LITERATURE CITED

1. I. L. Chertkov, Clin. Med., No. 5, 28 (1974).
2. L. N. Lemenova and I. L. Chertkov, Probl. Gematol., No. 5, 9 (1976).
3. I. L. Chertkov, L. N. Lemenova, and O. A. Mendelevich, Probl. Gematol., No. 1, 3 (1972).
4. G. Cudkowicz and M. Bennett, J. Exp. Med., 134, 83 (1971).
5. J. W. Goodman and S. C. Shinpock, Proc. Soc. Exp. Biol. (N.Y.), 129, 417 (1968).
6. J. W. Goodman and H. B. Wheeler, Transplantation, 6, 173 (1968).
7. C. J. Gregory, E. A. McCulloch, and J. E. Till, Transplantation, 13, 138 (1972).
8. E. A. McCulloch and J. E. Till, J. Cell Physiol., 61, 301 (1963).
9. J. E. Till, S. Wilson, and E. A. McCulloch, Science, 169, 1327 (1970).

CLASSES OF CELL SURFACE IMMUNOGLOBULINS DETECTED ON RAT LYMPHOCYTES BY ENZYMIC RADIOIODINATION

V. L. Yurin, I. M. Meinert, and A. N. Uspenskii

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It was shown by the methods of enzymic radioiodination, immunoprecipitation, and electrophoresis in sodium dodecylsulfate–polyacrylamide gels that splenic lymphocytes of normal rats carry on their surface immunoglobulins of two main classes: monomeric IgM (IgM_S) and $Ig(H_2L_2)$, the heavy chains of which are a little smaller than μ chains and differ from them in their antigenic properties. The class of cell surface Ig thus revealed is evidently equivalent to human IgD and to the IgD-like protein of the cytoplasmic membrane of mouse lymphocytes. The presence of small quantities of IgG on the surface of lymphocytes can be explained both by its cytophilic properties and by the immunological state of the experimental animals.

KEY WORDS: enzymic radioiodination; classes of lymphocyte surface Ig.

The view that the immunoglobulins (Ig) of the cell membrane are antigen-identifying receptors of B lymphocytes is generally accepted [13]. However, it is not yet known how the surface Ig are bound to the cell membrane, whether the method of binding is common to all or special to each type of surface Ig, and whether differences are found in the structure of the surface and secreted Ig. As a first step toward the solution of these problems the various types of lymphocyte surface Ig must be isolated and described.

The object of this investigation was to analyze the surface immune proteins detectable by "lactoperoxidase radioiodination" of the splenic lymphocytes of normal rats.

EXPERIMENTAL METHOD

August rats aged 2-3 months were used. A suspension of splenic lymphocytes containing more than 90% of viable cells (test with 0.2% trypan blue) was obtained by density fractionation in a bovine serum albumin concentration gradient [10] and washed repeatedly in isotonic physiological buffered (pH 7.2) saline (PBS). Samples containing 10^7 cells in 20 μl PBS were treated with 10 μl of a solution of lactoperoxidase (0.25 mg/ml), 5 μl $Na^{125}I$ (100-200 μCi, specific activity 100-150 mCi/ml), and 5 μl $Na^{127}I$ (0.15 mM). Enzymic iodination

Laboratory of Cytochemical and Molecular-Biological Bases of Immunogenesis, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow.

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was initiated with 10 μl 0.03% H_2O_2 at 30°C with vigorous mixing. After 3 min a further 10 μl 0.03% H_2O_2 was added, and 3 min later the reaction was stopped by the addition of 2 ml cold PBS. The cells were sedimented and washed three times with PBS. The efficiency of iodination was 20-30% [4]. The cell suspensions were pooled and treated with NP-40 detergent (final concentration 0.5%, 2 ml solution to 10^8 cells) for 10 min at 0°C. The nuclei were removed from the cell lysates by centrifugation (2000g, 15 min) and the supernatants were dialyzed against PBS (12 h, 4°C). To prevent proteolytic degradation of the Ig during solubilization and dialysis, trasylol (500-5000 units/ml) was used.

The radioiodinated Ig was isolated from the dialyzed and centrifuged (20,000g, 20 min) lysates by indirect immunoprecipitation. Usually 100-200 μl of lysate was treated with 25 μg rabbit antibodies against rat Ig (experimental precipitates) or 25 μg normal rabbit IgG_2 (control precipitates) and, after 1-2 h, with 50-100 μl donkey antiserum against rabbit Ig. Precipitation was carried out for 10-12 h at 4°C. Normal rabbit IgG_2 and donkey antiserum were first exhausted on rat Ig (18% Na_2SO_4 fraction of serum), fixed on cellulose.

As the first component of the antibodies in indirect immunoprecipitation rabbit antibodies against μ and χ chains of rat Ig were used. Anti-μ antibodies were obtained from the serum of rabbits immunized with myeloma or normal IgM, by isolating them and exhausting them on IgM and IgG_2 globulins respectively, fixed on cellulose. Anti-χ antibodies were isolated from the serum of rabbits immunized with IgG_2 from August rats on IgG_2 (August) cellulose and exhausted on cellulose with fixed IgG_2 of MSI/BL rats. The anti-χ antibodies thus obtained were active against the RL-2 allotypical determinants in the C region of the L chains, which consist to the extent of 95% of Ig molecules of August rats [8].

The immunological specificity of the antibodies and also the necessary parameters of immunoprecipitation were established in immunological reactions with ^{125}I-labeled rat and rabbit Ig [6] and electrophoretically in sodium dodecylsulfate (DSN)–polyacrylamide gels [5].

The experimental and control precipitates were washed with cold PBS (four or five times) and dissolved in 50-100 μl 0.125 M Tris–HCl buffer, pH 6.8, containing 3% SDS, 10% glycerol, 2% β-mercaptoethanol, and 6 M urea. The samples were heated for 3-5 min at 100°C and alkylated (3-4 mg iodoacetamide). The samples were dissolved without reduction in the same buffer solution but without β-mercaptoethanol. The reduced and unreduced materials were analyzed in 10 and 5% polyacrylamide gels, respectively, containing 0.1% SDS (10 and 5% SDS gels) [7]. At the end of electrophoresis the gels were cut into pieces 1 mm thick which were counted in the GC-30 (Intertechnique) gamma spectrometer.

Reduced and alkylated normal and myeloma rat IgM, IgG_2, and IgG_1, labeled with ^{125}I, were used as markers of the position of the L, γ, and μ chains in 10% SDS gels. Rat myeloma ^{125}I-IgM, partly reduced (1 mM dithiothreitol, 1 h, 20°C) and alkylated (10 mM iodoacetamide), was used as marker of the positions of the μ_2L_2 and μL subunits in 5% SDS gel [2].

EXPERIMENTAL RESULTS

Analysis of the enzymically radioiodinated surface immunoglobulins of rat splenic lymphocytes showed that their predominant component was material which corresponded in mobility in 5% SDS gel to IgM_S (μ_2L_2) subunits of IgM. Small quantities of IgG (H_2L_2) and material with mobility similar to that of the μL subunits of IgM also were found. The typical picture of distribution in 5% SDS of the lymphocyte surface Ig precipitated from NP-40 lysates by anti-χ antibodies is shown in Fig. 1a. If anti-μ antibodies were used for precipitation, on electrophoresis in 5% SDS gel only IgM_S (μ_2L_2) could be found on the lymphocyte surface (Fig. 1c). This description of the classes of the lymphocyte surface Ig was confirmed by an investigation of the size of the popypeptide chains composing them. For instance, the IgM_S material precipitated by anti-μ antibodies broke up after reduction into L and μ chains, identical in their mobility in 10% SDS gel with the L and μ chains of rat serum IgM (Fig. 1c,d), whereas the Ig material isolated from NP-40 lysates with the aid of anti-χ antibodies broke up after reduction into chains corresponding in size to L, γ, and μ chains (Fig. 1a,b). In that case, however, considerable heterogeneity of the labeled material was found in the "μ region" of the gel, and this was clearly revealed on comparison with the relatively homogeneous distribution of the μ chains isolated from lymphocyte surface IgM_S by anti-μ precipitation (Fig. 1b, d).

The heterogeneity of the popypeptide chains of the μ region observed in 10% SDS gel could be attributed either to proteolytic degradation of the μ chains during isolation of the surface Ig of the splenocytes or to the presence of heavy chains of a different class with somewhat higher mobility than the μ chains. The first suggestion, however, seems unlikely. For instance, the data given above (Fig. 1c) indicate absence of any marked

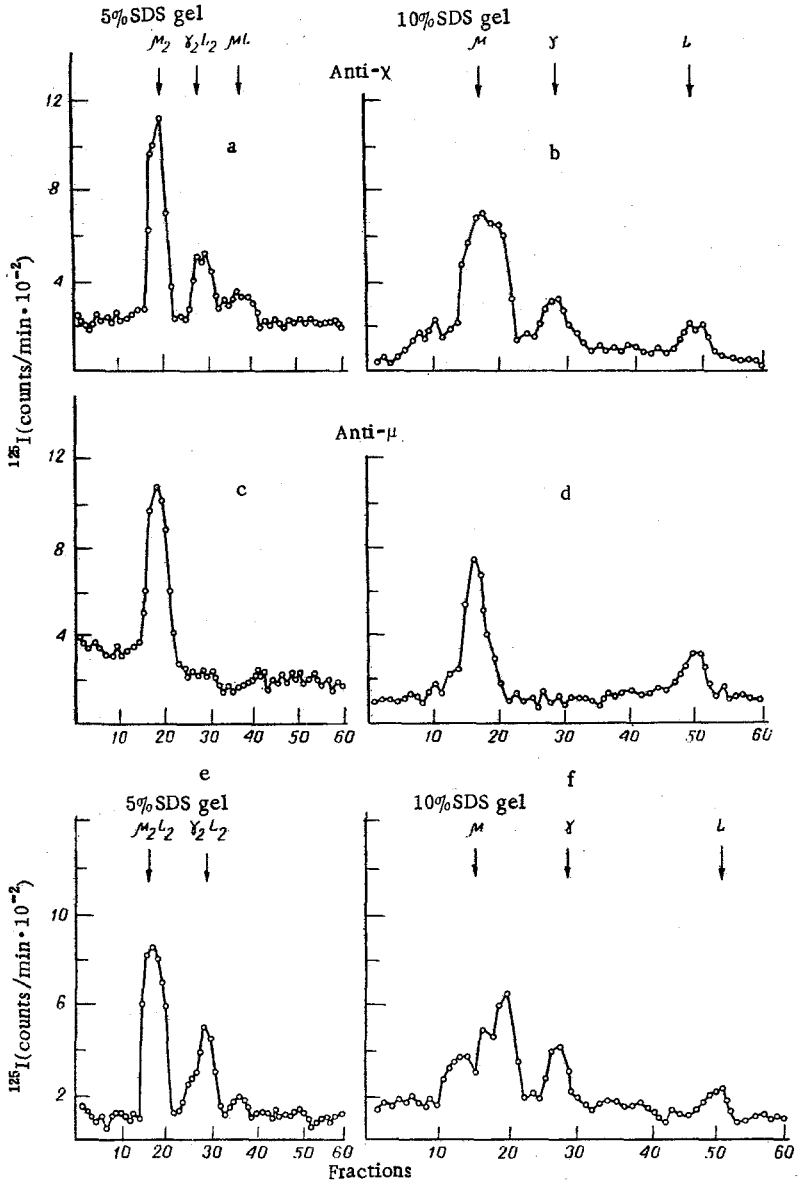

Fig. 1. Surface immunoglobulins of rat splenic lymphocytes: a and c) electrophoretic fractionation of lymphocyte surface Ig obtained by precipitation with anti-χ (a) and anti-μ (c) antibodies, in 5% SDS gel; b and d) distribution of reduced and alkylated lymphocyte surface Ig, obtained by precipitation with anti-χ (b) and anti-μ (d) antibodies, in 10% SDS gel; e and f) electrophoretic analysis of intact (e) and reduced and alkylated (f) lymphocyte surface Ig, obtained by precipitation with anti-χ antibodies of NP-40 lysates of cells from which monomeric surface IgM had previously been removed by anti-μ precipitation. Arrows mark positions occupied in parallel gels by ^{125}I-Ig markers. Results of analysis of control precipitates not shown, for they do not distort the distribution patterns of the surface IgG represented.

heterogeneity of the surface μ chains obtained by precipitation with antibodies against μ chains of both myeloma and normal rat IgM. Furthermore, no changes were found in the size of the μ chains in ^{125}I-μ_2L_2 and ^{125}I-μL (obtained by partial reduction of ^{125}I-IgM), which were added to the splenocytes and taken through the whole procedure of isolation and analysis of the surface Ig. At the same time, the hypothesis that there is a class of H chains with somewhat greater mobility than the μ chains was confirmed by the results of electrophoretic analysis of reduced anti-χ precipitates of lysates of cells from which the IgM_S had first been removed by anti-μ immunoprecipitation. As Fig. 1f shows, in experiments carried out under these conditions, H chains

smaller in size than μ chains and which, under ordinary conditions, were partly masked by them, could be detected in 10% SDS gel. The results of analysis in 5% SDS gel show that the surface Ig which contained in its composition this class of heavy chains evidently had a structure of the H_2L_2 type and, under these conditions, could not be separated from IgM_S (Fig. 1e).

The results thus show that in addition to monomeric IgM, another basic class of Ig is also present on the surface of rat splenocytes, and the heavy chains of this second class differ in their antigenic and physicochemical properties from μ chains and their mobility in SDS gel is lower than that of γ_1, γ_{2a}, and γ_{2b} chains. This Ig evidently does not belong to the IgA class either. In fact, as autoradiography shows, IgA is found on an extremely small number of rat spleen cells [14] whereas the class of Ig now described, according to rough calculations, accounts for at least 20-30% of the total surface Ig isolated. It is therefore perfectly probable that this Ig found on the surface of lymphocytes is a new class of rat immunoglobulins not previously described. This possibility is confirmed by results showing that the number of rat splenocytes binding anti-L antibodies is substantially greater than the total number of cells binding anti-μ, anti-γ, and anti-a reagents [11, 14]. This class of Ig could be equivalent to human IgD, found on the surface of a large number of peripheral blood lymphocytes of newborn infants and adults [6, 9]. IgD-like molecules with properties similar to those described in this paper have also been found recently on the surface of more than 30% of Ig-positive mouse spleen cells [1, 12]. These molecules have been shown to appear during antigen-independent differentiation of B lymphocytes immediately after IgM [12], and that some of the cells carry both classes of Ig [1]. The biological importance of the IgD-like receptor Ig for the function and further differentiation of B lymphocytes is unknown.

To conclude, the biochemical approach used in this investigation has revealed two basic and, evidently, specialized types of immunoglobulins on the cytoplasmic membrane of the lymphocytes of normal rats: a monomeric IgM and an IgD-like Ig, and if they are present in the serum, it is only in the form of minor components. The discovery of this IgG on the surface of lymphocytes may be associated both with cytophilic properties and with natural antigenic stimulation of the animals used.

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LITERATURE CITED

1. E. R. Abney, I. R. Hunter, and R. M. Parkhouse, Nature, 259, 404 (1976).
2. E. R. Abney and R. M. Parkhouse, Nature, 252, 600 (1974).
3. W. Hunter and F. Greenwood, Nature, 194, 495 (1962).
4. D. Haustein, J. Immunol. Meth., 7, 25 (1975).
5. J. Jensenius and A. Williams, Eur. J. Immunol., 4, 91 (1974).
6. W. Knapp, R. Bolhuis, et al., J. Immunol., 111, 1295 (1974).
7. U. K. Laemmli, Nature, 227, 680 (1970).
8. O. Rokhlin, T. Vengerova, and R. Nezlin, Immunochemistry, 8, 525 (1971).
9. D. Rowe, K. Hugl, et al., J. Exp. Med., 138, 965 (1973).
10. K. Shortman, N. Williams, and P. Adams, J. Immunol., 1, 273 (1972).
11. S. Strober, Transplant. Rev., 24, 84 (1975).
12. E. S. Vitetta, U. Melcher, et al., J. Exp. Med., 141, 206 (1975).
13. N. L. Warner, Adv. Immunol., 19, 67 (1974).
14. A. F. Williams, Eur. J. Immunol., 5, 883 (1975).