A RECEPTOR FOR ANTIBODY ON B LYMPHOCYTES

II. Immunochemical and Electron Microscopy Characteristics*‡

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The preceding article contains evidence for the existence on all B lymphocytes, but not on T lymphocytes, of a membrane-associated receptor for antibody (1). Antibody-binding lymphocytes were detected by a sensitive radioautographic technique using ^{125}I-labeled antigens. The present paper is devoted to a study of the type of antibody capable of attachment to the B cell and the distribution of this receptor on the cell surface. Data are presented to show that the receptor, although a marker for mature B cells (antibody-forming cell precursors), is absent from hematopoietic precursor cells and is lost during differentiation to antibody-forming cells.

Materials and Methods

Animals.—Mice of the highly inbred CBA strain were used as a source of cells in the majority of experiments (1). Tumors (*vide infra*) were serially transplanted in syngeneic mice. Their strain of origin, induction, and maintenance will be described elsewhere.[1] Five lines (plasma cell tumors MOPC-460, XCI and HPC-6, lymphoma SIAT.4, and mastocytoma P815) were obtained from Dr. A. W. Harris as continuous cultures in vitro.

Thoracic Duct Cannulation.—Cannulation was carried out by the method of Miller and Mitchell (2).

Thymectomy.—The procedure for preparing adult thymectomized irradiated bone marrow protected mice (TxBM)[2] was described in the previous paper (1).

Irradiation.—This was carried out as in reference 1.

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[1] Warner, N. L. Plasma cell tumor induction in NZB and NZB hybrid mice. Manuscript in preparation.

[2] *Abbreviations used in this paper:* BSA, bovine serum albumin; CoF, purified low molecular weight anticomplementory factor; FCS, fetal calf serum; FγG, fowl immunoglobulin G; HGG, human gamma globulin; SRBC, sheep red blood cells; TDL, thoracic duct lymphocytes; TxBM, adult thymectomized, irradiated, and marrow protected.

Preparation of Cell Suspensions.—Single cell suspensions of spleen, bone marrow, and thoracic duct lymphocytes (TDL) were obtained as outlined in the previous paper (1). Unless otherwise stated TDL from normal mice were used in all assays.

Antigens.—Fowl immunoglobulin IgG (FγG) was obtained from chicken serum as described previously (3). Before iodination it was absorbed extensively against mouse lymphocytes to remove nonspecific anti-mouse activity.

Iodination.—FγG was iodinated with ^{125}I (Radiochemical Centre, Amersham, England, catalogue No. IMS 3) according to the method of Byrt and Ada (4). A specific activity of approximately 50 μCi/μg FγG was used in all experiments with the exception of the electron microscope studies where a higher level of radioactivity viz. 150 μCi/μg was required. 5% bovine serum albumin (BSA) (Cohn fraction V, Armour and Company Ltd., Eastbourne, England) provided a source of carrier protein in the procedure.

Antisera.—Antisera to FγG were obtained from hyperimmune CBA mice as described in the previous paper (1) and heat inactivated before use. Their antibody activity and that of any fragments prepared from them was titrated by a hemagglutination technique using sheep red blood cells (SRBC) coated with fowl anti-SRBC IgG (3).

Assay for Antibody-Binding Cells.—A detailed description of the assay was presented in the previous paper (1). Briefly, single cell suspensions were incubated for ½ hr at 37°C in whole anti-FγG antiserum or in various antibody fractions obtained from it at a concentration of 50×10^6 cells/ml. The cells were then centrifuged once, the supernatant removed, and the cell pellet resuspended immediately in a solution of FγG-^{125}I. After a further incubation period of ½ hr at 4°C, the cells were washed free of excess radioactive material by centrifugation through gradients of fetal calf serum (FCS) (Commonwealth Serum Laboratories, Melbourne, Australia). Smears for radioautography were made in 100% FCS and processed as described previously (1). In general 500–1000 cells were counted per slide.

BSA Gradients.—In experiments designed to examine whether complement was required for binding of antibody to B cells, it was essential to avoid exposing the lymphocytes to a source of complement at any stage of the assay. Gradients for removal of excess FγG-^{125}I were therefore made with BSA not FCS. A stock solution of BSA, pH 5.1, was prepared according to the method of Shortman et al.[3] Before use it was diluted with unbuffered salt solution to a final concentration of 20% w/w (density 1.060 g/mm^3 at 4°C). Cells to be centrifuged through BSA were suspended in Dulbecco's phosphate-buffered saline (5) of mouse isotonicity. The osmolarity of this solution was 269 mOsM which is equivalent to 0.168 M NaCl.[4]

Electron Microscope Radioautography.—Cells for study were labeled with anti-FγG and FγG-^{125}I of high specific activity (viz. 150 μCi/μg FγG) as described above. After removal of excess radioactive antigen, they were divided into aliquots one of which was processed for standard light microscopy. The other was resuspended in 50% FCS and centrifuged in small cellulose nitrate Spinco tubes (3/16 × 1⅝ inch) (Spinco Division of Beckman Instruments, Inc., Palo Alto, Calif.). The loose pellet was fixed in the tube by layering over it 2.5% glutaraldehyde in 0.1 M cacodylate buffer. 5 min later the glutaraldehyde was removed and replaced with fresh fixative for a further 2 hr. The bottom of the tube containing the fixed cells was then cut off and placed in a large volume of buffer (0.1 M cacodylate) overnight. Finally the pellet was postfixed in 2% osmium tetroxide for 2 hr and dehydrated in a graded series of acetone. Absolute acetone dissolved the cap of cellulose nitrate protecting the pellet.

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[3] Shortman, K., N. Williams, and P. Adams. The separation of different cell classes from lymphoid organs. V. Simple procedures for the removal of cell debris, damaged cells, and erythroid cells from lymphoid cell suspensions. Submitted for publication.

[4] Williams, N., N. Kraft, and K. Shortman. The separation of different cell classes from lymphoid organs. VI. The effect of osmolarity of gradient media on the density distribution of cells. Submitted for publication.

This method allowed very small quantities of cells to be processed since losses due to mechanical handling of the pellet were avoided. After embedding in Araldite (Ciba Pharmaceutical Company, Summit, N. J.) thin sections (600–800 A) were cut with glass knives on an LKB Ultrotome III ultramicrotome (LKB Instruments Inc., Rockville, Md.). The sections were picked up with a fine glass probe and placed on a drop of glass distilled water on a collodion-coated slide. After drying, the slide was coated with a thin layer of carbon (approximately 50 A thick), dipped in Kodak NTE emulsion, and exposed for 10–14 days. The exposed slides were developed in Dektol (Kodak) and after fixation, the sections were stripped off and mounted on unsupported 100-mesh copper grids. The cells were examined unstained in a Philips EM 300 electron microscope (Philips Electronic Instruments, Mount Vernon, N. Y.) at 60 kv. In addition to radioautographs, conventional thin sections stained with uranyl acetate and lead citrate were examined.

Fractionation of Antisera.—A pool of hyperimmune CBA anti-FγG serum was separated into IgM- and IgG-containing fractions by gel filtration through Sephadex G-200 (Pharmacia Fine Chemicals Inc., Uppsala, Sweden). Electrophoretic separation of a slow migrating IgG fraction was made in starch Geon with a pH 8.5 Veronal buffer. Separation into IgG2- and IgG1-rich fractions was made by electrophoresis in 1% Ionagar pH 8.2 Veronal buffer on glass plates as previously described (6). Quantitative estimation of IgG1 and IgG2 content of these fractions was performed by the inhibition of precipitation technique using ^{125}I-labeled myeloma proteins and monospecific heavy chain antisera (6). Preparation of the Fab fragment by papain digestion was performed according to the method of Porter (7).

Immunoglobulin Preparations for Inhibition Studies.—A series of purified myeloma proteins were isolated by standard methods (8) from the sera of mice bearing plasma cell tumors. The immunoglobulin class of the proteins used is given in Tables II and III. The IgG fractions of several class specific rabbit anti-mouse immunoglobulin sera were isolated by electrophoresis in starch Geon (pH 8.5 Veronal buffer). Pepsin digestion (1:50 ratio pepsin to protein) was performed as previously described (9) followed by separation of the F(ab)'$_2$ fragment on Sephadex G-150 gel filtration.

RESULTS

Direct Binding of Fractionated Antisera to B Lymphocytes.—To determine whether the property of binding of an antibody to the B cell surface was shared by all immunoglobulin classes, a standard pool of mouse anti-FγG antiserum was fractionated by electrophoresis and gel filtration. The original antiserum and fractions obtained from it were incubated with normal TDL (12–20% of which are usually B cells [1]) followed by FγG-^{125}I. 13% of this particular TDL preparation bound the unfractionated antiserum (Table I). The binding capacity of the various fractions was then compared by using amounts containing similar antibody activity. Significant labeling occurred with the excluded IgM fraction from Sephadex G-200 gel filtration, although the quantity of antigen bound in the complex was less, as shown by mean grain counts.

Electrophoretic separation of the antiserum in agar gel, resulted in a relatively pure IgG2 fraction (containing IgG_{2a} and IgG_{2b}) and a faster migrating fraction which had approximately equal amounts of IgG1 and IgG2. Although both fractions gave significant binding, the percentage of cells with complexes, and the amount of complex as determined by grain counts, was greater with the fraction containing IgG1 antibody.

A total IgG fraction obtained from starch block electrophoresis also labeled a

significant number of TDL, whereas a similar concentration of the Fab fragment of this fraction gave no binding at all.

Inhibition of Antibody Binding to B lymphocytes by Purified Mouse Immunoglobulins.—Since it is relatively difficult to obtain completely purified mouse IgG subclasses by electrophoresis, a further examination of possible heterogeneity in B cell binding of subclasses was made by comparing the ability of purified mouse myeloma proteins to inhibit the binding of a standard antibody preparation. In these experiments, TDL were treated for 3 hr at 4°C with various amounts of purified immunoglobulins. At the end of this period anti-FγG antibody (titer 1:512) was added to a final concentration of 1 in 500 and incubation continued for a further ½ hr at 37°C. The cells were then centrifuged and resuspended in FγG-^{125}I in the usual way for radioautography. Control cells were treated in the same manner except immunoglobulin was excluded. In the first set of experiments, inhibition with immunoglobulins from various sources was examined. As shown in Table II labeling was completely blocked by all IgG1 samples used and partially blocked by IgG_{2b} and IgM myeloma proteins.

TABLE I

Direct Binding of Immunoglobulin Fractions to B Lymphocytes

Immunoglobulin fractions from anti-FγG antiserum*	Per cent TDL labeled with FγG-^{125}I	Mean grain counts per labeled cell‡
	%	
Whole anti-FγG antiserum	13	≫100
IgM	7.5	27
IgG2	5.5	38
IgG1 + IgG2§	10	90
IgG§‖	14	>100
Fab fragment‖	<1	<1

* Samples were diluted so that cells were exposed to comparable amounts of antibody activity. In the case of the Fab fragment, a comparable protein concentration was used.

‡ Obtained from one experiment. Similar results were obtained in two other experiments.

§ After reaction with FγG-^{125}I, cells were centrifuged through BSA (complement free) gradients.

‖ Containing all three IgG classes.

In contrast, IgG_{2a}, IgA, and light chain myeloma proteins at the same concentration (2 mg/ml) were without effect. A single immunoglobulin of each class was then selected and the degree of inhibition determined for various concentrations (Table III). At a concentration of 0.5 mg/ml blocking of labeling occured only with IgG1 myeloma protein. These results thus confirmed that IgG1 globulin was the most effective competitor with antibodies for the Fc receptor on B cells.

Complement Independence of Antibody Binding to B Lymphocytes.—The failure of the Fab fragment of antibody molecules to label B cells posed the question

TABLE II

Inhibition of Binding of Antibody to B Lymphocytes with Purified Mouse Immunoglobulin. Effect of Immunoglobulins from Different Tumors

Source of immunoglobulin	Type of immunoglobulin (2 mg/ml)	Per cent TDL labeled with anti-FγG· FγG-^{125}I
		%
–	–	15
–	–	16
–	–	13
HPC-77	IgG1	<1
HPC-81		<1
HPC-39		<1
HPC-3	IgG_{2a}	13
HPC-56		15
GPC-7		15
HPC-84	IgG_{2b}	3
HPC-71		3
MPC-86		6
HPC-1	IgA	14
HPC-38		17
HPC-76	IgM	5*
HPC-14	Light chains	14
HPC-4		16

* IgM protein only available from one tumor. This figure represents the mean of results from two experiments.

TABLE III

Inhibition of Binding of Antibody to B Lymphocytes with Purified Mouse Immunoglobulins. Effect of Varying Concentrations of a Single Immunoglobulin

Source of immunoglobulin	Type of immunoglobulin	Per cent TDL labeled with anti-FγG·FγG-^{125}I after incubation with various concentrations of immunoglobulin (mg/ml)			
		0.2	0.5	1	2
			%		
–	–		17		
HPC-39	IgG1	3.5	<1	<1	<1
HPC-3	IgG_{2a}	15	14	15	18
HPC-84	IgG_{2b}	16	12	8	4
HPC-1	IgA	14	20	16	14
HPC-76	IgM	–	14	–	6
HPC-14	Light chain	15	15	19	17

whether complement was required for binding to the receptor. Several lines of evidence suggesting that the process is complement independent have already been presented. For example, significant binding occurred with heat-inactivated antiserum at dilutions up to 1:500, and with the electrophoretically slow migrating fraction of IgG which was relatively complement free. Furthermore, IgG1 myeloma protein was the most effective inhibitor of labeling with anti-FγG. FγG-^{125}I, while, as shown in the previous paper (1), antisera raised in chickens, the IgG fraction of which does not fix mammalian complement (10), adhered to B cells as effectively as isologous antiserum. None of these findings, however, formally excluded possible involvement of C'3. To examine this question a potent inhibitor of C'3 (11), the purified low molecular weight anticomplementary factor (CoF) from the cobra *Naja naja* was used.

TABLE IV

Effect of Purified Cobra Venom Factor (CoF) on Binding of Antibody to B Lymphocytes

Pretreatment of anti-FγG antiserum*	Per cent labeling of TDL with FγG-^{125}I	
	1‡	2‡
	%	%
NIL	15	12
Nonactivated CoF	16§	10§
Activated CoF	13§	9§

* Dilution 1:500.

‡ Results from two separate experiments.

§ No difference in grain counts per cell was observed between cells treated with nonactivated and activated CoF.

CoF was obtained in lyophilized form[5] and dissolved in phosphate-buffered saline, pH 7.3, at a concentration of 500 units/ml. Activation was carried out by treatment with fresh rabbit serum at 37°C for 20 min. 5–20 units of activated or nonactivated CoF were then added to 0.2 ml of inactivated anti-FγG antiserum (titer 1:256, dilution 1:500) and incubated for a further 30 min at 37°C. At the end of this period the two samples of anti-FγG antibody were added to separate aliquots of 10^7 normal TDL. Incubation was continued for 30 min at the same temperature after which the cells were centrifuged and resuspended with FγG-^{125}I in the usual way.

As shown in Table IV, no significant reduction in labeling with FγG-^{125}I was observed even when the cells were exposed to antiserum treated with up to 20 units of activated CoF. This suggests that soluble immune complexes bind to B cells independently of complement components.

Relationship between Membrane-Associated Immunoglobulin and Receptors for Antibody on B Lymphocytes.—B lymphocytes are known to possess a high density of immunoglobulin molecules on their surface. The question therefore arose whether the receptor for the Fc portion of antibody was associated

[5] Obtained from Dr. C. G. Cochrane by Dr. Marc Feldmann.

with the immunoglobulin. To examine this, TDL were incubated with either normal rabbit IgG $F(ab)'_2$ or with the $F(ab)'_2$ fractions from rabbit anti-mouse immunoglobulin sera for 3 hr at 4°C. Pepsin-digested fragments were used instead of intact anti-globulins to avoid both nonspecific inhibition by rabbit anti-mouse–mouse immunoglobulin complexes and nonspecific labeling of rabbit anti-mouse–mouse anti-FγG complexes with ^{125}I antigen. At the end of this period anti-FγG antiserum was added to the suspension to a final concentration of 1:500 and incubation continued for a further ½ hr interval, this time at 37°C. The cells were then centrifuged and reacted with FγG-^{125}I in the usual manner. Thus the steps in this procedure were identical to those in the experiments where sucessful inhibition of antibody binding with myeloma proteins was obtained (*vide supra*). It is clear, however, from the data in Table V that none of the $F(ab)'_2$ fractions prevented access of antibody-antigen complexes to B cells

TABLE V

Failure of Rabbit Anti-Mouse Immunoglobulin $F(ab)'_2$ Fragments to Inhibit Binding of Antibody to B Lymphocytes

$F(ab)'_2$ fragments*	Per cent labeling of TDL with anti-FγG-FγG-^{125}I
	%
Normal rabbit IgG $F(ab)'_2$	16
anti-κ $F(ab)'_2$	14
anti-μ $F(ab)'_2$	16
anti-γ $F(ab)'_2$	19

* Used at a concentration of 1.8–2.4 mg/ml.

even when concentrations of protein similar to those required for effective blocking with immunoglobulins (Tables II and III) were used.

Electron Microscope Radioautography of B Lymphocytes Coated with anti-FγG·FγG-^{125}I.—The use of a radioautographic technique for detection of receptors for antibody on B cells permitted visualization of their surface distribution by electron microscopy. TDL from TxBM donors (containing approximately 70% labeled cells by light microscopy) were selected for ultrastructural study. Examination of stained specimens revealed that 65–70% of cells were lymphocytes. Of the remainder the majority had features of plasma cells or plasma blasts although a few granulocytes (4–5%) and occasional monocyte-like cells (1%) were also seen. In the radioautographs most of the lymphocytes were labeled regardless of size. They displayed a characteristic distribution of grains which took the form of discrete patches on their surface often scattered at irregular intervals (Fig. 1). Between patches the cell surface was free of grains. Occasional grains seen over the cell substance could not be distinguished from background. In contrast cells clearly identifiable as plasma blasts or plasma cells failed to bind labeled material (Fig. 2). A few transitional types with minimal amounts of

rough endoplasmic reticulum did so. Cell debris (Fig. 3) and the occasional monocyte-like cell (Fig. 4) were frequently labeled. In the former case, however, the silver was present as single grains which were obviously associated with the debris, while in the latter the grains were seen overlying lysosome-like cytoplasmic vacuoles, not surface structures.

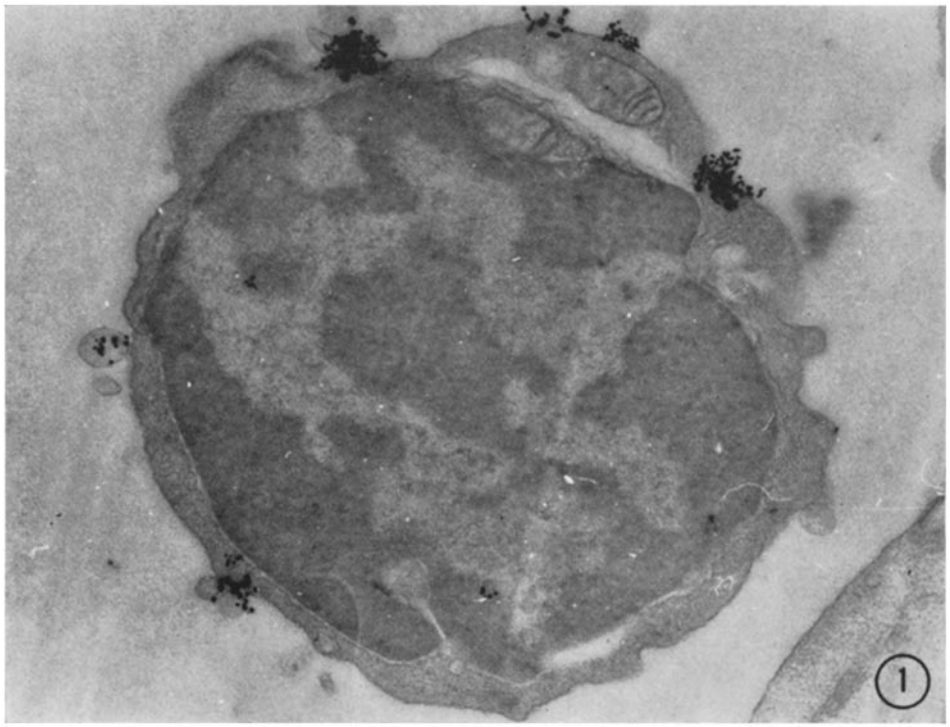

FIG. 1. Electron microscope radioautograph of a small lymphocyte from thoracic duct lymph of TxBM mice which had been exposed in vitro to anti-FγG·FγG-^{125}I. The cell shows dense patches of label at the cell surface but the major part of the surface is free of grains. Scattered single grains are present over the nucleus but these are consistent with the background label found in the section. Exposure 14 days, unstained section. × 21,000.

Relationship between Differentiation in B Cells and their Receptor for Antibody.—Modifications in surface alloantigens during lymphocyte differentiation have now been well documented (12). It was therefore of interest to determine whether changes occurred in the antibody-binding receptor during B differentiation. The possible presence of the receptor on stem cells was examined by use of a column technique designed to deplete cell populations of B lymphocytes carrying the receptor (13).

Polymethylmetaacrylic beads (Degalan V26, Degussa Wolfgang AG, Hanau am Main,

Germany) were coated with an antigen such as human gamma globulin (HGG) (Commonwealth Serum Laboratories) and poured into glass columns. Suspensions of spleen and bone marrow cells were incubated with rabbit anti-HGG serum at a concentration of 5×10^7 cells/ml for ½ hr at 37°C. They were then centrifuged once, resuspended in tissue culture Medium 199 containing 10% normal mouse serum, and poured into the columns (150×10^6 cells to each column). After a further 1 hr incubation at 4°C, during which cells such as mature B lymphocytes with antibody on their surface adhered to the antigen-coated beads, the

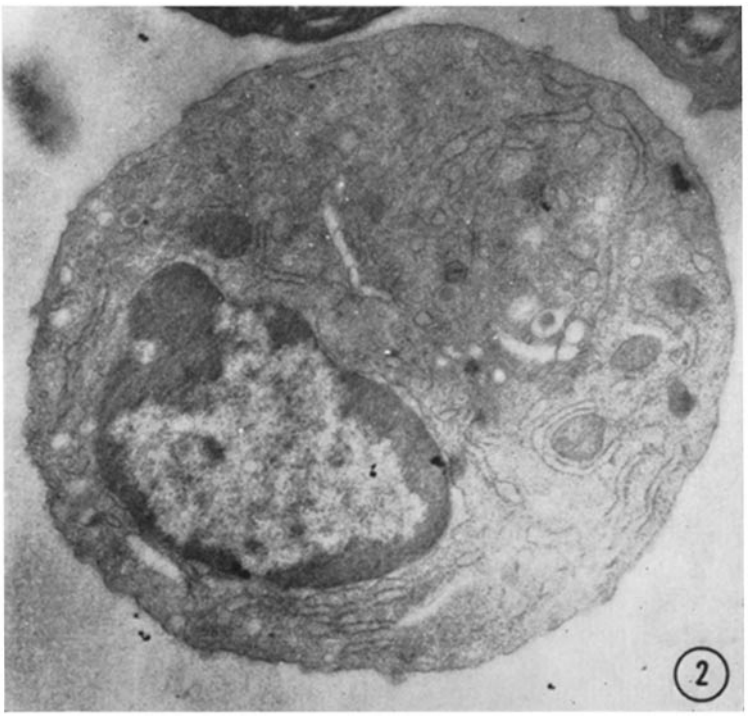

FIG. 2. Electron microscope radioautograph of an immature plasma cell from thoracic duct lymph of TxBM mice which had been exposed in vitro to anti-FγG·FγG-^{125}I. Dense patches of label are strikingly absent. Scattered single grains represent background. A neighboring lymphocyte (partially visible) is heavily labeled. Exposure 14 days, unstained section × 18,200.

effluent was collected and divided into two pools. The first pool was reacted with anti-FγG·FγG-^{125}I to check that B cell depletion had occurred. The second pool was counted and tested for its stem cell content by the colony-forming assay of Till and McCulloch (14). Graded numbers of column-treated cells and controls were injected intravenously into heavily irradiated (800 rads) syngeneic hosts. 7 days later the spleens were removed and placed in Bouin's fixative. Macroscopically visible colonies were counted and related to the number of injected cells.

It was predicted that column-treated cell suspensions would be markedly depleted of colony-forming activity if a significant number of hemopoietic stem

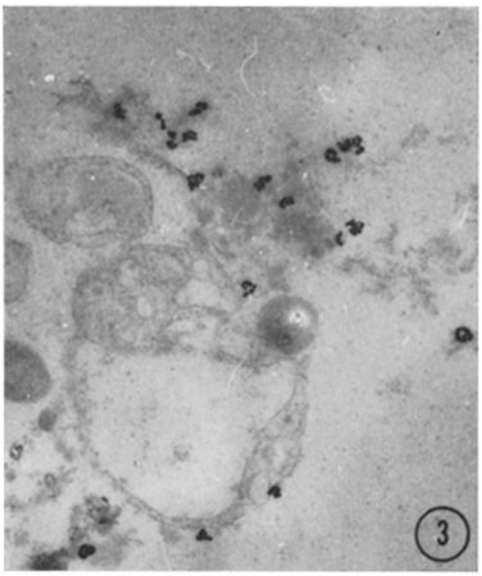

FIG. 3. Electron microscope radioautograph of cell debris from the same source as the cells seen in Figs. 1 and 2 showing an aggregation of grains clearly associated with the debris. Although numerous grains are present they do not form discrete patches like those found on labeled lymphocytes. Exposure 14 days, unstained section. × 18,000.

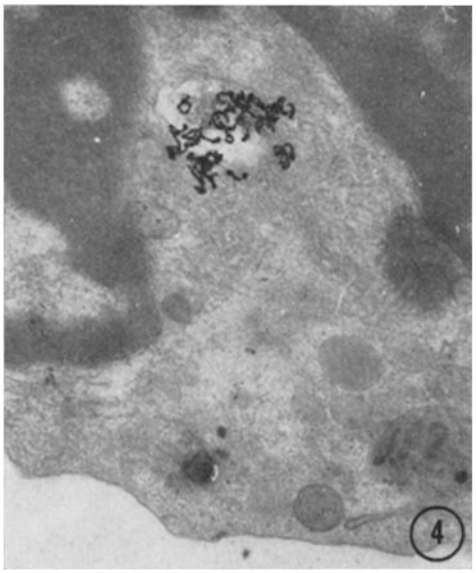

FIG. 4. Electron microscope radioautograph of a portion of a phagocytic thoracic duct cell from TxBM mice showing intracellular localization of labeled immune complex. A large multimolecular patch is present overlying a clear vacuole deep in the cytoplasm. Other lysosome-like vesicles are also present but are unlabeled. Exposure 14 days, unstained section. × 24,000.

cells carried the receptor for antibody. This was not found and in fact a three- to fourfold enrichment was observed instead (Table VI). In other words, most, if not all, precursor cells lack this membrane-associated receptor.

The electron microscopy findings already reported suggested that the receptor for antibody was absent from antibody-forming cells as well as from the precursors of B cells. Support for this was obtained by examining the antibody-binding properties of pure populations of malignant plasma cells. Tumor cells were obtained from either in vitro continuous cell culture lines or from in vivo transplanted tumors. Eight plasma cell tumors were studied, and all but one proved completely negative. The positive line, HPC-6, is a variant derived from an NZB IgA-secreting tumor and now only secretes light chains. It should, however, be noted that of the seven negative tumors, two also secreted only immunoglobulin light chains. One θ-positive lymphoma has been included as a negative control and three myeloid-type lines as positive controls, namely a mastocytoma (receptor for immune complexes previously described[6]), a myelomonocytic leukemia, and a reticulum cell sarcoma type A (Table VII).

TABLE VI

Failure to Detect the Receptor for Antibody on Precursors of B Lymphocytes

Source of cells	CFU activity*	
	CFU/10^5 cells	Total CFU‡
Spleen before column treatment	4.5	6750
Spleen after column treatment§	12.5	6000
Bone marrow before column treatment	28	42,000
Bone marrow after column treatment‖	120	48,000

* CFU = colony forming units.

‡ Six recipients per group. Data from one experiment. Two others gave similar results.

§ Yield was 32% of 150 × 10^6 cells put on column.

‖ Yield was 27% of 150 × 10^6 cells put on column.

DISCUSSION

Evidence has been presented for the existence on B lymphocytes of a membrane-associated receptor for antibody (1). The receptor was detected radioautographically by treating cells with antibody followed by the corresponding radioiodinated antigen. Formation of a stable antibody-antigen complex on the cell surface by this technique implied that attachment of antibody to receptor occurred via the C terminal end of the molecule. The ability of intact IgG antibody to bind and the failure of Fab fragments from the same source to do so (Table I) supported this notion. The fact that antibody adhered to the receptor

[6] Cline, M. J., and N. L. Warner. Immunoglobulin receptors on a mouse mast cell tumor. Submitted for publication.

by way of its Fc portion made it important, particularly from the functional point of view, to establish which heavy chain classes or subclasses the receptor could recognize. This question was examined in two different ways. In the first, various antibody fractions were tested directly for their ability to adhere to B lymphocytes. IgM and total IgG globulin both bound to an appreciable number of cells as did fractions enriched for IgG1 and IgG2 subclasses. A marked differ-

TABLE VII

Failure to Detect the Receptor for Antibody on Malignant Plasma Cells

Strain of origin	Tumors	Tumor type	Immunoglobulin synthesized and secreted	Binding of anti-FγG· FγG-^{125}I*
(BALB/c × NZB)F_1	HPC-64	Plasmacytoma	L chain	−
BALB/c	HPC-76	Plasmacytoma	IgM	−
BALB/c	MOPC-104E	Plasmacytoma	IgM	−
(BALB/c × NZB)F_1	HPC-32	Variant plasmacytoma	L chain	−
(BALB/c × NZB)F_1	HPC-38	Plasmacytoma	IgA	−
BALB/c	MOPC-460‡	Variant plasmacytoma	NIL	−
C3H	XCI‡	Variant plasmacytoma	NIL	−
BALB/c	SIAT.4‡	θ-positive lymphoma	NIL	−
NZB	HPC-6‡	Variant plasmacytoma	L chain	+§
(NZB × C57BL)F_1	T 171-3	Reticulum cell sarcoma (type A)	NIL	+
BALB/c	WEHI-3	Myelomonocytic leukemia	NIL	+
DBA2	P815‡	Mastocytoma	NIL	+

* Anti-FγG sera used here bound to 16% normal TDL.

‡ Lines maintained in continuous culture in vitro.

§ Plus sign means that 90–100% of viable tumor cells were labeled.

ence in percentage of binding cells and in grain counts per cell, however, was observed. Thus total IgG globulin labeled more heavily than did IgM, and IgG1-containing fractions more heavily than IgG2 fractions lacking IgG1. These findings suggested that the receptor displayed the highest avidity for IgG1 heavy chains.

Inhibition of antibody binding with purified immunoglobulins provided an alternative way of studying the types of immunoglobulin capable of attachment to the receptor (Tables II and III). Pretreatment of B cells, with excess IgG_{2a}, IgA, or light chain myeloma proteins failed to block the subsequent uptake of antibody, whereas IgG1 proteins and to a lesser extent IgG_{2b}, and IgM proteins

at the same concentrations did so. When the protein concentration of the solution was reduced fourfold (from 2 mg/ml to 0.5 mg/ml), IgG1 myeloma protein alone proved capable of inhibition. Taken together, the binding and blocking experiments suggest that the receptor on B cells has a marked predilection for IgG1 antibody. IgM and IgG_{2b} antibody may bind but with lower avidity and probably in insignificant amounts if IgG1 antibody is present in excess. The B cell and the macrophage with its receptor for IgG_{2a} antibody (15) thus have the potential to bind antibody of the major classes and all the IgG subclasses. It is interesting to speculate what role an array of receptors of this kind may play in antigen trapping in lymphoid tissue or antibody-mediated regulation of the immune response (1).

The need for an intact Fc piece for attachment of antibody to receptor made it essential to examine whether complement was required during formation of the bond. Data presented here (Table IV) and in the previous paper (1) strongly suggest that complement components play no role, at least in the case of soluble immune complexes. For example, binding occurred with inactivated antiserum at high dilution with chicken immunoglobulin which cannot fix mammalian complement (10) and with the electrophoretically slow migrating fraction of IgG which was relatively complement free. Particularly important, however, is the evidence (*vide supra*) which indicates that receptor recognizes the non-complement-fixing IgG1 class. Finally, lack of inhibition of binding by activated CoF excluded a possible role for heat stable C'3. The formation of antibody-antigen complexes on the B cell surface in the absence of complement is apparently contrary to the previous findings of Bianco et al. (16). In their system particulate complexes in the form of antibody-coated erythrocytes only made rosettes around B cells in the presence of C'3. The discrepancies between our system and theirs have already been discussed in the previous paper (1). Failure to implicate complement in the binding process, does not, however, exclude the possibility that the receptor itself might contain or be composed of a complement component. The use of specific antisera should resolve this problem.

The relationship between the receptor on B cells for antibody and surface immunoglobulin was examined by a competitive inhibition technique. Lymphocytes were treated with a variety of specific anti-immunoglobulin $F(ab)'_2$ fragments followed by antibody and radioiodinated antigen (Table V). None of the reagents used significantly blocked attachment of antibody to B cells. In preliminary experiments with the reverse procedure, preincubation of lymphocytes with immune complexes has failed to prevent labeling of B cells with the IgG-^{125}I fraction from a polyvalent anti-immunoglobulin serum.[7] The receptor for antibody and surface immunoglobulin (the receptor for antigen) would therefore appear to be discrete entities on the B cell membrane. Similar findings have been reported by Bianco and Nussensweig in their rosette system (17).

[7] Kindly provided by Miss H. Lewis (specific activity, 50 μCi/μg).

The use of a radioautographic technique for detection of antibody-binding lymphocytes enabled us to visualize distribution of the receptor for antibody by electron microscopy. Its morphology (Fig. 1) was identical to that described for antigen-binding receptors (18). Thus only a small proportion of the cell surface appeared to be involved in recognition of antibody, the remainder presumably being modified for other functions. Not all cells in the preparation examined (TDL from TxBM mice) possessed the receptor. The majority of lymphocytes whatever their size did so, but cells morphologically classifiable as plasma cells or plasma blasts were never labeled (Fig. 2).

The failure of plasmacytoid cells to bind antibody implied that the receptor for antibody is lost during differentiation of B cells into antibody-forming cells. Support for these observations was obtained from studies of various plasma cell tumors grown either in vivo or in vitro (Table VII). All of those tested lacked the receptor with the exception of HPC-6, a mutant line possessing the morphological characteristics of a lymphoma rather than a plasma cell tumor.

Binding of antibody to this tumor has been independently demonstrated by a rosette technique.[8] The absence of the receptor from other light chain–secreting plasma cells raises the interesting possibility that development of various tumors like HPC-6, which differ from their parent lines in a deficiency of immunoglobulin synthesis or secretion, may represent a true dedifferentiation from plasma cells to B cells. Further studies will require the use of other B cell–plasma cell discriminatory markers.

The possible presence of the receptor on precursors of B cells was examined in a different manner. Suspensions of bone marrow and spleen were passed through a column designed to deplete lymphocyte populations of cells binding antibody. When the cells emerging in the effluent were tested for stem cell activity in a colony-forming assay, significant enrichment was observed (Table VI). Thus most precursor cells have not yet acquired the receptor for antibody. Taken together, the studies on differentiation make this a marker for mature B cells. It is not present on stem cells and is lost when B cells commence secreting immunoglobulin. The recent demonstration that an appreciable population of mobilizable B cells are long-lived and can to some extent recirculate,[9] emphasizes the potential importance of a receptor of this kind in regulation of the immune response.

SUMMARY

Binding of antibody to the surface of B lymphocytes was shown to involve the Fc piece of the immunoglobulin molecule. This property was not shared equally by all immunoglobulin classes as revealed by direct binding and inhibi-

[8] Cline, M. J., J. Sprent, N. L. Warner, and A. W. Harris. 1971. Receptors for immunoglobulin on B lymphocytes and cells of a cultured plasma cell tumor. Submitted for publication.

[9] Sprent, J., and A. Basten. Manuscript in preparation.

tion studies. Total IgG globulin was found to label cells more heavily than IgM, and IgG1-containing fractions more heavily than IgG2 fractions lacking IgG1. The ability of various purified myeloma proteins to inhibit attachment of antibody to B cells was examined. Pretreatment of B cells with excess IgG_{2a}, IgA, or light chain proteins failed to inhibit, whereas IgG1 proteins and to a lesser extent Ig_{2b} and IgM proteins at the same concentrations did so. At lower protein concentrations, IgG1 myeloma protein alone retained the capacity to inhibit binding. The conclusion was reached that the receptor on B cells for antibody has a marked predilection for the IgG1 class. Although IgM and IgG_{2b} antibody may bind, they do so with lower avidity and probably in insignificant amounts if IgG1 antibody is present in excess.

No evidence was found to implicate complement in the binding process. For example, heat-inactivated sera at high dilution retained the ability to label B cells, while the use of purified low molecular weight anticomplementary factor, a potent inhibitor of C'3, did not interfere with the formation of the bond between antibody and cell surface.

The failure of anti-mouse immunoglobulin $F(ab)'_2$ fragments to prevent access of antibody to B cells implied that the antibody-binding receptor and antigen-binding (immunoglobulin) receptor are discrete entities on the B cell membrane. Despite this, a marked similarity between their surface distribution was observed on electron microscopy.

The antibody-binding receptor was shown to be a marker for mature B cells. It did not appear to be present on hematopoietic precursor stem cells and was lost during differentiation to antibody-forming cells.

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