MEMBRANE ASSOCIATED IMMUNOGLOBULIN IN PIG THYMOCYTES

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SUMMARY. The polypeptides and glycoproteins in plasma membranes of thymocytes and mesenteric lymph node cells have almost identical patterns in polyacrylamide gels. Thymocyte plasma membranes contain IgG which comprises about 0.3% of the total membrane protein. IgM has not been detected. Previous studies with lymph node lymphocyte membranes have shown 0.6% immunoglobulin, containing both γ and μ determinants. Therefore, in their overall polypeptide composition and the possession of immunoglobulin, the membranes from the two types of cells are strikingly similar to each other.

INTRODUCTION. The two major classes of lymphocytes which participate in immune responses differ from one another in the presence or absence of various surface markers. Thymus-dependent (T) cells possess θ or Tl antigens and have little or no surface immunoglobulin (1). Bursa or bone marrow-derived (B) cells have readily demonstrable surface immunoglobulin, and surface binding sites for C'3 and antigen-antibody complexes (2). However, an occasional thymoma cell line may contain both T cell (θ) and B-cell (immunoglobulin) markers (3). Although much of this work has been done in mice and humans, and it is not clear to what extent the results are more generally valid, T and B cells bearing surface markers similar to those described in mice have been found in pigs (4, 5).

There is disagreement about the presence, the amount and the origin of surface immunoglobulin on thymus cells (2, 6, 7, 8). In the present study, we have been able to detect immunoglobulin in purified plasma membrane fractions derived from pig thymuses.

MATERIALS AND METHODS

Preparation of Membrane. Thymuses were removed from pigs immediately after death and the membrane fraction isolated with modifications

of the method of Allan and Crumpton (9, 10). The major modifications involved the conditions of centrifugation. Intact cells, nuclei and mitochondria were pelleted in an International Model PR 6000 in 50 ml buckets $3750g_{average}$ for 90 mins. The microsomal pellet was obtained by centrifuging the supernatant from the nuclear spin in a Spinco SW 25.2 rotor at $40,000g_{average}$ for 90 mins. The resuspended microsomal pellet was fractionated on a discontinuous gradient of 40% w/w sucrose overlaid with 27% w/w sucrose.

Polyacrylamide gel electrophoresis and molecular weight estimations were done by the method of Neville (11, 12) using a slab gel apparatus,

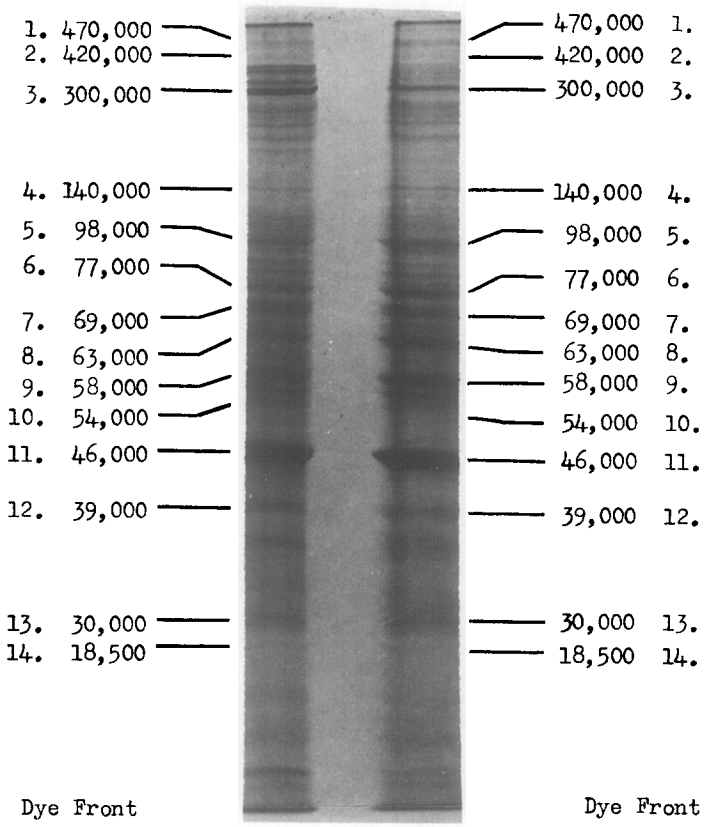

Figure 1.

Comparison of polypeptide chain composition of plasma membranes from a) young adult pig thymus cells, and b) mesenteric node cells. Each sample has been delipidated prior to electrophoresis. The figures next to the patterns are the molecular weights, in daltons, of the respectve polypeptide chains.

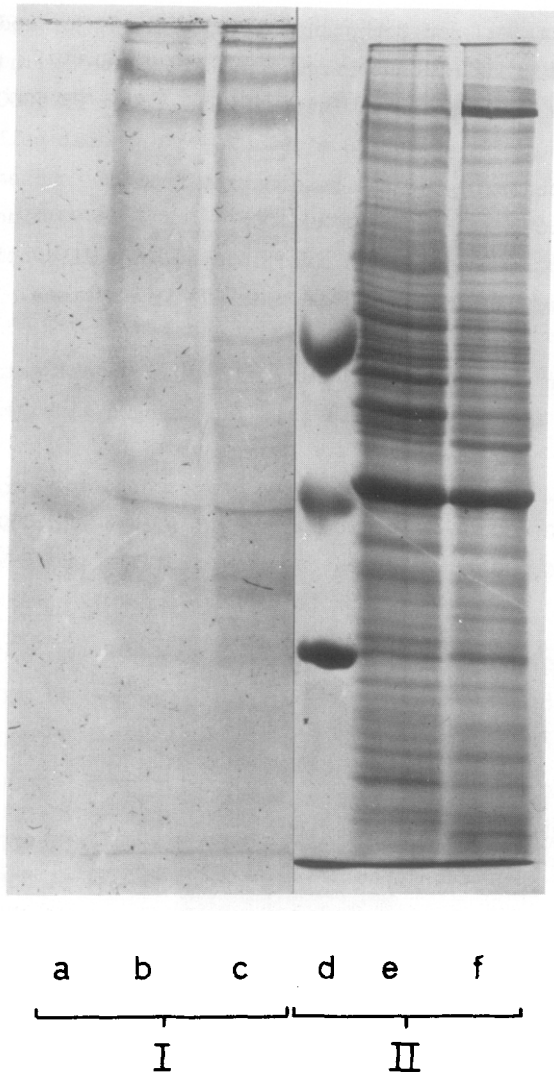

Figure 2.

Polypeptides and glycoproteins of pig mesenteric node lymphocyte and thymocyte plasma membranes. The electrophoretic separation was carried out in a single slab gel. After electrophoresis, the gel was divided and one half stained for carbohydrate (I) and the other half for protein (II). The photographs of the two halves have been juxtaposed by aligning the ovalbumin bands. a) Ovalbumin 10μg. b) Thymocyte membrane 250μg. c) Mesenteric lymphocyte membrane 250μg. d) Standard proteins: bovine serum albumin, ovalbumin, and carbonic anhydrase 5 μg of each. e) Mesenteric lymphocyte membrane, 100 μg. f) Thymocyte membrane 100μg.

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and the samples were stained for protein using Coomassie blue G-250 (13), or carbohydrate (14).

Immunological methods. Membranes were dissolved in solutions of 1%w/v sodium cholate and analysed for immunoglobulin using radial immunodiffusion (15); the agar gels contained 1% cholate and antiserum.

RESULTS AND DISCUSSION

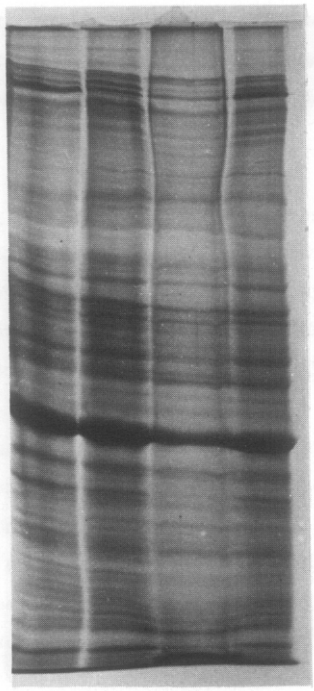
Membrane polypeptides. The polypeptide chain and glycoprotein composition of membranes from thymocytes and mesenteric lymphocytes are strikingly similar (Figs. 1 and 2); although there are several bands of similar mobility which vary in amount between the two membranes, there are no definite qualitative differences.

It is difficult to exactly align the polypeptides and glycoproteins, even when the electrophoretic separation has been done in a single slab of gel. Polypeptide 1 (molecular weight around 470,000 daltons) appears to be a relatively homogeneous glycoprotein. The next two lower molecular weight glycoproteins have a greater electrophoretic heterogeneity than the polypeptide chains in the corresponding portion of the gel, and may not be identical to any of the latter.

The polypeptide patterns of thymocyte membranes derived from whole thymus and from a suspension of washed cells (Fig. 3) and from young and newborn thymuses (Fig. 4d, e) are virtually indistinguishable from each other. An attempt was made to localise the immunoglobulin polypeptide chains by means of a co-migration experiment (Fig. 4a, b, c). There is an increase in staining of a band in the light chain region and a general loss of resolution in the heavy chain region. It may also be noted that pig light chains migrate as a pair of polypeptide chains (Fig. 4b).

Earlier studies have suggested an overall similarity among human thymocytes, pig thymocytes and pig mesenteric node membranes (9), and between thymocytes and peripheral lymphocytes of the calf (16). The method of electrophoresis which we have used (11, 12) is able to resolve a much larger number of polypeptide chains, and the similarity of the patterns persists. This similarity was unexpected because of the differences in cell populations of the two tissues. Approximately 5-10% of the lymphocytes from pig mesenteric lymph node have surface immunoglobulin (4), whilst 50% behave like T-cells (5).

Membrane-associated immunoglobulins. Three batches of membrane



a b c d

Figure 3.

Pig lymphocyte plasma membranes from intact thymuses (a,b,d) and washed cell suspension (c). All preparations contain 100μg of protein and are delipidated.

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prepared from intact thymuses of 3 month old pigs contained 0.2%, 0.2% and 0.4% respectively, of their total protein as immunoglobulin (Fig. 5). This appears to be largely IgG because it reacts with specific anti-γ but not with anti-μ antiserum (lower limit of detection around 63 μg ml^{-1} 19S IgM). This is in contrast to the demonstration of both γ and μ determinants in mesenteric lymph node membrane, using the same reagents and methods (S. I. Chavin, et al., unpublished experiments).

In order to look at the possibility that the membrane-associated immunoglobulin might be derived from extracellular fluids, two different types of membrane preparations were studied. A suspension of cells from young pig thymuses was washed and pelleted four times with large volumes of buffered saline; membranes prepared from these cells contained 0.08% of their total protein as immunoglobulin.

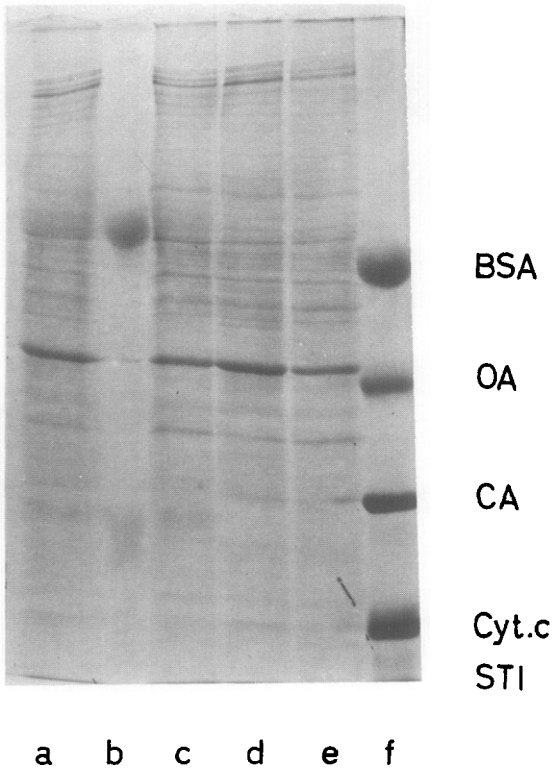

Figure 4.

Comparison of polypeptide chain patterns of young and newborn pig thymocyte plasma membranes. The membranes were prepared from intact thymuses, and each sample contained 100μg of protein.
a) Adult thymus plus 5μg of fully reduced pig IgM.
b) Fully reduced pig IgM, 5μg.
c) Newborn thymus plus 5μg of fully reduced pig IgM.
d) Newborn thymus. e) Adult thymus. f) Standard marker proteins, bovine serum albumin (BSA), ovalbumin (OA), carbonic anhydrase (CA), cytochrome c (Cyt c), and soy bean trypsin inhibitor (STI), 5μg each.

The reason for the decrease in immunoglobulin content is not known, but such a decrease was not seen in mesenteric lymph node cell membranes (S. I. Chavin, et al., unpublished experiments). This difference in the effect of washing between the thymus and lymph node membranes suggests that at least a fraction of thymocyte immunoglobulin may be more loosely associated with the membrane than is the lymphocyte membrane immunoglobulin.

The second type of membrane was made from intact thymuses of two unsuckled newborn piglets (killed three hours after birth) and contained 0.2% immunoglobulin (Fig. 6). Serum from these piglets contained

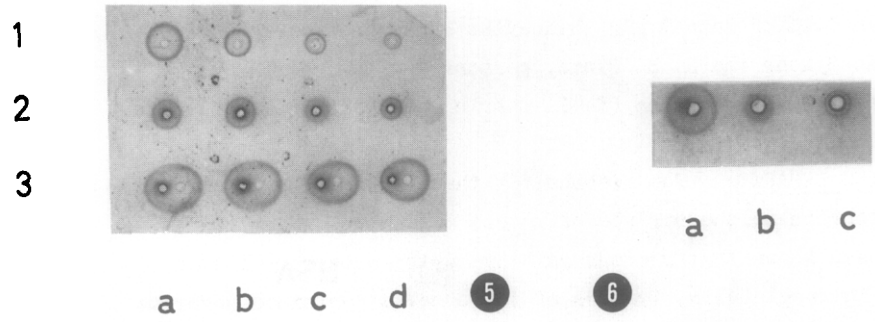

Figure 5.

Demonstration of pig thymocyte membrane-associated immunoglobulin using radial immunodiffusion. Row 1 contains pig IgG at the following concentrations, a) μg ml^{-1}: 1a) 200 1b) 100 1c) 50 1d) 25. Row 2 contains membrane dissolved in 1% sodium cholate at the following approximate protein concentrations: 2a) intact mesenteric node lymphocytes, 13.7 mg ml^{-1}, 2b) intact mesenteric node lymphocytes (different preparation), 1.3mg ml^{-1}, 2c) washed suspension of mesenteric node lymphocytes 1μg ml^{-1}, 2d) intact thymus thymocytes 1.3 mg ml^{-1}. Row 3. The first of each pair of wells contains the same membrane preparation as the corresponding well in Row 2; the second well of each pair contains pig IgG 200μg ml^{-1}. Row 3 demonstrates reactions of fusion which are analogous to reactions of identity in double diffusion plates. The gel contains a final concentration of 0.1% of sheep anti-pig IgG. The plate was washed, dried and stained after 12 days development, and photographed.

Figure 6.

Demonstration of membrane-associated immunoglobulins in thymocyte plasma membrane from newborn piglets and mesenteric lymph node lymphocyte membranes from young pigs. Details as in the legend to Figure 5. Plasma membranes from a) intact young pig mesenteric lymph nodes, 17 mg ml^{-1}; b) washed suspension of young pig mesenteric lymphocytes, 15 mg ml^{-1}; c) intact thymuses of newborn piglets, 18 mg ml^{-1}. The gel contains 0.1% sheep anti-pig IgG, reacting with light chains and γ chains. The plate was washed, dried and stained after 13 days, and then photographed.

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around 100 μg ml^{-1} immunoglobulin, i.e. about 1% of the amount in adult pig serum, confirming that the piglets had not suckled nor been subjected to <u>in utero</u> antigenic stimulation. Thus, the amount of thymocyte membrane-immunoglobulin is approximately the same in antigenically unstimulated newborn piglets, with very low levels of circulating immunoglobulin, as in 3-4 month old piglets.

These results show the presence of membrane-associated immunoglobulin in thymocytes, although surface immunoglobulin can be detected on

less than 0.1% of intact pig thymocytes by immunofluorescence (D. B. A. Symons and R. M. Binns, personal communication) or by immunoperoxidase labelling (S. I. Chavin, unpublished experiments)

CONCLUDING REMARKS. The reasons for the difficulties detecting immunoglobulin on the surface of intact thymus cells are not known. It has been shown that whereas rat thymocytes have very little surface-immunoglobulin, lysates of these cells contained moderate amounts of IgG_{2a}, IgG_{2b} and IgA, but very little IgM (17, 18). Since most of this immunoglobulin is presumably destined for secretion through the membrane, its detection in the isolated membrane fraction is to be expected. This membrane-associated immunoglobulin may be difficult to detect on the intact cell because it is relatively inaccessible to the various binding or labelling reagents which have been used. It should be emphasized that there are other membrane-associated sites or activities, such as polypeptide hormone receptors (19) and lectin-binding sites (20) which can be exposed and/or increased in number by variuos treatments of the cell surface.

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