ISOLATION AND PARTIAL CHARACTERIZATION OF LYMPHOCYTE SURFACE IMMUNOGLOBULINS*

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Knowledge of the nature of the receptor for antigen and its role in the specific activation of lymphocytes is essential to the elucidation of the mechanisms of immune recognition and differentiation. The fact that antibody formation (1–5) and cell-mediated immunity (6–9) can be inhibited by antiglobulin reagents suggests that this receptor shares antigenic determinants with immunoglobulin. Complete analysis of the lymphocyte receptor for antigen requires isolation of the molecule and direct measurement of its binding properties for antigen. Furthermore, the manner in which the receptor is attached to the cell surface and the mechanism by which combination with antigen activates immune differentiation must be ascertained.

As an initial step in this chain of biochemical proof, we have developed a method for the covalent attachment of radioactive iodine to accessible surface proteins of living lymphocytes (10, 11). A sufficient amount of radioisotope was incorporated to facilitate fractionation of these surface proteins by a variety of biochemical and immunological means. We previously reported the isolation of low molecular weight γM immunoglobulin from the surfaces of murine and neonatal human thymic lymphocytes (12). Uhr and his colleagues (13, 14) have independently employed a similar approach to isolate "7S" γM from splenic lymphocytes of BALB/c mice.

In the present communication we report the radioiodination, isolation, and partial characterization of surface immunoglobin from neonatal human thymic lymphocytes and a variety of murine lymphocyte populations. These lymphocyte populations were chosen to provide information on the nature of surface immunoglobulin associated with bone marrow–derived lymphocytes (B cells)[1] and thymus-influenced lymphocytes (T cells).

Materials and Methods

Cell Sources and Methods of Preparation.—Male and female CBA/H/Wehi and (CBA × C57BL)F_1 mice, weighing 20–25 g each, were used as a source of normal spleens and thymuses.

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[1] *Abbreviations used in this paper:* B cells, bone marrow–derived lymphocytes; HEPES, *N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid; MEM, Eagle's minimal essential medium; PHA, phytohemagglutinin; T cells, thymus-influenced lymphocytes.

Spleen cells and thoracic duct lymphocytes were obtained from congenitally athymic nu/nu mice to provide a source of B cells.[2] Thymus cells from CBA mice which had been activated by in vitro incubation with mitomycin-killed BALB/c spleen cells (15) were kindly provided by Dr. H. Wagner. Biopsy samples of human thymus were obtained from the Royal Childrens Hospital, Parkville, Australia. These were taken from five children ranging in age from 11 days to 10 yr who were undergoing cardiac surgery. Erythrocytes were removed by hypotonic lysis and suspensions of thymus and spleen lymphocytes were prepared as previously described (11).

Suspensions of in vitro cultures of murine lymphoma (S1AT.4), two thymomas (Wehi 22 and Wehi 105), were the generous gift of Dr. A. W. Harris. These cells did not secrete readily detectable amounts of immunoglobulin. Wehi 22 possessed the Θ antigenic marker[3] which has been taken to be characteristic of T cells (16). Before radioiodination the cells were treated as described elsewhere (11).

Radioiodination of Lymphocyte Surface Proteins.—External surface proteins were iodinated with carrier-free iodide-^{125}I (The Radiochemical Centre, Amersham, Buckinghamshire, England) using the lactoperoxidase technique reported previously (11). Cell suspensions were over 95% viable before labeling and no loss of viability occurred during the process of radioiodination. The quantity of iodide-^{125}I present was determined using a Packard Autogamma Spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) equipped with an NaI crystal detector.

Fractionation of Labeled Cell Surface Proteins.—Two methods were used for isolating ^{125}I-labeled lymphocyte surface proteins. In the first approach, labeled cells were solubilized in 9 M urea–1.5 M acetic acid (11). Proteins which were soluble in urea were fractionated by gel filtration on Sephadex G-25 (Pharmacia Fine Chemicals Inc., Uppsala, Sweden) in 6 M urea–1 M propionic acid to estimate the amount of macromolecular material which had been labeled. The solubilized proteins were equilibrated with physiological buffer (pH 8.0, 0.05 M Tris-HCl–0.15 M NaCl)[4] either by dialysis or by gel filtration on Sephadex G-25 equilibrated with this buffer. The second procedure for obtaining cell surface proteins soluble in physiological buffers was based upon previous observations that lymphocytes actively release surface proteins including immunoglobulin when incubated under tissue culture conditions (12, 17).[5] ^{125}I-labeled cells were suspended to a concentration of 5×10^6–1×10^7 cells/ml in Eagle's minimal essential medium (MEM) supplemented with nonessential amino acids (F-15, Grand Island Biological Co., Grand Island, N. Y.). The medium was buffered with 20 mM *N*-2-hydroxyethyl-piperazine-*N*-2-ethane sulfonic acid (HEPES, Calbiochem, Los Angeles, Calif.). This method has been described in detail elsewhere (17).

The labeled surface proteins which were soluble in physiological media were further fractionated by gel filtration on Sepharose 6B (Pharmacia Fine Chemicals Inc.) equilibrated with Tris-NaCl. This gel provides optimum resolution between γM immunoglobulin (mol wt 900,000) and γG immunoglobulin (mol wt 150,000) and enables the estimation of molecular weights in the range 7×10^4 to 2.2×10^6 daltons (18).

In some cases, fractions resolved on the basis of molecular size by gel filtration were concentrated by ultrafiltration and reiodinated to increase the sensitivity of subsequent analysis. The second iodination was performed using the chloramine-T oxidation procedure (19).

Identification and Isolation of Immunoglobulins.—Immunoglobulins were isolated from murine samples by coprecipitation with purified mouse γG immunoglobulin and rabbit antiserum to mouse immunoglobulin. Two antisera were used: one was purchased from Cappel

[2] All cells were resistant to treatment with anti-Θ serum. Sprent, J. Personal communication.

[3] Harris, A. W., and N. L. Warner. Personal communication.

[4] tris(hydroxymethyl)aminomethane (Tris)–NaCl.

[5] Wilson, J. D., G. J. V. Nossal, and H. Lewis. Metabolic characteristics of lymphocyte surface immunoglobulin. Submitted for publication.

Laboratories (Downington, Pa.) and reacted with both murine light chains and γ-chains. The second antiserum was made by immunizing rabbits with purified mouse γM immunoglobulin. This antiserum reacted with both L chain and μ-chain. Conditions for coprecipitation were determined such that more than 80% of the carrier was precipitated. Specificity controls consisted of replacing the rabbit antiserum with normal rabbit serum. A further control was performed to determine the amount of radioactivity which was passively trapped in a heterologous antigen-antibody precipitating system. The system consisting of *Limulus* hemocyanin and rabbit antiserum to this hemocyanin was employed for this purpose. Coprecipitation analyses of protein from human thymus were performed in an analogous fashion except that rabbit antiserum made against a mixture of human γM myeloma proteins was used. This antiserum reacted with light chains and μ-chain.

Resolution of Cell Surface Immunoglobulin into Polypeptide Chains.—Coprecipitated immunoglobulin was dissolved in 9 M urea and reduced and alkylated to cleave interchain disulfide bonds (20). Reduced alkylated samples and immunoglobulin markers were resolved into polypeptide chains by disc electrophoresis in acid urea (21).

RESULTS

We have previously shown by electron micrographic radioautography that proteins radioiodinated by this lactoperoxidase technique were confined to the surfaces of lymphocytes (11). As estimated by gel filtration on Sephadex G-25, 5–10% of radioiodinated protein from thymus lymphocytes and 10–20% of labeled protein from spleen lymphocytes was macromolecular. A comparable degree of radioactivity was retained after exhaustive dialysis of the urea soluble cell surface proteins. The relative amounts of immunoglobulin present in such dialyzed fractions of cell surface proteins are shown in Table I. The results given are the means of triplicate analyses. In all thymus and spleen lymphocyte preparations, approximately 5% of the high molecular weight protein was specifically precipitated. The thymoma Wehi 22 possessed substantial quantities of surface immunoglobulin. Thymoma Wehi 105 and lymphoma S1AT.4 lacked immunoglobulin detectable by this method. The human thymus taken from an 11 day old male possessed a quantity of immunoglobulin comparable to that observed for murine lymphocytes. Similar quantities of immunoglobin were isolated from the cells of four other human thymuses.

Polypeptide Chains of Cell Surface Immunoglobulin.—The type of polypeptide chain present in the specifically coprecipitated surface immunoglobin was ascertained by gel electrophoresis in acid urea. The precipitates were dissolved in 9 M urea and reduced and alkylated to cleave interchain disulfide bonds. Unlabeled carrier immunoglobin served as an internal marker for the position of light chains and γ-chains; γM was coprecipitated and treated in an identical fashion to provide a μ-chain marker. As Fig. 1 illustrates, surface immunoglobulin from normal mouse thymus (Fig. 1 *a*) and activated thymus lymphocytes (Fig. 1 *b*) possess both light and heavy polypeptide chains. The light chains migrate in the region characteristic of light chains of the standard proteins. The heavy chains resemble the μ-chain in their penetration of the polyacrylamide gel. The polypeptide chain pattern of immunoglobulin from the lymphoma Wehi 22 resembled that given in Fig. 1 *b*.

Similar results were obtained for all immunoglobulins isolated from thymus lymphocytes. Although a component probably corresponding to dimers of light chain was occasionally observed, polypeptide chains comparable to γ-chain in mobility were not present in readily detectable amounts. This observation was further strengthened in experiments in which radioiodinated immunoglobulin was first isolated by gel filtration on Sepharose 6B, concentrated, and reiodinated. Although the sensitivity of the assay was increased over 100-fold,

TABLE I

Immunologic Precipitation of Surface Immunoglobulin from Lymphoid Cells

Lymphocyte source	Specific precipitate	Ratio specific to control
	% of high molecular weight protein	
Thymus (C57 × CBA)F_1	4.1	4.2
Activated thymus (CBA)	4.5	5.3
Thymus (neonatal human)	4.4	5.2
Spleen (C57 × CBA)F_1	4.8	3.0
Spleen (nu/nu)	4.3	3.1
Thoracic duct (nu/nu)	5.9	5.3
Wehi 22 (thymoma)	14.9	15.1
Wehi 105 (thymoma)	0.3	1.1
S1AT.4 (lymphoma)	0.5	1.3
	precipitated	
Test globulins		
Mouse γG (^{125}I-labeled)	82.3	90
Human γM (^{125}I-labeled)	80.1	70

Under these conditions the amount of precipitate brought down by normal rabbit serum ranged from 0.9 to 1.6%. The amount of radioactivity passively adsorbed to the hemocyanin-antihemocyanin complex was within this range. Data are expressed as specific precipitates, i.e. the amount precipitated by the normal rabbit serum controls were subtracted from the experimental values.

no γ-chain was observed in immunoglobulin preparations derived from thymus lymphocytes (12).

Immunoglobulins isolated from the surfaces of lymphocytes taken from spleens or thoracic duct lymph differed from those described above in possessing definite quantities of γ-chain detectable by disc electrophoresis. Fig. 2 presents data obtained by subjecting specifically coprecipitated immunoglobulin from lymphocytes from CBA × C57 spleens to reduction and alkylation followed by disc electrophoresis under dissociating conditions. The immunoglobulin resolved here was initially isolated from the cell surface by active metabolic release of surface protein (17). For this reason, three curves are shown. Curve *a* presents the graph of material coprecipitated from the culture medium at time 0; curve *b* illustrates immunoglobulin precipitated after 1 hr of incubation;

curve *c* shows immunoglobulin isolated after 3 hr of incubation. Curve *a* is essentially negative. Definite quantities of immunoglobulin were released by 1 hr. Light chains and μ-type heavy chains were present, and the electropherogram is similar to that independently obtained by Bauer et al. (13) for surface immunoglobulin of splenic lymphocytes. Immunoglobulin released by 3 hr was present in greater quantity and contained moderate amounts of γ-chain. Results

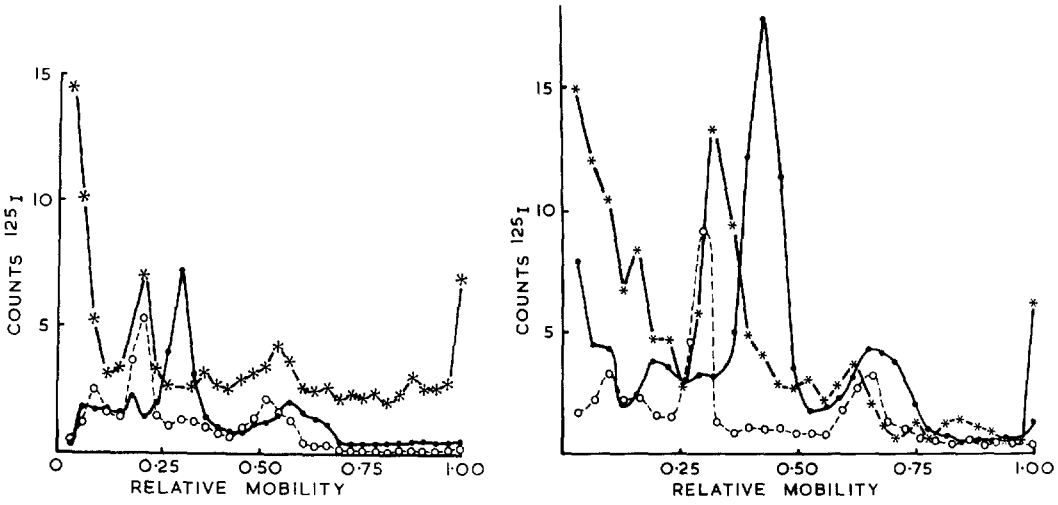

FIG.1 *a* FIG.1 *b*

FIG. 1. Comparison by disc electrophoresis in acid urea of ^{125}I-labeled polypeptide chains of surface immunoglobulins from mouse thymus lymphocytes with polypeptide chains of purified γM and γG immunoglobulins. Fig. 1 *a*: *–*, surface immunoglobulin polypeptide chains of thymus lymphocytes from CBA × C57 mice, cpm × 10^{-2}; ●—●, polypeptide chains of mouse γG immunoglobulin, cps × 10^{-1}; ○---○, polypeptide chains of human γM immunoglobulin, cps × 10^{-1}. 7% polyacrylamide. Relative mobilities of standard chains: light, 0.45–0.65; γ, 0.25–30; μ, 0.15–0.20. Fig. 1 *b*: *—*, surface immunoglobulin of activated thymus cells from CBA/H/Wehi mice, cpm × 10^{-2}; ●—●, polypeptide chains of mouse γG immunoglobulin, cps × 10^{-1}; ○--○, polypeptide chains of human γM immunoglobulin, cps × 10^{-1}. 6% polyacrylamide. Relative mobilities of standard chains: light, 0.55–0.75; γ, 0.40–0.48; μ, 0.27–0.33. All samples were specific coprecipitates which were reduced and alkylated in the presence of 9 M urea.

obtained when immunoglobulin was solubilized by acid urea treatment of cells were identical to those given in curve *c*. In an isolation and reiodination experiment similar to that described above, the predominant (>90%) heavy chain was found to be γ-chain. γM immunoglobulin, thus, is readily detectable on the intact cell by our surface-labeling reagent, although γG is the major immunoglobulin secreted by these cells.

Since normal spleen cells contain a mixture of T and B lymphocytes, experiments were carried out on spleen and thoracic duct cells of nu/nu mice which

provide pure populations of B cells. Electropheretic patterns of immunoglobulin chains obtained from the surfaces of these lymphocytes were similar to those described for normal spleens. Light chains were present as monomer and dimer and both μ-chains and γ-chains were detected. In terms of counts of polypeptide-^{125}I recovered, there was at least twice as much μ-chain as γ-chain.

The relative amounts of polypeptide chains of the cell surface immunoglobulin resolved by disc electrophoresis are given in Table II. Immunoglobulins iso-

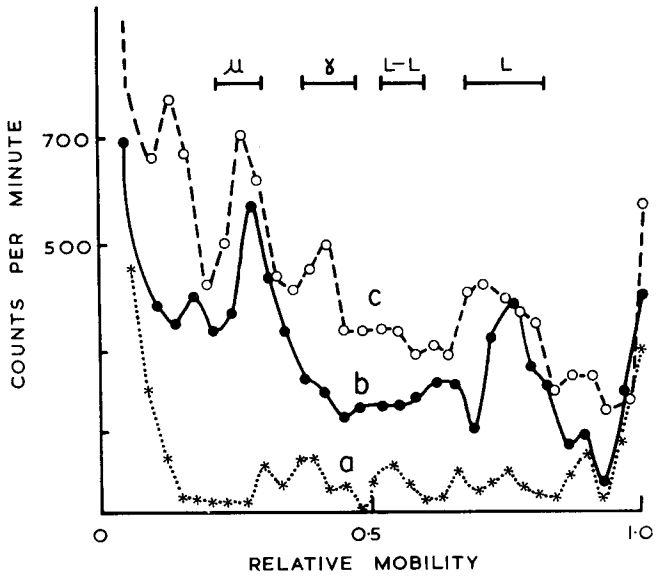

FIG. 2. Analysis of polypeptide chains of immunoglobulin isolated from the surfaces of spleen lymphocytes from CBA × C57 mice by disc electrophoresis in acid urea. Surface iodinated lymphocytes were incubated in tissue culture medium at 37°C and samples were specifically coprecipitated at time 0 (*a*), 1 hr (*b*), and 3 hr (*c*). The coprecipitates were dissolved in 9 M urea and reduced and alkylated. The bars indicate the positions of polypeptide chains from purified, coprecipitated immunoglobulins which were used as standards: μ, μ-chains; γ, γ-chain; *L–L*, light chain dimer; *L*, light chain. 6% polyacrylamide.

lated from thymus cell populations possessed light chains and μ-type heavy chains, whereas immunoglobulins from spleen and thoracic duct lymphocytes contained heavy chains resembling both μ- and γ-polypeptides in gel penetration. The ratio of μ-chains to γ-chains ranged between 1.7/1 and 3.5/1. The number of ^{125}I counts in heavy chains relative to those in light chains should provide a measure of the degree of accessibility of the polypeptide chains to the labeling reagent. The ratio of heavy chains to light chains in normal human and murine thymus (approximately 1/1) was low compared to the result which would be expected if this ratio strictly reflected mass of the individual chains (3.1/1). This ratio increases for activated thymus cells (1.95) and was close to

the theoretical value in Wehi 22. Immunoglobulins obtained from splenic lymphocytes of CBA × C57 and nu/nu mice were characterized by H/L ratios of approximately 1.6.

Estimate of the Size of Cell Surface Immunoglobulin.—The above results show that immunoglobulin polypeptide chains can be isolated from the surface of lymphocytes but give no information regarding the state of association of the intact immunoglobulin. For this reason, we carried out experiments to fractionate soluble cell surface protein on the basis of size using Sepharose 6B. This gel was chosen because it provides optimum resolution (18) of γM pentamer (mol wt 900,000) and γG immunoglobulin (mol wt 150,000). Fig. 3 presents the results obtained for cell surface proteins isolated from murine-activated thymus cells. Because of the great similarity we observed among gel filtration patterns of soluble surface proteins from all lymphocyte populations examined, this

TABLE II

*Labeled Polypeptide Chains from Cell Surface (Percentage of ^{125}I Radioactivity in Immunoglobulins)**

Lymphocyte source	μ	γ	Light	Ratio H/L
Thymus (mouse)	55	0	45	1.22
Thymus (man)	49	0	51	0.96
Thymus (in vitro activated)	66	0	34	1.95
Spleen (mouse)	47	15	38	1.64
Spleen (nu/nu)	43	17	40	1.55
Thymoma (Wehi 22)	74	0	26	2.85

* Identifications were made on the basis of electrophoretic mobility on polyacrylamide gel. The percentages given are the means of at least three gel experiments.

pattern may be taken as representative of the size distribution of human and murine lymphocyte surface proteins.

Immunoglobulin was located by performing coprecipitation analysis on the isolated fractions. We were able to detect immunoglobulin only in the fractions localized between the γM and γG markers as indicated by the bar. This antigenic activity corresponded to a visible shoulder which slightly preceded the γG marker. By comparison with these immunoglobulin standards and other markers (18), this component possesses a molecular weight ranging between 180,000 and 200,000. In other experiments the immunoglobulin fraction was concentrated and reiodinated. As shown in Fig. 4 immunoglobulin isolated in this fashion from neonatal human thymus chromatographed truly when subjected to a second analysis on Sepharose 6B. Over 80% of the reiodinated protein emerged from the column in fractions comparable to those initially pooled. The major component eluted slightly ahead of the γG marker and a shoulder of material characterized by a high molecular weight was also present. Since the second iodination was carried out using chloramine-T some aggregate was

present at the void volume. Free iodide emerged at the column volume. We have previously shown (12) that only μ-type heavy chains were present in surface immunoglobulins prepared from thymus lymphocytes by this approach.

These results indicate that γM immunoglobulins isolated from the surfaces of thymic and splenic lymphocytes exist as low molecular weight forms, rather than as the pentamer usually found in serum. The possibility must be considered that pentameric γM may be dissociated into 7S subunits by the iodination procedure. To test this possibility, human and murine γM myeloma proteins were

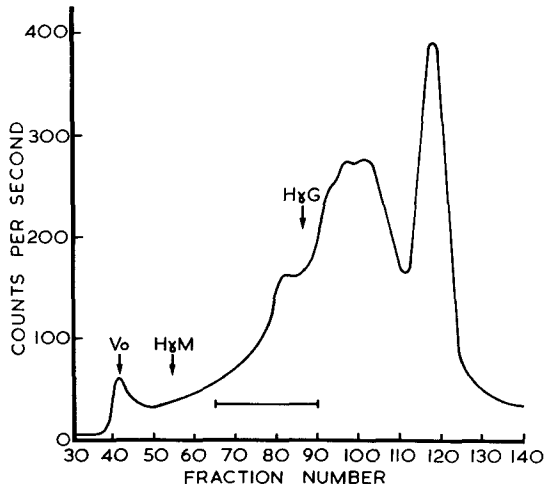

FIG. 3. Gel filtration on Sepharose 6B of radioiodinated surface proteins of thymus lymphocytes of CBA/H/Wehi mice. Cells were activated by in vitro incubation with allogeneic cells as described in reference 15. ^{125}I-labeled cells were solubilized in 1 M acetic acid–9 M urea. Soluble proteins were dialyzed against Tris-NaCl before chromatography. Elution buffer, Tris-NaCl; *Vo*, void volume; *HγM*, elution position of human γM immunoglobulin (mol wt 900,000); *HγG*, elution position of human γG immunoglobulin (mol wt 150,000); |—|, position of elution of surface immunoglobulin detected by specific coprecipitation with rabbit antiserum to mouse immunoglobulin.

iodinated using the lactoperoxidase method and chromatographed on Sepharose 6B. No conversion of the intact forms to the 180,000 mol wt subunit was observed under the conditions employed here.

Origin of Cell Surface Immunoglobin.—Since immunoglobulin present on the surface of lymphocytes may be of either exogenous or endogenous origin, we performed two types of experiments to distinguish between these alternatives. Firstly, we attempted to determine if purified immunoglobulins would adsorb to lymphocytes in sufficient quantity to be detected during our washing and extraction procedures. A human γM Waldenstrom protein (Frymac) and its 7S subunit and normal human and murine γG immunoglobulin were labeled with iodide-^{125}I using the lactoperoxidase technique. Thymus and spleen lympho-

cytes were incubated with these radioiodinated proteins and the degree of binding was assessed by radioautographic techniques (22) and radiation counting. After our standard washing procedure (11) the amount of radioactivity detected on cells was negligible. Our second approach was based upon the fact that lymphocyte surface proteins, including immunoglobulins, are actively released from the cell surfaces (17). We have previously shown that ^{125}I-labeled γG immunoglobulin from normal rabbit serum which was bound to

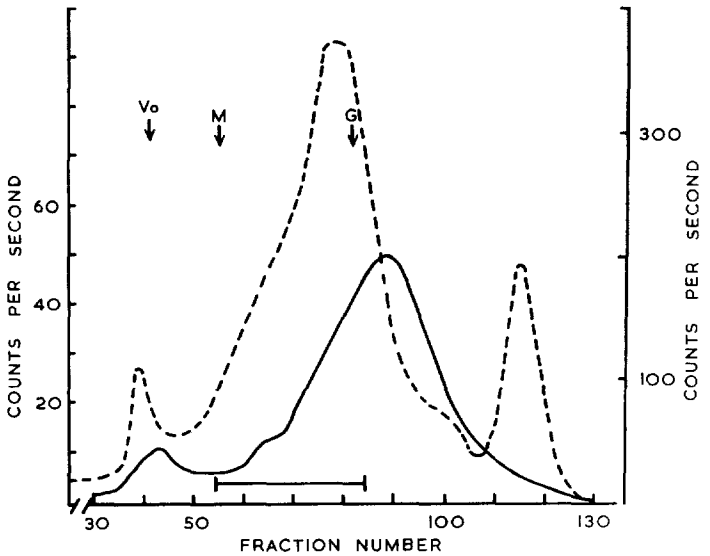

FIG. 4. Gel filtration on Sepharose 6B of ^{125}I-labeled surface proteins of lymphocytes from neonatal human thymus (11 day old male). —, cell surface was iodinated by the lactoperoxidase technique. Cells were solubilized in 9 M urea– 1 M acetic acid and soluble protein was dialyzed against Tris-NaCl. The fractions indicated by the bar were pooled, concentrated, and reiodinated using the chloramine-T procedure (19). ---, elution profile of reiodinated samples; *Vo*, void volume; *M*, elution position of human γM immunoglobulin (mol wt 900,000); *G*, elution position of human γG immunoglobulin (mol wt 150,000).

murine lymphocytes was virtually eliminated within 15 min if the labeled cells were incubated at 37°C under tissue culture conditions (17). Fig. 5 presents data obtained by incubating thymus and spleen lymphocytes which were radioiodinated by our technique and specifically coprecipitating immunoglobulin released into the medium as a function of time. The amount of immunoglobulin increased linearly for at least 2 hr for both cell populations and the amounts of immunoglobulin expressed as percentage of total released counts at each time point were indistinguishable. In similar experiments, thymus cells were labeled with ^{125}I, incubated for 2 hr, and the supernatant was recovered for coprecipitation analysis. The cells were then relabeled with ^{131}I and incubated a further 2 hr. The amount of ^{131}I-labeled surface immunoglobulin detected in

supernatants from the second phase of incubation was comparable to that detected as ^{125}I-labeled immunoglobulin in the first incubation phase.

DISCUSSION

In 1900 Ehrlich proposed that antibodies serve as the cell surface receptors for antigen (23). Recent studies have provided evidence supporting the essential truth of this prediction for the lymphocytes directly engaged in the synthesis of circulating antibodies, the B cells (2–4). Indirect evidence (1, 5, 6) also substantiates the conjecture that immunoglobulin serves as the antigen receptor on T lymphocytes which mediate cellular immune responses. In this

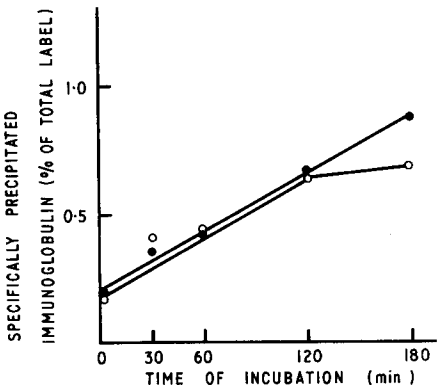

FIG. 5. Kinetics of the release of surface immunoglobulin from thymus and spleen lymphocytes of CBA × C57 mice. O—O, ^{125}I-labeled surface immunoglobulin of thymus lymphocytes; ●—●, ^{125}I-labeled surface immunoglobulin of spleen lymphocytes. Each point represents the mean value of two experiments; in each experiment, each point was done in triplicate.

paper, we have used the lactoperoxidase technique (10, 11) to incorporate iodide-^{125}I into proteins present on the surface of living lymphocytes in sufficient quantity to enable direct isolation of surface immunoglobulin. Lymphocytes taken from spleen, thoracic duct, and thymus possessed detectable quantities of surface immunoglobulin.

Both γM and γG immunoglobulins were isolated from splenic lymphocytes of C57 × CBA mice. A similar situation obtained with splenic and thoracic duct lymphocytes from congenitally athymic nu/nu mice which serve as a source of pure B lymphocytes. We consistently observed that the amount of μ-type heavy chain resolved on disc electrophoresis in acid urea was two to three times greater than the amount of γ-chain. Moreover, the γM immunoglobulin was isolated as a molecular species with a molecular weight of approximately 200,000, suggesting that the molecular formula is $L_2\mu_2$. Since the 19S form of the human Waldenstrom macroglobulin Frymac was not dissociated by iodina-

tion using the lactoperoxidase technique we conclude that the 7S form isolated from the surface represents the native state of the molecule.

We examine three populations of thymus lymphocytes in order to obtain information on surface immunoglobulins of thymus-derived lymphocytes. Lymphocytes taken from CBA × C57 mouse thymus and neonatal human thymus possessed 7S γM immunoglobulin similar to that isolated from B cells. Similar results were achieved with CBA thymus cells which were activated by in vitro incubation with mitomycin-treated BALB/c spleen cells. In no case did we observe γ-chain to be present on thymus cell populations. The lack of γG on thymus cells was confirmed by experiments in which the "7S region" of Sepharose-6B gel fractionation of surface-radioiodinated thymus lymphocytes was concentrated and reiodinated to increase the sensitivity of the detection system.

The observation that ^{125}I-labeled cells release surface immunoglobulin when incubated in tissue culture medium and exhibit similar behavior after being relabeled with ^{131}I indicates that the immunoglobulin detected is actually produced within the cell populations under consideration. Inasmuch as the release of cell surface proteins is an active process requiring cellular respiration and protein synthesis (17) the release of cell surface immunoglobulin and its reappearance at the cell surface may well represent turnover of these molecules. We would emphasize that the rate of secretion of immunoglobulins by plasma cells is at least two orders of magnitude greater than that of the release process studied here. We have previously discussed possible regulatory events mediated through this process (17).

The amount of immunoglobulin isolated from thymus and B cell populations was very similar when normalized to percentage of high molecular weight cell surface protein. This observation makes it unlikely that the immunoglobulin isolated from thymus cells represents contamination by a small fraction of B lymphocytes. Using our observations that immunoglobulin comprized approximately 5% of such protein and possessed a mol wt of 150,000–200,000, plus the measurements of Allan and Crumpton (24) on the mass contributions of porcine lymphocyte membranes, we calculate that each cell possessed 1×10^5–3×10^5 immunoglobulin molecules. The fractionation patterns obtained by Allan and Crumpton (24) for deoxycholate-soluble porcine membrane proteins on Sepharose 6B were similar to those we obtained for soluble high molecular weight fractions from human and murine lymphocytes.

The isolation of γM and γG immunoglobulins from B cells is consistent with a number of studies employing fluorescent (25) or radioactively labeled antiglobulin reagents (26, 27) to detect μ- and γ-chains on lymphocyte surfaces. Moreover, the fact that γM is present as a 7S molecule concurs with observations by Klein and Eskeland on lymphocytic leukemia cells (28) and Uhr and his colleagues on murine splenic lymphocytes (13, 14). Radioiodinated antiglobulin reagents readily detect immunoglobulin on B cells but require extensive exposure to detect immunoglobulin chains on T cells by radioautography (26, 27,

29). These observations have prompted the hypothesis that B cells have a high density of surface immunoglobulin, whereas T lymphocytes possess a much lower density of immunoglobulin (27, 29). The fact that we have isolated approximately equal amounts of γM immunoglobulin from B cells and a variety of thymus cell populations suggests that the well-confirmed difference in labeling between T cells and B cells represents relative accessibility of surface immunoglobulins rather than absolute number of molecules. The observation by Allan and Crumpton that isolated membranes from porcine thymus and lymph node lymphocytes possess equivalent numbers of binding sites for phytohemagglutinin (PHA) but that the living cells do not respond with equal vigor to this mitogen (30) is consistent with our proposal of differential accessibility of

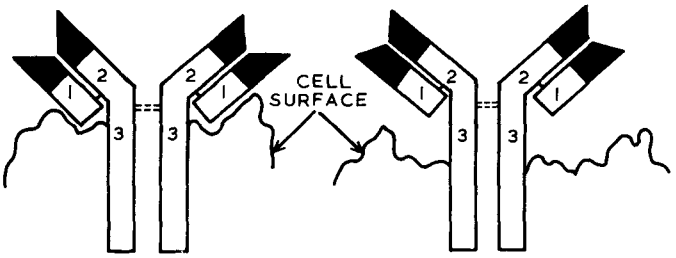

FIG. 6. Hypothetical model for the presentation of immunoglobulin upon the lymphocyte surface. The diagram on the left illustrates a situation where the Fc portion of the μ-chain is surrounded by surface components. This condition may hold for thymus-influenced lymphocytes. The diagram on the right presents a situation in which all of the Fab piece and most of the Fc region of the heavy chain are exposed. This situation occurs with activated thymus cells and bone marrow–derived lymphocytes. The dark regions represent variable regions of the light and heavy polypeptide chains which share the combining site for antigen. 1, constant region of light chain; 2, Fd piece; 3, Fc region of heavy chain.

surface receptors. This concept of accessible surface immunoglobulin is illustrated in Fig. 6. Our data on the ratio of labeled heavy chain to labeled light chain suggest that light chains are more accessible to the radioactive iodine than are the μ-chains on normal thymus cells. However, relatively more μ-chain was expressed by the activated thymus cells and by the thymoma Wehi 22. This implies that the $L_2\mu_2$ molecule is attached to the cell surface via the μ-chain and that some other surface components may obstruct the approach of labeling reagent. Steric problems would be acute in the case of antiglobulin reagents because these are the same dimensions as the target molecule and bind to accessible antigenic sites which comprise a small portion of the target. As represented in Fig. 6, an antiglobulin molecule would be unable to approach the heavy chain specific determinant on the Fc portion of the μ-chain under these conditions. Antibodies to light chain could bind to the type-specific determinant on the constant half of the light chain and possibly antibodies specific for the Fd piece might attach. Since the antibody to light chain is comparable in size to the cell surface γM unit, major geometric constraints could be in-

volved in binding more than one antiglobulin per surface immunoglobulin. Such a model may account for the observation that murine T cells bind antibodies to the κ-chain much better than they do antiglobulins specific for heavy chains (27, 29). Our approach may obviate many of the steric difficulties involved above because the enzyme need not contact the surface immunoglobulin directly to catalyze iodination of accessible tyrosines. The lactoperoxidase, activated by H_2O_2, oxidizes iodide to iodine radical which can act directly upon the tyrosyl residue. Furthermore, iodine formed by the combination of two iodine radicals can substitute onto the phenolic ring of tyrosine at neutral or alkaline pH (31). Thus, lactoperoxidase-catalyzed iodination may occur via the diffusion of a small reactive molecular species (mol wt 125 or 250) which can readily penetrate where bulky macromolecular reagents (mol wt 150,000) cannot. Identification of immunoglobulin in our system occurs during the isolation procedure. We should emphasize that delineation of the structural properties of the lymphocyte surface possibly entails further complexity because these cells possess an uncharacterized cell coat or glycocalyx (32).

Functional studies suggest that the receptor on T cells resembles the immunoglobulin receptor on B cells in specificity and antigenicity. Basten et al. (5) have elegantly shown that specific elimination of T cells by radioactive antigen is inhibited by treatment with antibodies to light chain. Functions of T cells which can be inhibited by antiserum to light chains include graft-*versus*-host reaction (6), "helper" activity (7), and immunocytoadherence (8).[6] Moreover, Greaves and Hogg (8) have presented evidence that μ-chain as well as light chain contributes to the binding site in immunocytoadherent reactions of θ-positive lymphocytes. In addition, Dwyer et al.[7] have shown that antigen-binding cells in the human thymus can be inhibited by antisera to light chains and μ-chains. Our data substantiate these indirect studies.

The fact that γM immunoglobulin occurs on the surface of lymphocytes raises an interesting evolutionary possibility. This class of immunoglobulin, expressed as both 19S and 7S forms, arose early in vertebrate phylogeny (33–35) and statistical evidence suggests that the μ-chain has had a conservative evolutionary history (36). A strong selective factor in maintaining this ancient immunoglobulin molecule may have arisen from its probable role as a surface receptor for antigen. The finding of 7S γM immunoglobulin on T lymphocytes and "primitive" lymphoid tumor cells (28, 37) probable recapitulates the fact that γM molecules emerged phylogenetically before the appearance of plasma cells, the end stage of lymphocyte development (38). The presence of large amounts of 7S γM in the serum of lower vertebrates (33–35) may represent the overproduction of surface immunoglobulin.

[6] Marchalonis, J. J., R. E. Cone, and R. T. Rolley. Antigen binding by thymus-influenced lymphocytes: inhibition by antiserum to immunoglobulin light chain. Submitted for publication.

[7] Dwyer, J. M., N. L. Warner, and I. R. Mackay. Specificity and nature of the antigen combining sites on fetal and mature thymus lymphocytes. *J. Immunol.* In press.

The presence of accessible immunoglobulins on the surface of T and B lymphocytes coupled with the body of observations that antisera to immunoglobulin chains inhibit immune functions by these cells establish a strong circumstantial case for the hypothesis that antibody serves as the cellular receptor for antigen. The experimental approach developed here renders feasible the isolation of cell surface immunoglobulin and conceivably direct measurement of the binding properties of these molecules.

SUMMARY

Immunoglobulins were isolated from the surfaces of lymphocytes from a variety of lymphocyte populations including murine and human thymus lymphocytes and murine spleen and thoracic duct lymphocytes. Cell surface proteins were labeled with iodide-^{125}I by lactoperoxidase-catalyzed iodination, and recovered in solution either by solubilization in dissociating solvents or active metabolic release. Immunoglobulins were identified and isolated by immunological coprecipitation. The polypeptide chain structure of immunoglobulins isolated from lymphocyte surfaces was analyzed by polyacrylamide gel electrophoresis of reduced, alkylated samples in acid urea. Human and murine thymus lymphocytes possessed only IgM immunoglobulin on their surfaces. This protein contained light chains and μ-type heavy chains and was characterized by a molecular weight of approximately 200,000. Murine splenic lymphocytes from CBA × C57 animals and congenitally athymic (nu/nu) mice possessed both IgM and IgG on their surfaces. The ratio of μ-chain to γ-chain was about 3/1. The presence of IgM on thymus lymphocytes probably does not reflect trace contamination by B lymphocytes because comparable quantities of IgM were isolated from both cell populations. Metabolic turnover data suggest that this immunoglobulin is synthesized by the cell population studied.

These results provide direct evidence for the presence of immunoglobulins composed of light and heavy polypeptide chains on the surfaces of lymphocytes of all classes.

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