SURFACE IMMUNOGLOBULIN HEAVY CHAINS OF MURINE SPLENOCYTES AND THYMOCYTES ARE DIFFERENT*

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SUMMARY: ^{125}I-labeled surface immunoglobulins were isolated from murine splenocytes and thymocytes and analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Surface immunoglobulin from splenocytes was shown to consist of light chain and two distinct heavy chains. In contrast, surface immunoglobulin from thymocytes comprised light chain and a single heavy chain. The thymocyte surface heavy chain was electrophoretically distinct from both splenocyte heavy chains. These results indicate that thymocytes surface immunoglobulin is not the product of bone marrow derived lymphocytes. In addition to immunoglobulin a cell surface component was isolated from both cell types which bound to antigen-antibody complexes.

INTRODUCTION: Cell surface immunoglobulin of lymphocytes has recently been isolated and characterized (1,2). The approach used in these studies consisted of labeling proteins exposed on the cell surface with radioactive iodide by the enzyme lactoperoxidase and subsequently isolating immunoglobulin from solubilized labeled surface proteins by immunochemical methods. Cell surface immunoglobulin of B lymphocytes is predominantly 7S IgM which dissociates after disulfide bond reduction into two components, one with a mobility of serum immunoglobulin μ heavy chain, the other with a mobility of serum immunoglobulin light chain (1,2). Immunoglobulin of thymus and T lymphocytes has also been characterized by some workers (1, 3, 4) using these approaches. They found that T lymphocyte immunoglobulin consists of light

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Abbreviations: B, bursal equivalent derived; T, thymus derived; SDS, sodium dedecyl sulfate; FCS, fetal calf serum; PBS, phosphate-buffered saline, pH 7.3, containing 0.05 M sodium phosphate - 0.15 M NaCl; nCSP, nondialysable cell surface protein; Ig, immunoglobulin.

chain, and heavy chains which have a mobility similar to that of the μ heavy chain of serum IgM immunoglobulin. Since B and T lymphocyte surface immunoglobulin are different in their capacity to bind to macrophages (5), Marchalonis and Cone (1) suggested that some structural differences may exist between them. In this communication we present evidence that two separable types of heavy chains exist on the surface of murine B lymphocytes, whereas only one heavy chain was found on T lymphocytes. This chain differs from both heavy chains of B lymphocytes in regard to electrophoretic mobility in SDS polyacrylamide gels. In addition we have identified a surface component of B and T lymphocytes which has the property of binding antigen-antibody complexes.

MATERIALS AND METHODS: Splenocytes and thymocytes of 6-8 weeks old female CBA mice were prepared as described elsewhere (6). Cell debris and clumps were removed from thymocytes by settling the cell suspension over FCS (6). Erythrocytes were removed from splenocytes using the method of Boyle (7), and dead and clumped cells by the method of von Boehmer and Shortman (8). More than 95% viable (eosin dye exclusion) splenocytes and thymocytes were labeled with ^{125}I without any loss of viability by a modification of the lactoperoxidase method developed for labeling cultured T lymphoma cells (9), whereby the reaction was reinitiated by repeated additions of lactoperoxidase and hydrogen peroxide at regular time intervals (per 10^7 cells: 5μl 0.15 mM ^{127}I-iodide and 5μl 0.05 mM ^{125}I-iodide, 100 μCi/μl: 3 additions of lactoperoxidase, 4 additions of hydrogen peroxide). Electronmicrographic radioautography showed that the radioactive label was associated with the outer surface of the cells. Radioiodinated cells were washed once with PBS (1 ml per 10^7 cells) and labeled cell surface proteins solubilized by extraction with 1% Nonidet-P40 (9) or by metabolic release (9) in Dulbecco's modified Eagle's medium without FCS for 5 hrs. (0.7 ml medium per 10^7 cells). The labeled cell surface material was dialyzed, and then centrifuged at 12,000 g for 30 minutes. Immunoglobulin was isolated from the nCSP by a specific precipitation system which consisted of mouse IgM and rabbit antiserum to mouse IgM. Control precipitation system: fowl IgG and rabbit antiserum to fowl IgG (9, 10, 11). The coprecipitates were washed 3 - 4 times with PBS, and counted in a manual deep well gamma counter. Precipitates were then dissolved in a

buffer containing 10% glycerol, 5% mercaptoethanol, 3% SDS in 0.125 M tris-HCI, pH 6.8, which was 6M in urea, and then analyzed by polyacrylamide gel electrophoresis (10% acrylamide, 0.25% bisacrylamide) in a discontinuous buffer system according to the method of Laemmli (12). The length of gel was approximately 10 cm and each slice was 2 mm long. Reproduceability of the gels was such that differences of one slice were not significant, but differences of 2 - 3 slices were highly significant.

RESULTS AND DISCUSSION: Radioiodinated cell surface proteins of splenocytes and thymocytes were solubilized either by extraction with Nonidet-P40 or by metabolic release. After dialysis and centrifugation, immunoglobulin was isolated from the nondialysable cell surface proteins (nCSP) by immunological precipitation. Counts per minute per 10^7 cells associated with washed precipitates were as follows. Nonidet-P40 extracts of splenocytes: 1.7×10^7 in nCSP; $21 \pm 1.4 \times 10^4$ specific; $5.3 \pm 0.5 \times 10^4$ control. Metabolically released material from splenocytes: 2.6×10^6 in nCSP; $8.3 \pm 0.5 \times 10^4$ specific; $2.7 \pm 0.2 \times 10^4$ control. Metabolically released material from thymocytes: 1.5×10^6 in nCSP; $4.4 \pm 0.2 \times 10^4$ specific; $1.8 \pm 0.1 \times 10^4$ control. As previously described by Cone and Marchalonis (6) only very small amounts of immunoglobulin were detected in Nonidet-P40 extracts of radioiodinated thymocytes (2.2×10^7 in nCSP; $6.0 \pm 0.1 \times 10^4$ specific; $5.5 \pm 0.2 \times 10^4$ control).

The precipitates were dissolved in SDS buffer containing mercaptoethanol in order to cleave disulfide bonds, and then analyzed by polyacrylamide gel electrophoresis. As shown in Fig. 1, the gel patterns obtained for specifically precipitated material from Nonidet-P40 extracts (Fig. 1A) and metabolically released material (Fig. 1B) from the same population of radioiodinated splenocytes were very similar. In both cases two clearly distinct heavy chains were found. The electrophoretic mobility of one heavy chain corresponded exactly to the μ chain standard, whereas the mobility of the other chain was faster. Moreover, only one heavy chain was detected analyzing the precipitated material metabolically released from thymocytes. This chain migrated slightly faster than the μ chain standard and did not correspond in mobility to either of the heavy chains of immunoglobulin of the splenocytes. The heavy chain of thymocyte surface immunoglobulin migrated consistently 2 - 3 slices in front of the more slowly moving splenocyte heavy chain,

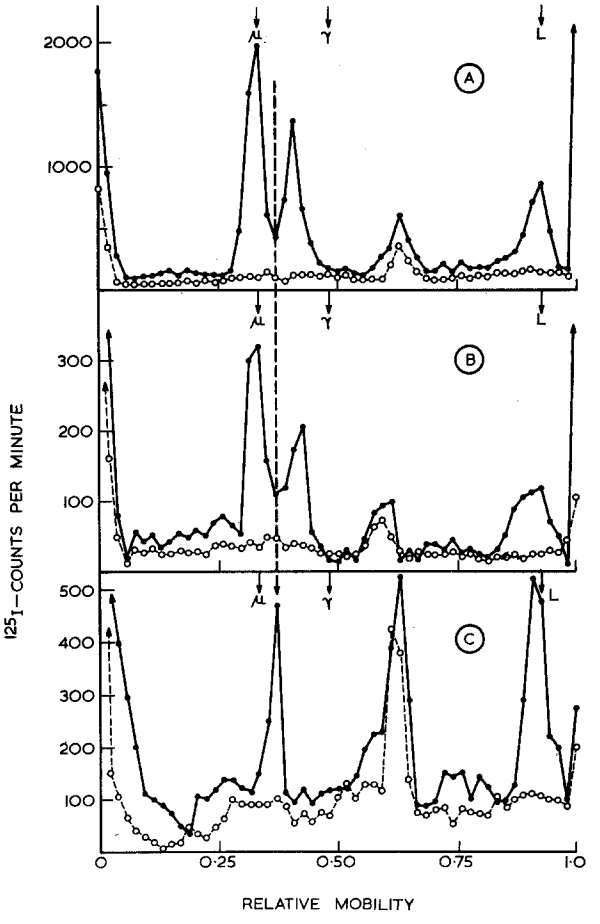

Fig. 1. Polyacrylamide gel electrophoresis (10% polyacrylamide) in SDS of ^{125}I-labeled polypeptide chains of surface immunoglobulin of splenocytes and thymocytes. A, Ig chains of splenocytes extracted with 1% Nonidet-P40; B, Ig chains of thymocytes metabolically released; ●, specific precipitates; O, control precipitates; μ, γ and L refer to positions of standard mouse immunoglobulin chains.

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but an equal distance behind the faster moving splenocyte heavy chain (Fig. 1, dotted line). This finding was confirmed in five independent experiments.

In addition to the heavy chain(s) and light chain all three patterns show a component with an electrophoretic mobility of 0.6. The mobility of this component was consistent with a molecular weight of 40,000 - 45,000. The fact that the component was also precipitated by the control

precipitation system (legend to Fig. 1) shows that this peak represents a cell surface molecule which has the property of binding antigen-antibody complexes. Since these properties are similar to those expected for the cell surface receptor which binds to the Fc portion of IgG-antibodies in antigen-antibody complexes (13) we would like to stress that this component is a possible candidate for the Fc-receptor on B and T lymphocytes. The magnitude of this peak was always small compared with the size of the immunoglobulin peaks in the case of splenocytes but large in the case of thymocytes. A component with the same properties was found on spleen cells from congenitally athymic ("nude") mice (G. Warr, unpublished observations) and T lymphoma cells (10, 11).

The surface immunoglobulin extracted by Nonidet-P40 lysis of splenocytes (fig. 1A) is predominantly of B cell origin, as very little immunoglobulin was detected in Nonidet-P40 lysates of T cells (see above). The presence of two distinct surface immunoglobulin heavy chains on murine B lymphocytes has been recently reported by other workers (14, 15, 16, 17). The nature of the second heavy chain of B lymphocytes, which has a faster mobility than that of the μ chain standard, is not yet clear. It has been proposed (15, 16) that this chain is the murine counterpart of the human δ chain. Our data provide no evidence on the antigenic properties of this chain, because our anti IgM serum had activity against both μ and light chains. The second heavy chain may also differ from the slower moving heavy chain in carbohydrate content (17). Our data comparing the ratios of the two heavy chains of immunoglobulin extracted with Nonidet-P40 (fig. 1A) and metabolically released during 5 hrs. (fig. 1B) exclude the possibility of a precursor-product relationship, as already mentioned by Abney and Parkhouse (16). It should be pointed out that our data, as well as those of other workers, do not at present resolve the question of whether both types of heavy chains are present on the same cell or on different cells.

In contrast to the B lymphocyte situation, only a single heavy chain, with electrophoretic mobility intermediate between the two B cell heavy chains, was found on thymocytes. As described elsewhere, the heavy chain of surface immunoglobulin isolated from a series of different T lymphoma cells (10) and human thymus lymphocytes (18) was also slightly faster in its mobility than μ chain standard. Whether the observed difference in mobility is

due to carbohydrate or other structural differences is not yet clear. We would like to stress that the finding of one type of T lymphocyte immunoglobulin heavy chain with an electrophoretic mobility different to both heavy chains of B lymphocytes and serum immunoglobulin M is the first physico-chemical evidence for a structural difference between T and B lymphocyte immunoglobulin. In addition, this finding excludes the possibility that the thymocyte immunoglobulin detected in our experiments is cytophilically adsorbed from serum or derived from contaminating B lymphocytes or plasma cells.

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