than the lymphocyte membrane.

MATERIALS AND METHODS

Portions (8–17 g wet wt) of the thymus were removed from 1–12 year old children during open-heart surgery and were immediately cooled to 2 °C. Plasma membrane was prepared within 3–24 h by using an identical procedure with that described previously for pig mesenteric lymph node[8]. The cells were disrupted by

using a hand-operated mincer with a screw-driven, close-fitting plunger (Climpex, London NW7). This procedure required a minimum amount of 6-8 g wet wt of thymus and is applicable only to those tissues that contain some connective tissue. The plasma membrane fraction was subsequently isolated by differential centrifugation and by centrifuging through a sucrose density gradient. On two occasions thymuses collected with 18 h of the death of very young children (up to 2 weeks old) were used to prepare plasma membrane; the properties of the purified membrane did not differ significantly from that prepared from fresh tissue.

Polyacrylamide disc gel electrophoresis was carried out as previously described[9]. Samples of membrane (1 mg of protein) were dissolved by heating (100 °C for 5 min) in 5 % sodium dodecyl sulphate (0.15 ml); 40 and 75 μl of the solution were used for protein and carbohydrate analyses, respectively. Electrophoresis was performed in columns of 7.5 % polyacrylamide gel in 0.1 M sodium phosphate buffer (pH 7.2), containing 0.1 % sodium dodecyl sulphate for 17 h at 4.5 mA/tube. Gels were stained for protein with Naphthalene Black 10B in methanol–water–acetic acid (3:6:1, by vol.) and for carbohydrate with periodate-Schiff reagent[10], and were calibrated with proteins of known molecular weight.

Other materials and analysis were as described by Allan and Crumpton.[8]

RESULTS AND DISCUSSION

Table I shows the chemical and enzymic compositions of the human thymocyte membrane fraction. Electron micrographs of sections of the purified membrane were indistinguishable from those of the pig lymphocyte membrane (Plate 4 of ref. 8) and showed a population of predominantly smooth membrane vesicles some of which contained amorphous material and a few ribosomes. As noted previously for the lymphocyte membrane, a number of small, membrane-bounded, electron-dense bodies of unknown origin were revealed. Nuclei, mitochondria and rough endoplasmic reticulum were, however, not detected. This interpretation of the micrographs was confirmed by the small amounts of RNA and DNA found in the membrane fraction

TABLE I

COMPOSITION OF THE PLASMA MEMBRANE FRACTION OF HUMAN THYMUS CELLS

Values were determined as already described[8]. Amounts of RNA and 5′-nucleotidase represent the average of eight preparations whereas the amounts of other components represent the average of at least two preparations. Enzyme activities are expressed as μmoles of product liberated per mg protein per h.

Component	*Amount*
Phospholipid (μg/mg protein)	540
Cholesterol (μg/mg protein)	197
Cholesterol/phospholipid molar ratio	0.75:1
Carbohydrate (μg glucose/mg protein)	73
RNA (μg/mg protein)	25
DNA (μg/mg protein)	0
5′-Nucleotidase	3.9
Acid phosphatase	0.22
Glucose 6-phosphatase	0.02

(Table I), and by the low activities of marker enzymes for lysozomes (acid phophatase, EC 3.1.3.2) and endoplasmic reticulum (glucose 6-phosphatase, EC 3.1.3.9). On the other hand, the relatively high value for the cholesterol/phospholipid molar ratio (Table I) suggested, by analogy with the results of Coleman and Finean[11], that the preparation consisted mainly of plasma membrane. Furthermore, the specific activity of 5'-nucleotidase (EC 3.1.3.5.) which is a recognised marker of plasma membrane, was enriched 16-fold in the membrane fraction compared with the tissue homogenate. One disadvantage of the method of preparation used, was the occlusion of material within membrane vesicles during the disruption of the cells. This was, however, considered not to represent a major source of contamination and it was concluded that the purified membrane fraction represents primarily the plasma membrane of human thymus cells. This conclusion is supported by the properties of antisera prepared by immunizing rabbits with the membrane in Freunds' complete adjuvant (M. J. Crumpton, D. Thomas, J. G. Woodrooffe and H. Zola, unpublished observations). These antisera agglutinated human lymphocytes, failed to agglutinate platelets, possessed low titres of erythroagglutinins and had high titres of antibodies against the surface of lymphoid cells, as determined by immunofluorescence. Antibodies against human serum proteins were not detected by immunoelectrophoresis. No evidence was obtained for the binding of antibody by rat glomerula basement membrane; consequently, the plasma membrane fraction was most probably not contaminated with reticulum and capillary basement membranes (*cf.* ref. 7).

The yield of plasma membrane varied from 3.4 to 13.6 mg protein per 10 g wet wt of thymus and appeared to be related to the texture of the tissue in that lower yields were obtained from the tougher samples that probably contained larger amounts of connective tissue. Between 10 and 15 % of the 5'-nucleotidase activity of the thymus homogenate was recovered in the plasma membrane fraction.

The chemical and enzymic compositions of the human thymocyte plasma membrane were similar to those of the plasma membranes of pig thymus and mesenteric lymph node cells[8]. The human thymocyte membrane resembled, however, that of pig thymocytes in possessing a lower 5'-nucleotidase activity than the lymphocyte membrane. Also, as noted previously for the pig thymocyte membrane, different preparations of the human membrane showed a large variation in 5'-nucleotidase activity (within the range 2.6-7.2 μmoles of P_i liberated per mg protein per h), whereas the activity of the lymphocyte plasma membrane never varied by more than 20%. This variation in activity did not appear to be related to the age of the child or to the yield of plasma membrane fraction; it is, however, possible that it depends on the particular part of the thymus used (isthmus and/or one or both lobes) or on the relative proportions of cortical and medullary cells.

Human thymocyte plasma membrane that had been heated in sodium dodecyl sulphate in order to promote dissociation and to inactivate proteases[12,13], was electrophoresed in polyacrylamide gel and stained for protein or carbohydrate. When gels were stained for protein about 20 bands of differing intensities (Fig. 1A) were revealed; a turbid broad band that did not stain for protein and that moved faster than cytochrome *c* was also detected. After staining for carbohydrate a very much simpler pattern was obtained consisting of about seven faint bands and a broad, intense-staining band near the bottom of the gel (Fig. 2B). If it is assumed that the intensity of staining with periodate–Schiff reagent is proportional to the amount of

carbohydrate present, then the major portion of the carbohydrate of the human thymocyte plasma membrane is associated with the fast-running band. So far as could be judged from comparing gels that had been run at the same time and then stained for protein or carbohydrate, each of the faint carbohydrate bands corresponded

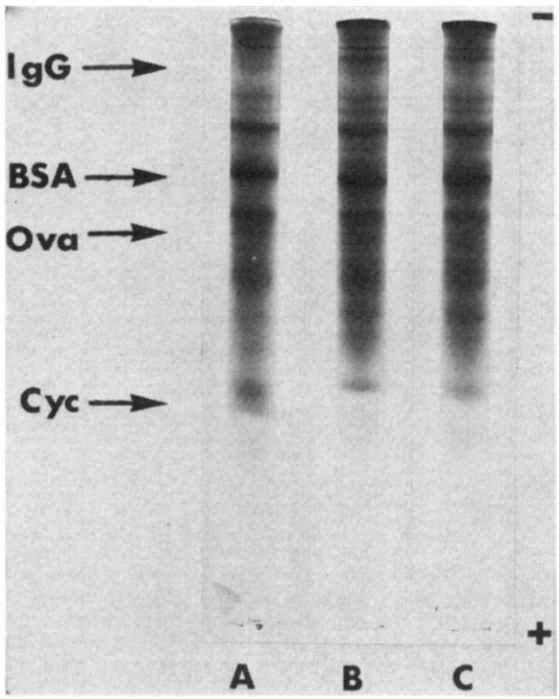

Fig. 1. Protein patterns of plasma membranes of human thymocytes (A), pig thymocytes (B) and pig lymphocytes (C) after polyacrylamide gel electrophoresis in sodium dodecyl sulphate. Gels were photographed in transmitted light. Although this procedure revealed the majority of the stained bands, it failed to show a turbid, unstained band that moved faster than cytochrome *c* and that probably represents lipid; also, the presence of stain on the top of Gels B and C was exaggerated by the photographic process. The arrows indicate the positions of the centres of the bands given by marker proteins; pig immunoglobulin G (IgG; mol. wt, 151000), bovine serum albumin (BSA; mol. wt, 69000), ovalbumin (Ova; mol. wt, 43000) and cytochrome *c* (Cyc; mol. wt, 12380).

to a protein band and, thus, represented a glycoprotein. On the other hand, the intense carbohydrate band corresponded to the turbid band that did not stain for protein. This band probably represents glycolipid, since it was not detected in gels of lymphocyte plasma membrane extracted with aqueous *n*-butanol which removed 98 % of the lipid phosphorus and 62 % of the neutral sugar of the membrane[14]. Lenard[15] and Bender *et al.*[13] have reported a band with similar properties in gels of human erythrocyte membranes and have also suggested that the material is glycolipid.

The protein pattern of the human thymocyte plasma membrane showed an overall similarity to those of the plasma membranes from pig thymus and mesenteric lymph node cells (Fig. 1). A number of differences were, however, detected. A comparison of the patterns of the faintly-staining carbohydrate bands of the various membranes also revealed some differences (Fig. 2). At least some of the carbohydrate

differences appeared to coincide with the protein variations; for example, the pig thymocyte and lymphocyte membranes possessed a prominent protein (Fig. 1B and C) and carbohydrate-staining band (Fig. 2A and C) with a slightly slower mobility than immunoglobulin G whereas this band was barely detected in the human thymo-

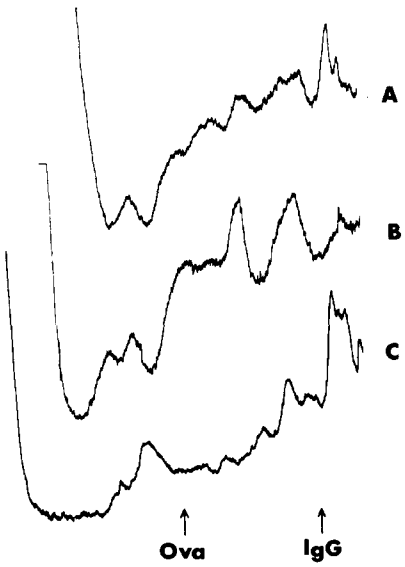

Fig. 2. Densitometer tracings of carbohydrate patterns of plasma membranes of pig thymocytes (A), human thymocytes (B) and pig lymphocytes (C) after polyacrylamide gel electrophoresis in sodium dodecyl sulphate. The direction of electrophoretic migration is from right to left. After staining for carbohydrate, gels were stored in methanol–water–acetic acid (3:6:1, by vol.) and were scanned using a Joyce–Loebl double-beam recording microdensitometer (Model E12 MK 111B). The large absorption at the left of the tracings probably represents glycolipid. The most intensely staining region of the glycolipid bands of the different membranes occupied approximately the same positions, but in the case of the gels of the thymocyte membranes shown (A and B) these regions possessed diffusely-staining, trailing edges. The arrows indicate the positions occupied by pig immunoglobulin G (IgG) and ovalbumin (Ova).

cyte membrane (Fig. 1A and 2B). Some of the variations particularly those detected in the carbohydrate patterns appeared, however, to be based on quantitative (*i.e.* intensity of staining) rather than qualitative (*i.e.* position of band) differences. Similar results to those shown in Figs 1 and 2 have been obtained on a number of occasions using different membrane preparations. It was concluded that the plasma membranes of human thymus, pig thymus and pig mesenteric lymph node cells differ in their glycoprotein compositions. Membrane glycoproteins have been reported to be exposed on the cell surface and have been ascribed important roles in mediating cell adhesion, cell recognition and histocompatibility[16–19]. If these suggestions are correct then it seems likely that the different biological activities of pig and human thymus and pig lymph node cells, and the different properties of their homologous antisera are related to the variation in glycoprotein composition of the plasma membranes.

The results of the above comparisons also suggest that species variation (human and pig) is not associated with a gross change in the protein composition of the surface membrane from cells of the same tissue. This interpretation agrees with

the results of recent studies on the protein compositions of erythrocyte membranes from various species[20,21]. In contrast, Carraway and Kobylka[22] have reported that there is no apparent common pattern of proteins among the erythrocyte membranes of different species.

ACKNOWLEDGEMENTS

We are indebted to Mr. A. D. Barnes, Queen Elizabeth Hospital, Birmingham, Dr. E. Lockey, National Heart Hospital, London and Professor J. F. Soothill, Institute of Child Health, London for human thymus tissue. The expert technical assistance of Miss Judy Auger and Mr. P. B. Gillett is gratefully acknowledged.

REFERENCES

1 E. M. Lance, P. J. Ford and M. Ruszkiewicz, *Immunology*, 15 (1968) 571.
2 S. Sell, R. A. Mascari and P. J. Goldblatt, *J. Immunol.*, 105 (1970) 1569.
3 H. Zola, B. Mosedale and D. Thomas, *Transplantation*, 9 (1970) 259.
4 R. J. Perper, S. C. Lyster, R. E. Monovich and B. E. Bowersox, *Transplantation*, 9 (1970) 447.
5 A. E. Sloboda and J. Landes, *J. Immunol.*, 104 (1970) 185.
6 W. A. Sterling, L. R. Elveback and R. G. Shorter, *Transplantation*, 10 (1970) 297.
7 H. E. Taylor, *Transplant. Proc.*, 2 (1970) 413.
8 D. Allan and M. J. Crumpton, *Biochem. J.*, 120 (1970) 133.
9 D. Allan and M. J. Crumpton, *Biochem. J.*, 123 (1971) 967.
10 R. M. Zacharius, T. E. Zell, J. H. Morrison and J. J. Woodlock, *Anal. Biochem.*, 30 (1969) 148.
11 R. Coleman and J. B. Finean, *Biochim. Biophys. Acta*, 125 (1966) 197.
12 J. V. Maizel, in K. Habel and N. P. Salzman, *Fundamental Techniques in Virology*, Academic Press, New York, 1969, p. 334.
13 W. W. Bender, H. Garan and H. C. Berg, *J. Mol. Biol.*, 58 (1971) 783.
14 D. Allan, J. Auger and M. J. Crumpton, *Nature New Biol.*, 236 (1972) 23.
15 J. Lenard, *Biochemistry*, 9 (1970) 1129.
16 G. M. W. Cook, *Biol. Rev.*, 43 (1968) 363.
17 M. C. Glick, C. Comstock and L. Warren, *Biochim. Biophys. Acta*, 219 (1970) 290.
18 S. B. Oppenheimer, M. Edidin, C. W. Orr and S. Roseman, *Proc. Natl Acad. Sci. U.S.*, 63 (1969) 1395.
19 A. R. Sanderson, P. Cresswell and K. I. Welsh, *Nature New Biol.*, 230 (1971) 8.
20 J. Lenard, *Biochemistry*, 9 (1970) 5037.
21 H. Knufermann, H. Fischer and D. F. H. Wallach, *FEBS Lett.*, 16 (1971) 167.
22 K. L. Carraway and D. Kobylka, *Biochim. Biophys. Acta*, 219 (1970) 238.