PLASMA MEMBRANES OF BURSAL, THYMIC AND CIRCULATING LYMPHOCYTES HAVE UNIQUE PROTEINS

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Received October 30,1972; revised November 13,1972

Summary

The protein subunit composition of plasma membranes of chicken bursal, thymic and circulating lymphocytes was determined by polyacrylamide gel electrophoresis. Two of the proteins common to bursa and thymus were not expressed in the circulating lymphocytes. Bursal membranes had 4 unique proteins, 2 of which were still expressed in the circulating lymphocytes. Thymic membranes had 3 unique proteins, one of which was still expressed in the circulating lymphocytes. Membranes of circulating lymphocytes had one unique protein.

The division of the immune systems of birds and mammals into two functionally different compartments is well documented (1). Lymphocytes derived from the thymus (T lymphocytes) are responsible for cellular immunity and lymphocytes derived from the bursa of Fabricius of chickens or from the bone marrow of mammals (B lymphocytes) are responsible for humoral immune responses. Studies with antilymphocyte serum have shown that the outer surfaces of bursal and thymic lymphocytes are immunologically distinct, and thus apparently contain components unique to each cell type (2-4). Additional immunologic studies have led to the discovery of specific markers for bursal and thymic lymphocytes. The theta antigen (5) is a marker for mammalian T lymphocytes, while immunoglobulins are markers for B lymphocytes (6).

Since polyacrylamide gel electrophoresis has been used as a sensitive and reliable method of separating and identifying the protein components of membranes (7-10), we have used this method to determine the similarities and differences in protein composition of the plasma membranes (PM) of the several types of chicken lymphocytes in a search for unique proteins which may serve as additional markers for B and T lymphocytes. When protein subunits having the same mobilities were found in PM from more than one cell type, we considered them to be common to those cell types. Protein subunits common to several membranes on the basis of their behavior in sodium dodecyl sulphate-polyacrylamide gel electrophoresis may be identical (10), but this assumption would have to be verified by extensive chemical and immunologic studies.

Bursal, thymic and circulating lymphocytes were obtained from lymphoid leukosis- and Marek's disease-free White Leghorn chickens which had been maintained in filtered air under positive pressure (11). Bursal and thymic tissues were taken from 4-5 week old birds. Circulating lymphocytes were isolated from the blood of 7-14 week-old birds by the Stage I method of Noble et al (12). Plasma membranes were prepared by the method of Perdue and Sneider (13) except the membrane fractions were separated on discontinuous rather than continuous gradients (14). The membrane fractions were characterized (15). The fractions used in this study were comparable to the "B" fraction of Perdue and Sneider.

The membranes were prepared for electrophoresis by solubilizing them in 5% sodium dodecyl sulphate and 2% 2-mercaptoethanol in a boiling water bath for 5 minutes. Samples of 100 µl solubilized membrane (ca. 4 mg protein/ml) were mixed with 100 µl 0.2 M sodium phosphate buffer, pH 7.2, in 40% (w/v) sucrose; 5 µl 0.04% Bromphenol Blue was added prior to electrophoresis. Fifty to 100 µg protein of

these samples were applied to each gel column (7.5 cm x 0.5 cm) consisting of 7.5% (w/v) polyacrylamide, 0.1 M sodium phosphate buffer, pH 7.2, and 0.1% sodium dodecyl sulphate (16). The same buffer was used in the electrode vessels. Electrophoresis was performed at room temperature for 5 hrs at a constant current of 4.5 mA per tube. Under these conditions Bromphenol Blue moved approximately 6 cm through the gel. The gels were stained in 0.05% Coomassie Brilliant Blue R 250 in 12.5% trichloracetic acid for 5 hrs and destained in 10% trichloracetic acid (17). The gels were allowed to fade and then stained according to the method of Weber and Osborne (18). Scannir of the gels at 625 nm produced the same profiles regardless of the method of staining.

Thirty-two proteins were resolved from bursal membranes, 31 from thymus and 30 from circulating lymphocytes (Fig. 1). Twenty-eight of them were common to bursa and thymus and 26 were common to all three lymphocyte populations. Fraction 27 was selected as a reference protein because of its relatively high concentration and characteristic shape in the gel profiles (Fig. 1). The mobilities of the other membrane proteins and proteins of known molecular weights (MW) were determined relative to the reference protein both by examination of gel profiles (Fig. 1) and direct measurement of the gels. Relative mobilities (Rf) and estimated MW (19) for the proteins of all three lymphocyte types are listed in Table 1. The relative amounts of the subunits were determined by estimating the areas under the peaks in the gel profiles. The relative amount of each subunit was constant for each type of lymphocyte as illustrated in Fig. 1 with the exception of fractions 35 and 36 which varied considerably.

It has been demonstrated previously with polyacrylamide gel electrophoresis that membranes are composed of heterogenous

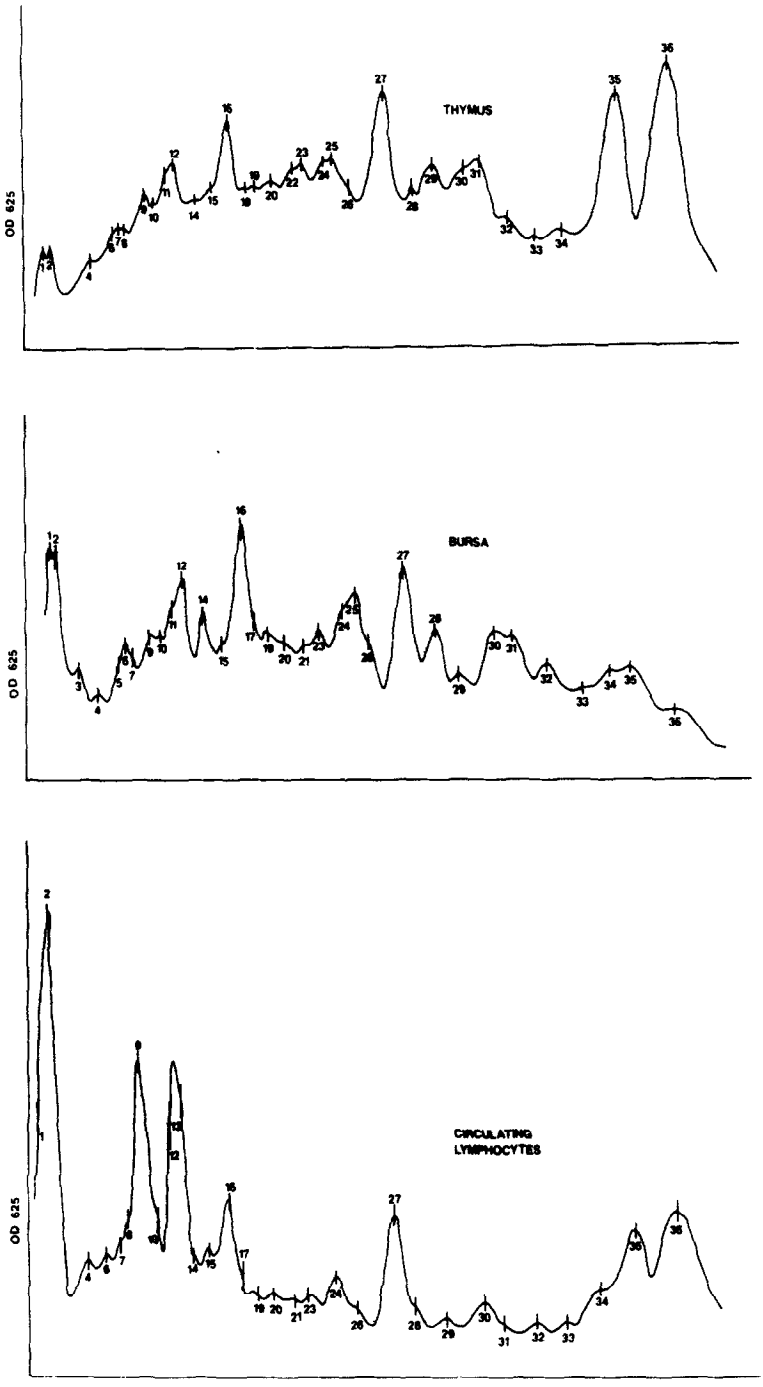

Fig. 1. Sodium dodecyl sulphate-polyacrylamide gel electrophoretograms of plasma membranes of chicken bursal, thymic and circulating lymphocytes. The gels were stained with Coomassie Blue (Ref. 16) and scanned at 625 nm with a Gilford Model 240 linear transport (Gilford Instrument Labs, Inc., Oberlin, Ohio). The vertical marks on the gel profiles correspond to the positions of the discs as determined by direct measurement of the gels. The numbers correspond to fraction numbers in Table 1.

Table 1. Relative mobilities (Rf) and estimated molecular weights (See Ref. 19) of the proteins of chicken bursal, thymic and circulating lymphocyte (Circ. Lymph.) plasma membranes separated by polyacrylamide gel electrophoresis. Rf were determined by the formula: distance of fraction from origin/distance of fraction 27 from origin x 10^3. Rf values are $\bar{X} \pm$ s.e.m. (no. of observations).

Fraction No.	Molecular Weight (x 10^3 daltons)	Rf		
		Bursa	Thymus	Circ. Lymph.
1	165	56 ± 7(3)	61 ± 9(2)	56 ± 3(5)
2	160	72 ± 4(4)	81 ± 5(4)	86 ± 4(3)
3	153	114 ± 11(4)	-	-
4	137	175 ± 11(4)	191 ± 3(4)	202 ± 17(6)
5	130	236 ± 3(4)	-	-
6	127	256 ± 3(4)	252 ± 3(4)	247 ± 5(4)
7	124	276 ± 0(2)	270 ± 5(3)	274 ± 9(7)
8	118	-	289 ± 12(4)	304 ± 7(3)
9	113	325 ± 4(4)	336 ± 7(4)	346 ± 7(7)
10	108	353 ± 1(3)	362 ± 10(4)	368 ± 4(5)
11	104	388 ± 3(4)	393 ± 6(4)	-
12	101	412 ± 2(4)	417 ± 6(4)	416 ± 5(7)
13	97	-	-	449 ± 5(7)
14	94	475 ± 3(4)	476 ± 7(4)	479 ± 4(4)
15	90	513 ± 1(2)	516 ± 6(4)	516 ± 6(7)
16	84	569 ± 7(4)	566 ± 2(4)	566 ± 6(7)
17	78	604 ± 1(2)	-	601 ± 6(4)
18	75	-	619 ± 6(4)	-
19	71	640 ± 5(4)	647 ± 7(4)	635 ± 5(5)
20	69	688 ± 3(4)	693 ± 6(4)	694 ± 5(4)
21	66	730 ± 6(4)	-	727 ± 6(4)
22	64	-	748 ± 5(4)	-
23	62	777 ± 2(4)	775 ± 1(4)	781 ± 7(7)
24	57	834 ± 3(4)	837 ± 7(4)	843 ± 5(7)
25	54	869 ± 8(4)	871 ± 6(4)	-
26	52	908 ± 3(4)	910 ± 2(3)	902 ± 4(8)
27	46	1000 ± 0(4)	1000 ± 0(4)	1000 ± 0(7)
28	42	1082 ± 4(4)	1073 ± 22(4)	1057 ± 8(7)

29	37	1158 ± 12(4)	1148 ± 5(4)	1147 ± 9(7)
30	33	1238 ± 5(4)	1223 ± 21(4)	1234 ± 14(7)
31	31	1291 ± 6(4)	1276 ± 15(4)	1295 ± 30(5)
32	27	1361 ± 20(4)	1364 ± 18(4)	1401 ± 8(7)
33	24	1467 ± 2(2)	1441 ± 14(4)	1462 ± 0(1)
34	22	1531 ± 19(3)	1517 ± 33(4)	1565 ± 21(7)
35	19	1629 ± 18(4)	1627 ± 16(4)	1685 ± 14(7)
36	14	1761 ± 30(4)	1750 ± 40(4)	1860 ± 39(7)

populations of proteins (7-10,20) which differ with each class of membrane (21-23). When PM of rat liver, kidney and erythrocytes were compared, 7 proteins were common to liver and kidney and 3 of them also were present in erythrocyte membranes (10). Simon et al. (7) found 3 major proteins common to rat liver PM and erythrocyte membranes, and 2 of them had the same MW (48,000 and 27,000) as the proteins Neville and Glossman (10) found in rat liver, kidney and erythrocyte. More similarity in the protein pattern has been found between PM of various organs than between PM and the intracellular membranes of the same organ (10). Lenard (9) has found that the electrophoretic patterns of the erythrocyte membrane proteins from 5 species are similar and have at least 8 major proteins in common. Since the different populations of chicken lymphocytes are related so closely ontogenetically it is not surprising to find they have 26 proteins in common.

Three major proteins were found in the PM of all 3 lymphocyte populations. They are fractions 12, 16 and 27 which have MW of 101,000, 84,000 and 46,000 respectively. When a protein subunit is present in excess of about 3% of the total membrane protein, as each of these 3 fractions was, its primary function is probably to confer structural integrity to the membrane rather than to serve as

a catalytic protein (10). Although it is not imperative that avian PM have "structural" proteins of the same MW as mammalian PM, it is interesting that three of the major proteins common to the erythrocyte membranes of 5 species had MW of 108,000, 86,000 and 45,000 (9). Common proteins of 27,000-28,000 daltons (7,10,24) and 90,000 daltons (7,8) also have been reported and though proteins of these MW were common to all 3 chicken lymphocyte types, they were not major protein fractions. The most frequently observed MW class of mammalian PM protein has been 45,000 - 48,000 which corresponds with our reference peak. Our data are in agreement with the speculation that all PM have a major protein of about 45,000 - 48,000 daltons (10).

The minor proteins of chicken lymphocyte PM are especially interesting because of the apparent uniqueness of several of them to one or more of the lymphocyte populations. Fractions 11 and 25 were found only in bursal and thymic PM. They were evidently lost (or severely reduced to amounts not resolved by the procedures used) during the process of maturation and entry of the lymphocytes into the circulatory system. Fractions 3 and 5 were present only in bursal PM and fractions 18 and 22 only in thymic PM, all four proteins being lost during maturation. Fraction 9 was found in thymic and not in bursal PM but was not lost during maturation as it was also detected in PM of the circulating lymphocytes. Likewise, fractions 17 and 21 were present in bursal and not thymic PM but were not lost during maturation. The distinction between fractions 17 and 18 and between 21 and 22 needs to be made with some caution as 18 and 22 in thymus could essentially be the same proteins as 17 and 21 in the bursa respectively except that their mobilities have been increased slightly by some minor change. The Rf values of these proteins, although close to each other, are significantly

different and should be considered different until evidence to the contrary is presented. Since fractions 17 and 18 both have been found in PM of lymphoid tumors (15), it seems that they are indeed different proteins. One protein, fraction 13, was unique to the PM of circulating lymphocytes. Whether this protein is associated exclusively with either bursal derived or thymus derived lymphocytes has not been determined.

These observations indicate that the PM of immature lymphocytes of bursa and thymus, organs which both regress during maturation, contain proteins not found in mature circulating lymphocytes. Since they appear in organs which involute early in life it is tempting to consider that these proteins are foetoproteins of the lymphoid system. Chemical and immunologic characterization should be done to further establish the separate identities of these unique proteins as they should find use not only as markers for bursal and thymic lymphocytes but also as immunologic agents for various procedures such as the production of specific antilymphocyte serum for use in immunologic bursectomy and thymectomy.

This work was supported by the National Poultry Research Foundation and General Research Support Grant FR-5-SO1-RR 05601-04 from the National Institutes of Health. R. L. Doak is a recipient of a Damon Runyon Research Fellowship. Journal Series 969 from the Institute of Comparative Medicine, University of Georgia.

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