

RECEPTORS FOR AGGREGATED IgG ON MOUSE LYMPHOCYTES  
THEIR PRESENCE ON THYMOCYTES, THYMUS-DERIVED, AND BONE  
MARROW-DERIVED LYMPHOCYTES\*

BY CLARK L. ANDERSON AND HOWARD M. GREY

(From the National Jewish Hospital and Research Center, Denver, Colorado 80206)

(Received for publication 27 December 1973)

Since the original report by Boyden and Sorkin (1, 2) that membranes of macrophages bind "cytophilic" antibody, numerous studies have described a similar phenomenon in other cell types; viz., mast cells (3); neutrophils (4); murine tumor cells of the mastocytoma (5) and lymphoma types (6-8); various human tumor cells (9, 10); monocytes (11); and lymphocytes (12-14). Studies of immunoglobulin binding to lymphocytes by Basten et al. (12, 13) in mice and by Dickler and Kunkel (14) in humans have shown that binding is characteristic of B cells and not of T cells. However, more recently it has been claimed that thymus-derived lymphocytes also bear receptors for immunoglobulin (15-17).

The methods used to study antibody binding by cell membranes have generally utilized antigen-antibody complexes, employing either a particulate antigen such as sheep erythrocytes or a radiolabeled antigen such as [<sup>125</sup>I]chicken  $\gamma$ -globulin. Non-specifically aggregated IgG, which has been shown to possess biological properties similar or identical to antigen-antibody complexes (18), has also been used (9, 14). These Ig aggregates, formed either with a bifunctional reagent such as BDB<sup>1</sup> or with heat, are distinctly advantageous in that preparation is simple and different immunoglobulin classes and subclasses can be used to determine the specificity of immunoglobulin interaction with cells.

In the present study, using heat- and BDB-aggregated mouse IgG, we have shown that both B and T lymphocytes bear receptors for immunoglobulin. Some of the characteristics of this binding phenomenon are described, and the relative numbers of receptors on various cell types are compared.

*Materials and Methods*

*Preparation of Cells.*—Spleens and thymuses were obtained from 5-7-wk old male and female normal Balb/c mice. They were dissected free of extra-organ tissue under magnification and were teased apart with forceps in cold HBSS. Debris and cell aggregates were allowed to

\* Supported by National Institutes of Health Grant AI-09758 and Allergy Disease Center Grant AI-10398.

<sup>1</sup> *Abbreviations used in this paper:* agg-141, aggregated IgG2b (MOPC-141); BDB, bis-diazotized benzidine; BSA, bovine serum albumin; CGG, chicken  $\gamma$ -globulin; HBSS, Hanks' balanced salt solution; HGG, human  $\gamma$ -globulin; PBS, phosphate-buffered saline; SDS, sodium dodecylsulfate; SIg, surface immunoglobulin;

settle out for 10 min at 4°C. The cells were then washed three times in HBSS. Viability was 90% or greater. For the isolation of macrophages female Balb/c mice were injected intraperitoneally with 1.5 ml of a sterile 10% solution (wt/vol) of proteose peptone (Difco Laboratories, Detroit, Mich.) in saline (19). 3 days later macrophages were harvested by washing the peritoneal cavities with cold HBSS containing 0.03 M sodium azide and 10 U/ml heparin. Cells were washed three times in HBSS with azide. 80–85% of cells were large mononuclear phagocytic cells. Cells of both the murine mastocytoma P815 and the theta-positive lymphoma, L5178Y, maintained in ascitic form in DBA/2 mice, were obtained by paracentesis and washed three times in HBSS. In order to obtain allogeneically activated thymus cells (20, 21), male BDF<sub>1</sub> (C57/B16 × DBA/2) mice were lethally irradiated with 1,100 R from a Co<sup>60</sup> source and within 2 h were infused intravenously with 50–60 × 10<sup>6</sup> thymocytes from 5–7-wk old DBA/2 mice. On day 7 cells from recipient spleens were teased and washed in cold HBSS.

*Purification of Proteins.*—Mouse myeloma proteins MOPC 141 (IgG2b), MOPC 195 (IgG2b), MOPC 21 (IgG1), and S 121 (IgA) were purified by starch block electrophoresis. In the case of S 121 further purification was obtained by gel filtration on Sephadex G-200. Cohn Fraction II of normal chicken serum was exhaustively absorbed with Balb/c thymus and spleen cells to remove heterophile activity. Absorbed CGG did not bind to mouse lymphocytes when tested by indirect immunofluorescence. Bovine albumin powder, fraction V from bovine plasma was obtained from Metrix (Armour and Co., Ltd., Eastbourne, England) lot J39404 and used without further purification. Human  $\gamma$ -globulin was obtained by DEAE chromatography of Cohn Fraction II (obtained from the American Red Cross). Papain digestion of mouse IgG and separation of Fab and Fc fragments was performed as previously described (22).

*Aggregation of Proteins.*—Myeloma proteins MOPC 141, MOPC 195, and CGG at concentrations of 2 mg/ml in PBS were heated in a 63° C water bath until barely visible opalescence was obtained. For CGG the time required for aggregation was less than 1 min, for MOPC 195 about 3 min, and for MOPC 141 10–11 min. Alternatively, these proteins and others used in this study which did not readily form soluble aggregates upon heating were aggregated with BDB according to the method of Ishizaka (18). It was found that by varying the relative concentrations of protein and BDB, large soluble aggregates could be obtained. BDB and heat-aggregated preparations of the same protein behaved similarly with respect to lymphocyte-binding capacity.

Aggregates were radioiodinated with <sup>125</sup>I by a modification of the chloramine T method (23). Specific activities ranged from 6–9  $\mu$ Ci/ $\mu$ g. Labeled aggregates were diluted to a concentration of 5  $\mu$ g/ml in 10% heat-inactivated calf serum/HBSS and were centrifuged at 3,000 rpm for 20 min immediately before use.

The extent of aggregation was evaluated by Sepharose 4B gel filtration. For covalently linked aggregates 3% polyacrylamide gel electrophoresis in SDS was also performed (8). Polyacrylamide gels were either stained with Coomassie blue or in the case of radiolabeled proteins were fractionated on an autogel divider (Savant Instruments, Inc., Hicksville, N. Y.) and counted in an automatic gamma scintillation counter.

*Binding Assay and Autoradiography.*—Cells prepared as described above were resuspended in 10% heat-inactivated calf serum/HBSS. To 10–20 × 10<sup>6</sup> cells aliquoted into 5-ml polystyrene tubes was added 0.1 ml of the centrifuged radiolabeled aggregated protein solution (at 5  $\mu$ g/ml), an amount calculated to be in aggregate excess. The mixture, in a total volume of 1 ml, was incubated in a 37° C water bath for 1 hr. To test for inhibition of binding, the cells were first incubated for 10 min at 37° C with 0.1 ml of unlabeled inhibitor protein at a concentration 500 times that of the radiolabeled test aggregate. The radiolabeled aggregate was then added and incubation at 37° C continued for 1 hr. The cell suspension was then diluted to 4 ml with cold medium and was centrifuged at 1,000 rpm for 10 min. The cell pellet was washed three times in medium, resuspended gently in 0.2 ml of medium, and smeared onto

gelatin-coated microscope slides. Slides were allowed to air dry, fixed for 30 min in 1% glutaraldehyde-PBS, washed for 30 min in distilled water, and again air dried. Fixed and dried slides were dipped in NTB-2 emulsion and allowed to dry vertically. After varying periods of exposure, slides were developed and stained with Giemsa. Autoradiographs were analyzed by bright field microscopy. Between 200-300 cells were scored for the number of grains lying over or contiguous with the cell. In thymocyte and splenocyte preparations, only small mononuclear cells were scored, eliminating insofar as possible monocytes, macrophages, and polymorphonuclear cells. In macrophage preparations, only large mononuclear cells with vacuolar cytoplasm were scored. Small lymphocytes and neutrophils were excluded.

To study cell surface redistribution of bound [ $^{125}$ I]agg-IgG, lymphocytes were incubated at 0° C for 1 h with 0.1 ml of the radiolabeled aggregated preparation. Unbound Ig was then removed by washing the cells three times with medium following which the cells were incubated at 37° C for varying periods of time. The cells were again washed and autoradiographs were made. Labeled cells were sorted into two groups: those with grains evenly situated about the cell perimeter and those with definite asymmetry of grain distribution.

To determine the percentage of SIg $^+$   $\theta^+$  and "Null" (SIg $^-$ ,  $\theta^-$ ) lymphocytes which bound [ $^{125}$ I]agg-141, washed spleen cells were incubated first with [ $^{125}$ I]agg-141 for 15 min at 37° C. After three washes in cold HBSS, the cells were incubated either in a direct staining procedure with fluoresceinated rabbit antimouse Ig antiserum or in an indirect procedure with anti-theta antiserum (kindly supplied by Dr. John J. Cohen) as the first layer followed by fluoresceinated antimouse Ig antiserum as the second layer. The antiserum incubations were performed at 0° C for 30 min in the presence of 0.03 M sodium azide. Cells were then prepared for autoradiography as described above except that absolute methanol was used as a fixative rather than glutaraldehyde. Small round cells were scored for both fluorescence and grains. The exposure times used in this experiment were chosen so that only cells with a relatively large number of agg-141 receptor sites would be scored as positive.

#### RESULTS

*Binding of Radiolabeled Aggregated IgG to Lymphocytes.*—To determine the extent of binding of aggregated Ig to lymphocytes, cells were incubated for 1 h at 37°C with radiiodinated (6–9  $\mu$ C/ $\mu$ g) heat-aggregated mouse IgG2b myeloma protein MOPC-141 ([ $^{125}$ I]agg-141). This protein was selected because it readily formed soluble aggregates when heated at 63°C. Cell suspensions of mouse thymus and spleen were incubated with 0.5  $\mu$ g of [ $^{125}$ I]agg-141 and prepared for autoradiography. A representative experiment is shown in Fig. 1, A. After 9 days of exposure about 70% of spleen lymphocytes contained 6 or more grains, a grain count in considerable excess of background and significantly greater than that obtained with spleen cells that had been preincubated with unlabeled aggregated MOPC-141 before the addition of the radiolabeled preparation. After a very long exposure (5 mo) autoradiographs of spleen cells showed 75% of highly labeled small lymphocytes with more than 30 grains/cell and another 10% having 16–30 grains/cell (Fig. 1, B). Only 1% of control cells (preblocked with unlabeled MOPC-141) had 16 grains/cell or more. Therefore, about 85% of normal spleen cells bound significant amounts of [ $^{125}$ I]agg-141. Since only 40–50% of spleen cells were B cells as judged by immunofluorescence for surface Ig, these data indicate that 30–40% of non-B cells, presumably T cells, also have the capacity to bind aggregated Ig. This point is discussed in greater detail in a subsequent section.

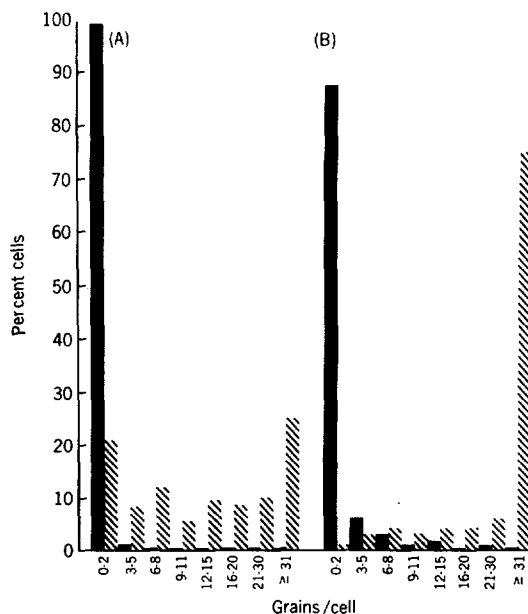


FIG. 1. Splenocytes were exposed to [ $^{125}$ I]agg-141 for 60 min at 37°C (hatched bars). Cells were also incubated with 500-fold excess of unlabeled agg-141 before incubation with [ $^{125}$ I]agg-141 (solid bars). The grains associated with 200 small mononuclear cells were counted and the number of grains per cell was plotted against per cent of cells so labeled. The grain count distribution of an autoradiograph exposed for 9 days is shown in A, whereas B represents the grain count distribution after a 5-mo exposure period.

Thymus cells were similarly studied for their capacity to bind [ $^{125}$ I]agg-141 (Fig. 2). After a 9-day exposure, 21% of thymocytes had 6 or more grains and 31% had 3 or more grains, whereas none of the control cells had more than 2 grains/cell. After 5 mo exposure, the number of specifically labeled cells increased to 45–50%. There was a suggestion of a bimodal distribution with one population having a peak at 6–8 grains/cell and another population with more than 20 grains/cell. The percentage of thymus cells that bound agg-141 varied considerably from one experiment to another depending on both the exposure time of the autoradiographs and the particular preparation of agg-141. In general, with exposure times of 3–4 wk, 10–20% of thymocytes bound agg-141.

*Specificity of Binding of Agg-141 to Lymphocytes.*—Several parameters of the binding of agg-IgG to lymphocytes were investigated to obtain information regarding the specificity of the interaction. First, the importance of aggregate size itself was studied. In order to study what degree of aggregation was necessary for binding, a preparation of BDB-aggregated MOPC-141 was fractionated on a Sepharose 4B column. A protein peak was obtained in the void volume followed by a broad plateau which extended from the void volume to the position

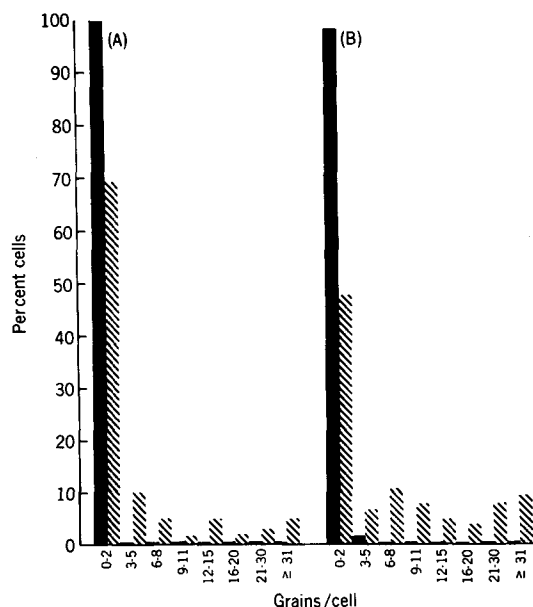


FIG. 2. Thymocytes were exposed to [ $^{125}\text{I}$ ]agg-141 for 60 min at 37°C (hatched bars). Cells were also incubated with 500-fold excess of unlabeled agg-141 before incubation with [ $^{125}\text{I}$ ]agg-141 (solid bars). The grains associated with 200 small mononuclear cells were counted and the number of grains per cell was plotted against per cent of cells so labeled. The grain count distribution of an autoradiograph exposed for 9 days is shown in *A*, whereas *B* represents the grain count distribution after a 5-mo exposure period.

of IgG monomer at which point another peak was observed. Two tubes, one close to the void volume and one close to the monomer peak, were chosen for further study. These two samples were iodinated, tested for binding to spleen and thymus lymphocytes, and analyzed for degree of aggregation by polyacrylamide electrophoresis in SDS. Table I contains the binding data together with the molecular weight information. Monomer 141 demonstrated no significant binding to either spleen or thymus lymphocytes. The fraction eluted just ahead of the monomer peak (Fr 2) had a mean aggregate size of three. Under conditions that showed unfractionated BDB agg-141 binding to 40% of splenic lymphocytes and 8% of thymocytes, the low molecular weight aggregates bound to only 5% of spleen cells and 1% of thymocytes. Fraction 1 of the Sepharose 4B column had a mean aggregate size of about 15 and bound to 31% of spleen cells but only 2% of thymocytes. It is obvious from these studies that the degree of binding to lymphocytes is directly proportional to aggregate size and that the optimal size is greater than 15 and probably is of a size large enough to be present in the void volume of the column, i.e.,  $\geq 20,000,000$ .

Next, purified preparations of other aggregated proteins were tested for their capacity to bind to mouse lymphocytes. For this purpose a nonimmunoglobulin,

TABLE I  
*Lymphocyte Binding of Aggregates of Different Molecular Size*

Preparation	Mean aggregate size	Percentage of positive cells	
		Spleen	Thymus
Unfractionated	~100 (1 to > 100)*	40	8
Sepharose Fr. 1	15 (6-25)	31	2
Sepharose Fr. 2	3 (1-6)	5	1
Monomer	1	0	0

\* Range of aggregate size.

bovine serum albumin; a nonmammalian Ig, chicken  $\gamma$ -globulin; and a mouse Ig not known to possess cytophilic properties, IgA, were used. All proteins were aggregated with heat or BDB and were shown by SDS polyacrylamide electrophoresis to have aggregates comparable in size with agg-141. These aggregates were then tested for lymphocyte binding by performing autoradiography on mouse lymphocytes either incubated directly with the radioiodinated preparations or incubated first with unlabeled aggregated preparations and then with [ $^{125}$ I]agg-141. Table II illustrates the capacity of the radiolabeled preparations to bind to spleen cells and thymocytes. Besides BSA, mouse IgA, and CGG, radiolabeled aggregates of MOPC-141, MOPC-21 (IgG1), and human IgG were also tested. Whereas no significant binding occurred with IgA, BSA, or CGG, both IgG1 and HGG gave results fairly comparable with those obtained with [ $^{125}$ I]agg-141. The ability of unlabeled aggregated proteins to inhibit the binding of [ $^{125}$ I]agg-141 to lymphocytes was next studied, and the data are presented in Table III. All unlabeled proteins were added before the addition of [ $^{125}$ I]agg-141, in 500-fold excess to the radiolabeled preparation. The homologous protein, agg-141, virtually abolished the capacity of the labeled preparation to bind to lymphocytes in a fashion similar to that previously demonstrated in Figs. 1 and 2. Another IgG2b protein, MOPC-195, was also an effective inhibitor, as was the IgG1 protein, MOPC-21. Since only a single large concentration of unlabeled protein was used, subtle differences in the ability of these proteins to inhibit [ $^{125}$ I]agg-141 binding to lymphocytes may have been present but not detected by this experiment. On the other hand, aggregates of BSA, CGG, and the Fab fragment of MOPC-195 had no inhibitory capacity. Attempts to form soluble aggregates of the Fc fragment of an IgG2b protein have not been successful to date so it has not been possible to evaluate the binding capacity of this fragment. Partial inhibition was obtained with the IgA

TABLE II  
*Specificity of Binding of [<sup>125</sup>I]Aggregated Proteins to Mouse Lymphocytes*

Exp.	Aggregate	Percentage of positive cells	
		Spleen	Thymus
		%	%
1	IgG2b (MOPC 141)	45	11
	IgA (S 121)	0	0
	BSA	4	0
	CGG	1	0
2	IgG2b (MOPC 141)	57	12
	IgG1 (MOPC 21)	34	5
3	IgG2b (MOPC 141)	38	7
	HGG	40	2

TABLE III  
*Inhibition of Binding of [<sup>125</sup>I]Agg-141 to Mouse Lymphocytes by Unlabeled Protein Aggregates*

Exp.	Inhibiting aggregate	Percentage of positive cells	
		Spleen	Thymus
		%	%
1	None	26	15
	IgG2b (MOPC 141)	1	0
	IgA (S 121)	19	9
	BSA	37	16
	CGG	20	23
2	None	45	17
	IgG2b (MOPC 195)	2	0
	Fab (MOPC 195)	43	12
3	None	50	17
	IgG1 (MOPC 21)	11	0

preparation. This might have been due to a relatively weak capacity of IgA to bind to the IgG receptor site on lymphocytes, or alternatively, due to trace contamination of this preparation with IgG.

From these data it can be concluded that lymphocyte binding of agg-141 is specific in that it is a property unique to the aggregated form of the protein and is not shared by the aggregates of several other unrelated proteins such as BSA, CGG, and IgA. It is, however, a reaction which is shared with closely related proteins such as another IgG2b protein, IgG1, and another mammalian IgG protein, HGG. Although the data are consistent with the binding site being localized to the Fc fragment, a direct demonstration of this is thus far lacking.

*Effect of Temperature on the Binding of [<sup>125</sup>I]Agg-141 to Lymphocytes.*—The capacity of spleen and thymus cells to bind [<sup>125</sup>I]agg-141 was tested at 0 and 37°C. As shown in Table IV, except for a single experiment with spleen cells, binding at 37°C was considerably greater than at 0°C. Optimal binding was observed after 15 min incubation at 37°C, whereas no clear plateau of binding was obtained at 0°C, even after 4 h of incubation. Binding experiments were also performed at 13 and 25°C. Somewhat inconsistent results were obtained at these temperatures. In some experiments the degree of binding was equal to that observed at 37°C, whereas in others a moderate decrease in the binding capacity was observed.

*Distribution of Agg-IgG Binding Sites on the Surface of Lymphocytes.*—The distribution of grains on the surface of lymphocytes that contained receptors for agg-IgG was studied, since it was apparent that under the standard conditions of incubation at 37°C, many cells had an asymmetric distribution of grains

TABLE IV  
*Effect of Temperature on the Binding of [<sup>125</sup>I] Agg-141 to Mouse Lymphocytes*

Temperature °C	Exp:	Percentage of labeled cells					
		Spleen			Thymus		
		1	2	3	1	2	3
0		8	49	27	1	7	0
37		60	54	—	30	18	15

reminiscent of the “capping” phenomenon. To study the temperature requirements for this phenomenon, both thymus and spleen cells were incubated with [<sup>125</sup>I]agg-141 at 0°C for 1 h, washed, incubated at 37°C for varying lengths of times up to 120 min, after which the cells were rapidly cooled to 0°C and prepared for autoradiography. Although few thymocytes bound [<sup>125</sup>I]agg-141 at 0°C, as shown in Table V, the proportion of labeled cells with eccentrically placed grains was high before any 37°C incubation and was not increased by warming. In contrast, capping of labeled spleen cells increased from about 20% to over 80% by incubation at 37°C. It is obvious from these data that the majority of spleen cells undergo redistribution of receptor sites from a random distribution to a capped one following incubation at 37°C. The situation with thymocytes is more difficult to assess since an asymmetric distribution of grains was observed under all conditions of study. No conclusion can be made as to whether the observed caps on thymocytes represented a redistribution of binding sites or whether the binding sites were asymmetrically disposed on the cell surface before their binding agg-IgG.

*Comparison of Agg-IgG Binding by Different Cell Types.*—To determine the



TABLE V  
Capping of [<sup>125</sup>I]Agg-141 on the Surface of Mouse Lymphocytes

Time at 37°C	Percentage of labeled cells capped	
	Spleen	Thymus
<i>min</i>	%	%
0	20	75
10	17	71
20	61	—
30	78	—
60	83	73
110	86	—

relative degree of binding of agg-IgG by various types of cells, the following experiment was performed. The mouse cells chosen for study were: mastocytoma P815; macrophages obtained from peritoneal exudates; the theta-positive DBA/2 lymphoma L5178Y; Balb/c spleen cells; Balb/c thymocytes; and spleen cells obtained from irradiated BDF<sub>1</sub> mice 7 days after they were injected with parental DBA/2 thymocytes. These preparations were all incubated with [<sup>125</sup>I]agg-141. Macrophages were incubated at 0°C to prevent phagocytosis, whereas all the other cell types were incubated at 37°C. Autoradiographs were developed after varying exposure times in order to have significant numbers of grains for each cell type. Grain counts were then normalized for time of exposure and expressed as grains/cell/day of exposure. Fig. 3 illustrates these results. The data are plotted as the cumulative percentage of cells having a certain grain count or more. Macrophages and the P815 mastocytoma bound the most [<sup>125</sup>I]agg-141 with an average of 40–50 grains/cell/day. An average of 20–30 grains/cell/day was seen on labeled allogeneically activated thymus cells. Spleen lymphocytes had a small population of high binding cells with the bulk of cells having an average of 2–3-grains/cell/day. The positive population of thymocytes had about 0.6 grains/cell/day and the L5178 lymphoma was somewhat higher with about 1 grain/cell/day.

In order to determine the relative binding capacity of subpopulations of splenic lymphocytes, the following double-labeling experiment was performed. Cells were first incubated with [<sup>125</sup>I]agg-141 for 15 min at 37°C. After being washed they were then incubated with either fluorescein-labeled rabbit anti-mouse Ig or anti-theta antiserum followed by antimouse Ig. This allowed enumeration of agg-141-positive cells simultaneously with SIg as a B-cell marker and theta as a T-cell marker. The data are shown in Table VI. After 4 days of exposure about 60% of lymphocytes bound agg-141. At this time approximately 80% of B cells, as measured by their being SIg<sup>+</sup>, bound agg-141; 30% of theta-positive cells bound agg-141; and 55% of “null” cells (SIg<sup>-</sup>, θ<sup>-</sup>) bound agg-141.

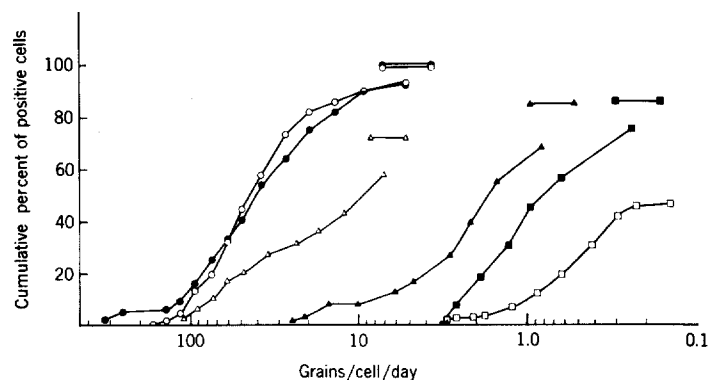


FIG. 3. Cells of various types were incubated at 37°C for 60 min (macrophages with azide at 0° C rather than 37° C) and autoradiographs were prepared. Grains/cell/day are plotted against the cumulative percentage of cells with a certain grain count or greater on the vertical axis. (○—○), macrophages; (●—●), P815 mastocytoma; (△—△), allogeneically activated T cells; (▲—▲), normal spleen cells; (■—■), L5178Y lymphoma; and (□—□), normal thymus. Horizontal lines above each curve represent the maximum percentage of positive cells obtained.

TABLE VI

*Distribution of Agg-IgG-Binding Cells Between SIg<sup>+</sup>, θ<sup>+</sup>, and Null Spleen Cells*

	Percentage of cells	
	[ <sup>125</sup> I]agg-141 positive	[ <sup>125</sup> I] agg-141 negative
	%	%
SIg <sup>+</sup>	33	6
SIg <sup>-</sup>	27	34
SIg <sup>+</sup> θ <sup>+</sup>	33	31
SIg <sup>-</sup> θ <sup>-</sup>	14	11
θ <sup>+</sup>	11	25
(SIg <sup>+</sup> θ <sup>+</sup> - SIg <sup>+</sup> )		

## DISCUSSION

Utilizing a sensitive autoradiographic assay to detect binding of <sup>125</sup>I-labeled heat-aggregated mouse IgG to various cell types, we have detected receptors for agg IgG on 70–85% of mouse spleen cells and 20–45% of thymus. Moreover, it has been possible to demonstrate similar receptor sites on the theta-positive lymphoma cell line, L5178, as well as on spleen cells obtained from lethally irradiated F<sub>1</sub> hybrid mice infused with parental thymocytes. Simultaneous labeling of surface Ig, theta antigen, and receptor sites for agg-IgG has demonstrated that the majority of B lymphocytes possess a relatively high density of receptor sites for aggregated IgG. A subpopulation of theta-positive cells also possess relatively large numbers of receptors as does a subpopulation of SIg<sup>-</sup>,

$\theta^-$ , null cells. There are two aspects of our study which need to be focused upon before discussing these results in relation to previous studies. First, as shown in Fig. 3, there is a great deal of heterogeneity in the number of agg IgG receptor sites when different cell types are compared. For instance, there is approximately a 100-fold difference in binding capacity between thymocytes and macrophages or P815 mastocytoma cells. Even within a tissue such as the spleen there is at least a 10-fold difference in receptor density when individual cells are compared. Second, besides having to prolong exposure times to detect cells with low receptor density, it was also necessary to use large sized aggregates. Our data indicate that optimal binding was obtained with aggregates in or near the exclusion volume of a Sepharose 4B column, i.e., of a size 20 million or more. This finding concurs with the data of Dickler and Kunkel (14) who found that aggregates of greater than 300S had to be used to obtain optimal binding to human B cells.

These factors—heterogeneity of numbers of receptors on different cells, importance of aggregate size, and sensitivity of the detection system—may, at least in part, explain why our results differ from those of others whose work has indicated that IgG receptor sites were limited to bone marrow derived lymphocytes. The reports of Basten et al. (12, 13) which indicated that binding of Ig was a property limited to B lymphocytes (although 2–4% of C57/B16 thymocytes were also positive) does not permit an analysis of aggregate size since undefined ratios of antigen and antibody were used to form the aggregates assayed for in their systems. With respect to sensitivity of assay, species and tissues studied, their system was similar to that we employed. Bentwich et al. (24) used similar sized aggregates, but a different, perhaps less sensitive, test system (immunofluorescence). Also their studies were done on human peripheral blood lymphocytes, whereas ours were performed on mouse spleen and thymus. It is difficult to assess which of these several differences between laboratories may account for the higher degree of B-cell specificity of the agg IgG receptor in the Bentwich et al. study (2–4% of T cells, as measured by their capacity to form rosettes with sheep erythrocytes, also bound agg-IgG) and the lack of such specificity in our study. The possibility that in peripheral blood the agg IgG receptor is primarily associated with B cells, whereas in the spleen it is not, is considered a real one since in a previous study on the occurrence of surface immunoglobulin and complement receptors (25), it was found that whereas in peripheral blood the complement receptor was found almost exclusively on SIg<sup>+</sup> cells, in the spleen there was a significant population of cells that were SIg<sup>-</sup> complement receptor positive.

As mentioned above, although our results are at variance with previous reports indicating that only B cells bear receptor sites for IgG, there are other studies which support our data that lymphoid cells other than B cells also possess such a receptor. Previous studies with the theta-positive lymphoma L5178 (8) as well as with another theta-positive lymphoma (6) have shown such

receptor sites on these cells. Secondly, using a rosette assay, Yoshida and Anderson (15) have also shown that a high percentage of activated T cells form rosettes with sheep cells coated with IgG antibody. Orr and Paraskevas (16) also have data suggestive of the presence of receptor sites for IgG on the surface of T cells. Moreover, Webb and Cooper (17) have demonstrated the capacity of thymocytes and blood lymphocytes from bursectomized birds depleted of B cells to form rosettes with sheep cells coated with IgM antish sheep erythrocyte antibody, indicating receptors for IgM on the surface of non-B lymphocytes. Experiments to test for the presence of aggregated IgM receptor sites in our system have not as yet been possible due to the lack of suitable aggregated IgM preparations. Work by Greenberg et al. (26, 27) studying antibody-dependent, cell-mediated cytotoxicity suggests that a non-B, non-T lymphoid cell with sedimentation velocity characteristics of nonphagocytic monocytes is the effector cell in this reaction. It is likely that some of the non-B non-T cells that we have detected by our aggregated IgG assay are similar to the cytotoxic cells that Greenberg describes.

The difference between the density of receptor sites on normal spleen cells as compared with spleen cells obtained from animals injected with semi-allogeneic parental cells was striking. Whether this represents an increase in receptor density following activation of T cells or is merely a characteristic of any proliferating lymphocyte population has not as yet been determined, and experiments to further investigate this phenomenon are underway.

Finally, the existence of IgG receptors on some T cells accentuates the problem of evaluating the presence of small amounts of T-cell-associated Ig as a possible synthetic product of T cells. Moreover, the use of this receptor as a means of eliminating B cells from a mixed population of lymphocytes must be done with the understanding that a receptor-bearing subpopulation of non-B cells, perhaps functionally important, may also be removed.

#### SUMMARY

An autoradiographic binding assay employing  $^{125}\text{I}$ -labeled heat-aggregated mouse IgG2b myeloma protein (MOPC 141) was used to demonstrate receptors for IgG on 20–45% of Balb/c thymocytes and on 70–80% of splenocytes. Binding could also be shown with heat or BDB aggregates of another IgG2b (MOPC 195), with IgG1 and with human  $\gamma$ -globulin, but not with aggregated chicken  $\gamma$ -globulin, IgA, BSA, nor with aggregated Fab fragments of IgG2b. Optimum binding was obtained at 37°C. Detection of binding was dependent upon aggregate size with complexes of more than 100 IgG molecules being optimal, aggregates of 6–25 detecting splenocytes but not thymocytes, and aggregates of less than 6 binding to a negligible extent. Comparison of grain counts on various cell types showed mastocytoma cells (P815) and macrophages averaging 40–50 grains/cell/day, allogeneically activated thymocytes 20–30, splenocytes 2–3, L5178 lymphoma cells 1, and positive thymocytes 0.6 grains/cell/day. Double

labeling experiments for surface Ig,  $\theta$ -antigen, and agg IgG receptor on mouse spleen cells indicated that a relatively high density of receptor was present on about 80% of B cells, 30% of T cells, and 60% of SIg<sup>-</sup>,  $\theta$ <sup>-</sup>, null cells.

## REFERENCES

1. Boyden, S. V., and E. Sorkin. 1960. The adsorption of antigen by spleen cells previously treated with antiserum in vitro. *Immunology*. **3**:272.
2. Boyden, S. V. 1964. Cytophilic antibody in guinea-pigs with delayed-type hypersensitivity. *Immunology*. **7**:474.
3. Tigelaar, R. E., N. M. Vaz, and Z. Ovary. 1971. Immunoglobulin receptors on mouse mast cells. *J. Immunol.* **106**:661.
4. Lay, W. H., and V. Nussenzweig. 1968. Receptors for complement on leukocytes. *J. Exp. Med.* **128**:991.
5. Cline, M. J., and N. L. Warner. 1972. Immunoglobulin receptors on a mouse mast cell tumor. *J. Immunol.* **108**:339.
6. Harris, A. W., A. D. Bankhurst, S. Mason, and N. L. Warner. 1973. Differentiated functions expressed by cultured mouse lymphoma cells. II.  $\theta$  antigen, surface immunoglobulin and a receptor for antibody on cells of a thymoma cell line. *J. Immunol.* **110**:431.
7. Shevach, E., R. Herberman, R. Lieberman, M. M. Frank, and I. Green. 1972. Receptors for immunoglobulin and complement on mouse leukemias and lymphomas. *J. Immunol.* **108**:325.
8. Grey, H. M., R. T. Kubo, and J.-C. Cerottini. 1972. T cell immunoglobulins. Presence of a receptor site for IgG and absence of large amounts of "buried" Ig determinants on T cells. *J. Exp. Med.* **136**:1323.
9. Dickler, H. B., F. P. Siegal, Z. H. Bentwich, and H. G. Kunkel. 1973. Lymphocyte binding of aggregated IgG and surface Ig staining in chronic lymphocytic leukemia. *Clin. Exp. Immunol.* **14**:97.
10. Tønner, O., and S. Thunold. 1973. Receptors for immunoglobulin Fc in human malignant tissues. *Scand. J. Immunol.* **2**:207.
11. Huber, H., and H. H. Fudenberg. 1968. Receptor sites on human monocytes for IgG. *Int. Arch. Allergy Appl. Immunol.* **34**:18.
12. Basten, A., J. F. A. P. Miller, J. Sprent, and J. Pye. 1972. A receptor for antibody on B lymphocytes. I. Method of detection and functional significance. *J. Exp. Med.* **135**:610.
13. Basten, A., N. L. Warner, and T. Mandel. 1972. A receptor for antibody on B lymphocytes. II. Immunochemical and electron microscopy characteristics. *J. Exp. Med.* **135**:627.
14. Dickler, H. B., and H. G. Kunkel. 1972. Interaction of aggregated  $\gamma$ -globulin with B lymphocytes. *J. Exp. Med.* **136**:191.
15. Yoshida, T. O., and B. Anderson. 1972. Evidence for a receptor recognizing antigen complexed immunoglobulin on the surface of activated mouse thymus lymphocytes. *Scand. J. Immunol.* **1**:401.
16. Orr, K. B., and F. Paraskevas. 1973. Cell surface associated gamma globulin in lymphocytes. V. Detection of early cytophilic complexes reacting with T and B lymphocytes. *J. Immunol.* **110**:456.

17. Webb, S. R., and M. D. Cooper. 1973. T cells can bind antigen via cytophilic IgM antibody made by B cells. *J. Immunol.* **111**:275.
18. Ishizaka, K., and T. Ishizaka. 1960. Biologic activity of aggregated  $\gamma$ -globulin. II. A study of various methods for aggregation and species differences. *J. Immunol.* **85**:163.
19. Cerottini, J.-C., and E. R. Unanue. 1971. Genetic control of the immune response of mice to hemocyanin. *J. Immunol.* **106**:732.
20. Cerottini, J.-C., A. A. Nordin, and K. T. Brunner. 1970. In vitro cytotoxic activity of thymus cells sensitized to alloantigens. *Nature (Lond.)* **227**:72.
21. Sprent, J., and J. F. A. P. Miller. 1971. Activation of thymus cells by histocompatibility antigens. *Nat. New Biol.* **234**:195.
22. Grey, H. M., J. W. Hirst, and M. Cohen. 1971. A new mouse immunoglobulin: IgG3. *J. Exp. Med.* **133**:289.
23. Klinman, N. R., and R. B. Taylor. 1969. General methods for the study of cells and serum during the immune response: The response to dinitrophenyl in mice. *Clin. Exp. Immunol.* **4**:473.
24. Bentwich, Z., S. D. Douglas, F. P. Siegel, and H. G. Kunkel. 1973. Human lymphocyte-sheep erythrocyte rosette formation: Some characteristics of the interaction. *Clin. Immunol. Immunopathol.* **1**:511.
25. Ross, G. D., E. M. Rabellino, M. J. Polley, and H. M. Grey. 1973. Combined studies of complement receptor and surface immunoglobulin-bearing cells and sheep erythrocyte rosette-forming cells in normal and leukemic human lymphocytes. *J. Clin. Invest.* **52**:377.
26. Greenberg, A. H., and L. Shen. 1973. A class of specific cytotoxic cells demonstrated in vitro by arming with antigen-antibody complexes. *Nat. New Biol.* **245**:282.
27. Greenberg, A. H., L. Shen, and I. M. Roitt. 1973. Characterization of the antibody-dependent cytotoxic cell. A non-phagocytic monocyte? *Clin. Exp. Immunol.* **15**:251.