Immunoglobulins Cytophilic for Human Lymphocytes, Monocytes, and Neutrophils

DAVID A. LAWRENCE, WILLIAM O. WEIGLE, and HANS L. SPIEGELBERG

From the Scripps Clinic and Research Foundation, La Jolla, California 92037

ABSTRACT The cytophilic activity of human myeloma proteins of different classes and subclasses for lymphocytes, monocytes, and neutrophils was investigated. Binding of both unaggregated immunoglobulins (Ig) and Ig aggregated with rabbit $F(ab')_2$ anti-Fab fragment sera was determined. Lymphocytes bound unaggregated IgG1 and IgG3 proteins, but none of the proteins of the other classes. In contrast, after aggregation, IgG of all subclasses and IgE proteins bound to lymphocytes; aggregated proteins of the other classes did not bind. Monocytes bound unaggregated IgG1 and IgG3 better than IgG4 whereas the binding of proteins of other classes was insignificant. Neutrophils bound unaggregated IgG1 and IgG3 proteins and, in addition, IgA1, IgA2, secretory IgA, and IgG4 proteins. After aggregation, the neutrophils bound more Ig of all classes; however, the differences between the amounts bound remained similar to the amounts of unaggregated proteins. The native structure of the Ig molecule is necessary for the maintenance of complete activity, because Fc fragments bound less than intact Ig, and reduction and alkylation abolished cytophilia. The Fc receptors on all cell types tested showed no specificity for any of the respective cytophilic IgG subclasses; however, neutrophils appear to have separate receptors for IgG and IgA proteins.

INTRODUCTION

In 1960, Boyden and Sorkin demonstrated that radioiodinated antigens could be bound to normal spleen cells which had previously been incubated with specific antisera (1). The term "cytophilic" immunoglobulins (Ig) [1] was coined for these cell-bound antibodies in immune sera. The physical and biological properties of cytophilic antibodies were further investigated by Berken and Benacerraf (2), who reported that cytophilia is restricted to certain Ig classes, that the Ig binding site for macrophages is located on the Fc fragment, and that the binding is not complement dependent. Ig have been shown to be cytophilic to macrophages (2–5), neutrophils (6), basophils (7), mast cells (8), and recently also to lymphocytes (4, 9). Cytophilic antibodies are involved in opsonization, a promotion of phagocytosis, and additional biological significance of cytophilic Ig was shown by the finding that IgE antibodies gave rise to allergies by binding to basophils and mast cells and inducing release of vasoactive substances from these cells after reaction with antigen (8). Cytophilic Ig may also enhance humoral and cellular immune responses as a function of their binding to macrophages (10) and/or thymus-derived (T) lymphocytes (11) which, in turn, could aid presentation of antigen to potential antibody-producing cells and increase immune clearance; in addition, they may be involved in delayed hypersensitivity (12, 13).

To date, most investigations of cytophilic Ig have involved macrophages or neutrophils and have employed a "rosette" technique in which antibody-coated red cells bind to phagocytes with Fc receptors (2, 4–6). Use of a rosette formation inhibition assay has shown that human IgG1 and IgG3 proteins are cytophilic for monocytes (5) and neutrophils (6). More recently, cytophilic properties have also been studied by measuring macrophage uptake of radiolabeled Ig (3), or release

This is Publication No. 842 from Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, Calif.

Dr. Lawrence is supported by U. S. Public Health Service Training Grant 5T1-GM683. Dr. Weigle is supported by U. S. Public Health Service Research Career Award 5K6-GM6936.

Received for publication 29 July 1974 and in revised form 8 October 1974.

[1] *Abbreviations used in this paper:* B, bone marrow-derived; BSA, bovine serum albumin; BSS, balanced salt solution; FCS, fetal calf serum; Ig, immunoglobulin; NGG, normal human IgG; S-IgA, secretory IgA; T, thymus-derived.

of histamine from basophils (8), or release of β-glucuronidase from neutrophils (14). Nine classes and subclasses of human Ig have been identified, but little is known about their individual cytophilic capabilities, particularly with lymphocytes. The present investigation was undertaken to further characterize the cytophilic properties of the different Ig by studying their ability to bind to lymphocytes, monocytes, and neutrophils. Human myeloma proteins and normal immunoglobulins were labeled with ^{125}I, and the uptake of both unaggregated or aggregated proteins was studied.

METHODS

Myeloma protein. 19 myeloma proteins, three macroglobulins, normal IgG, and secretory IgA were studied. The myeloma proteins consisted of four IgG_1, four IgE, two each of the IgG2, IgG3, IgG4, IgA1, and IgA2, and one IgD protein. The IgG myeloma proteins of slow electrophoretic mobility were isolated by DEAE-cellulose chromatography with 0.005 M phosphate buffer, pH 8.0. Normal human IgG (HGG) and myeloma proteins of fast gamma mobility were isolated with a 0.015 M phosphate buffer, pH 8.0. IgA myeloma proteins were isolated by Pevikon block electrophoresis, followed by Sephadex G-200 gel filtration. The isolated human secretory IgA was kindly provided by Dr. T. Tomasi. The IgD myeloma protein was isolated as previously described (15). IgE myeloma proteins were purified by DEAE-cellulose chromatography with a 0.015 M phosphate buffer, pH 8.0, followed by Sephadex G-200 gel infiltration. IgM macroglobulins were isolated by repeated euglobulin precipitations, followed by Sephadex G-200 gel filtration. The isolated proteins were analyzed by immunoelectrophoresis and radio immunoelectrophoresis with sheep anti-whole human serum or rabbit anti-HGG. Only traces of contaminating β and α_2 proteins were detected in the IgA and IgM preparations, respectively. In order to minimize the amount of normal IgG in the IgG myeloma proteins, only sera from patients having 4 g or more of myeloma protein/100 ml serum were used. Class and light chain type were determined by double diffusion in agarose with specific rabbit or goat antisera. The IgG subclasses were identified with specific rabbit antisera (16).

$F(ab')_2$, Fab, and Fc fragments of IgG myeloma proteins were prepared as previously described (17). Mild reduction and alkylation was performed exactly as described by Rhodes (18). The IgM proteins Og[2] and Vi, the IgA_2 protein Kr, and the IgG_3 protein Ca were dissolved in 0.1 M Tris buffer and reduced with 0.7 mM dithiothreitol for 1 h at room temperature, after which the proteins were alkylated by addition of 3.0 mM twice recrystallized iodoacetamide and incubated for 1 h at 0°C. The proteins were then dialyzed against 0.15 M NaCl, pH 7.0, and iodinated for binding studies.

Anti-Fab fragment antisera. Hyperimmune rabbit antisera to human light chains and Fab fragments from a previous experiment involving rabbits tolerant to human gamma globulin Fc fragments were used (19). The globulin serum fraction was then precipitated with 50% saturated ammonium sulfate and digested with pepsin at pH 4.0 according to the method of Nisonoff, Wissler, Lipman, and Woernley (20). A small amount of the precipitate which formed during digestion was removed and the supernate dialyzed against phosphate-buffered 0.15 M NaCl, pH 7.0. No Fc determinants were demonstrable in this preparation when analyzed with a sheep anti-rabbit IgG antiserum. The $F(ab')_2$ fragments still specifically precipitated isolated Bence Jones proteins or myeloma proteins of either K or L type when analyzed by double gel diffusion in agarose at a concentration of 10 mg protein $F(ab')_2$ fragments/ml.

Isolation of lymphocytes, monocytes, and neutrophils. Lymphocytes, monocytes, and neutrophils were isolated from the peripheral blood of individual healthy human donors. Lymphocytes were purified by Ficoll-Hypaque gradients and nylon fiber columns as described by Mendelsohn, Skinner, and Kornfeld (21). Monocytes were obtained by the method of Huber and Fudenberg (5), which utilizes a 28% bovine serum albumin (BSA) gradient. Neutrophils were isolated by the technique described by Henson (22). The neutrophil preparation was actually a granulocyte preparation; it contained approximately 2–6% eosinophils but will be referred to as "neutrophil" because neutrophils represented the majority of the cells. The final cell preparations of lymphocytes, monocytes, and neutrophils were 95–99, 80–90, and 90–98% pure, respectively, as determined by differential cell counts. All cell preparations were 95–99% viable as determined by trypan blue exclusion.

Binding assay of ^{125}I-labeled proteins. Suspensions of 5×10^6 cells in 0.1 ml of balanced salt solution (BSS) containing 0.25% BSA and 0.7 mM EDTA were put into plastic tubes on ice; 0.7 mM EDTA was consistently used in the incubation medium, because it has been reported to lower nonspecific binding (23). ^{125}I-labeled proteins were added in a volume of 10 μl, and the cells were gently mixed for 30 min at 4°C. After incubation the cells were washed twice with 100% fetal calf serum (FCS) by layering 1 ml of cell suspension on 6 ml of FCS and centrifuging at 250 *g* for 10 min. FCS washes were used because when cell counts after each wash were determined and plotted against number of washes, the plot was linear after two washes; whereas when BSS was used, 4–6 washes were required. To eliminate contribution of small but variable amounts of radioactive proteins sticking to the tubes, the plastic tubes were discarded after each wash and the cells transferred to a new tube. After washing, the final cell pellet was suspended in 1 ml of BSS and again transferred to a new tube before being assayed in a well type scintillation counter. The results were expressed as picograms of immunoglobulin per 10^6 cells. All analyses were performed in duplicate, sometimes in triplicate analyses, which usually did not vary by more than ±10%; if a greater variation was observed the results were discarded. As a control for nonspecific sticking to the cells, human and bovine serum albumin were similarly labeled and tested. In inhibition studies, various amounts of unlabeled proteins in 5-μl volumes were added to the cells, and the mixtures were preincubated for 30 min at 4°C. Binding of unaggregated and aggregated ^{125}I-labeled proteins was investigated. The ^{125}I-labeled proteins, 4 μg/ml, were deaggregated by ultracentrifugation in a Beckman SW 65 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) at 40,000 *g* for 150 min. Aggregation was accomplished by use of rabbit $F(ab')_2$ anti-human Fab fragment sera. Cells were incubated with the ^{125}I-labeled proteins for 30 min at 4°C, after which antibodies (0.1–1,000 μg/0.1 ml) were added and the mixture incubated for 30 min longer.

Radioiodination and autoradiography. Proteins were

[2] The proteins are identified by abbreviation of the patient's name.

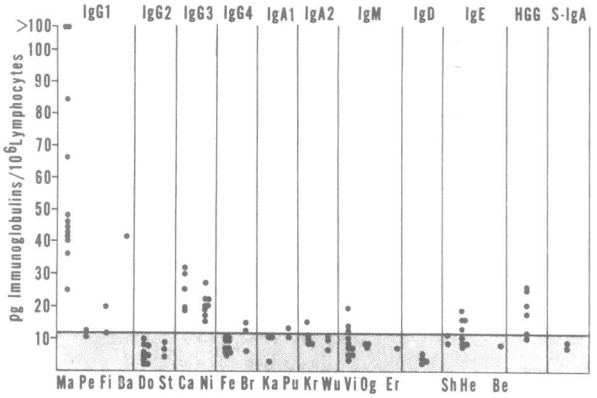

FIGURE 1 Binding of ^{125}I-labeled Ig to human lymphocytes. Each column represents uptake of either HGG, S-IgA, or a myeloma protein identified by abbreviation of patient's name and each point, the mean of duplicate determinations with an individual's lymphocyte preparation. The shaded area indicates the highest binding of IgG2 proteins below which binding is considered insignificant.

labeled in small volumes with ^{125}I (ICN Corp., Chemical & Radioisotopes Div., Irvine, Calif.) by a chloramine-T procedure (24). The specific activity of the different preparations varied from 27 to 50 μCi/μg. The autoradiographic method employed was a slight modification (25) of that described by Davie and Paul (26). Autoradiographic exposure time was 10–14 days.

RESULTS

Binding of unaggregated immunoglobulins to human lymphocytes, monocytes, and neutrophils. The amounts of unaggregated ^{125}I-labeled myeloma proteins, HGG, and secretory IgA (S-IgA) that bound to lymphocytes at 4°C are shown in Fig. 1. The highest amount of cell-bound IgG2 protein was arbitrarily selected as the baseline below which binding was considered insignificant because (*a*) consistently low levels of IgG2 proteins were bound, (*b*) there was little variation in amount bound, and (*c*) IgG2 proteins were tested with all isolated cell preparations. According to this baseline, lymphocytes bound significant amounts of unaggregated IgG1 and IgG3 proteins, but none of the proteins of the other classes. There was a relatively large variation among individual myeloma proteins within subclasses and among cells from different donors. The uptake varied from 10–210 pg for IgG1 and 17–33 pg for IgG3 proteins. One IgE protein did show a slight amount of binding (19 pg was the greatest amount bound), but two other IgE proteins did not bind. There were no binding differences between proteins with either κ or λ type light chains. The HGG which bound probably represented IgG1 and IgG3 protein binding, for these proteins constitute approximately 80% of IgG's heterogenous population. It was not unexpected, therefore, that HGG binding was similar to IgG1 and IgG3 binding. One of the IgG1 proteins (Ma) had an exceptionally high binding capacity.

The binding of unaggregated Ig to monocytes is shown in Fig. 2. As with lymphocytes the IgG1 and IgG3 proteins bound best; however, monocytes bound more IgG1 (20–430 pg) and IgG3 proteins (27–221 pg) than lymphocytes. A similar variation in the amount of each myeloma protein bound was also observed, and protein Ma also bound exceptionally well to monocytes. In contrast to lymphocytes, IgG4 proteins bound to monocytes, although to a lesser degree

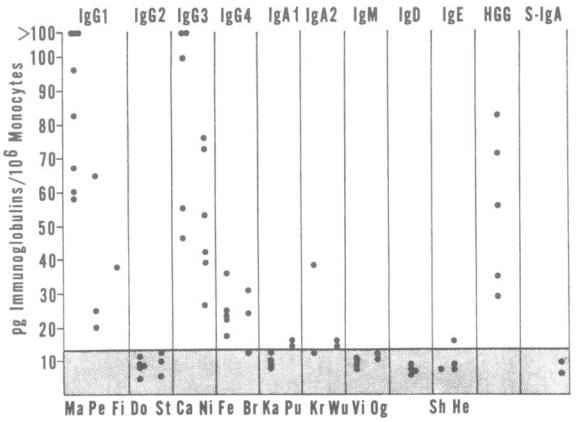

FIGURE 2 Binding of ^{125}I-labeled Ig to human monocytes. Each column represents uptake of either HGG, S-IgA, or a myeloma protein identified by abbreviation of patient's name and each point, the mean of duplicate determinations with an individual's monocyte preparation. The shaded area indicates the highest binding of IgG2 proteins below which binding is considered insignificant.

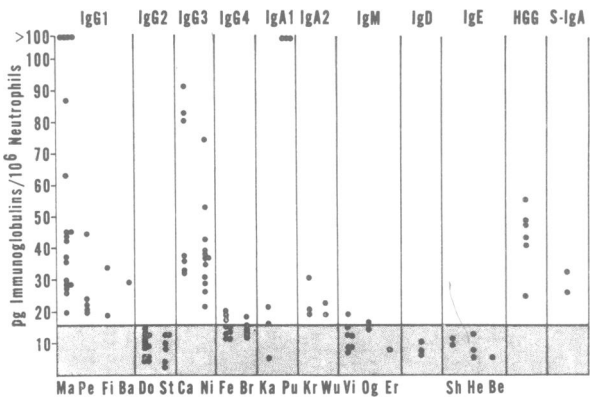

FIGURE 3 Binding of ^{125}I-labeled Ig to human neutrophils. Each column represents uptake of either HGG, S-IgA, or a myeloma protein identified by abbreviation of patient's name and each point, the mean of duplicate determinations with an individual's neutrophil preparation. The shaded area indicates the highest binding of IgG2 proteins below which binding is considered insignificant.

TABLE I

*Inhibition of ^{125}I-Labeled Human IgG (HGG) Binding to Human Neutrophils**

Inhibitor‡	% Inhibition
IgG1	
Ma§	68
Pe	46
Fi	44
IgG2	
Do	14
St	9
IgG3	
Ni	53
Ca	48
IgG4	
Fe	25
Br	27
HGG	51

* Inhibition determinations were done by incubating 5 × 10^6 neutrophils with inhibitor for 30 min at 4°C, adding 40 ng of [^{125}I]HGG, and then incubating the mixture for 30 min at °C. The figures represent the mean of duplicate determinations and the results are representative of a number of similar experiments.

‡ 4 μg of inhibitor was used.

§ Proteins are identified by abbreviation of patient's name.

than IgG1 and IgG3 proteins. HGG bound to monocytes in quantities similar to most IgG1 and IgG3 proteins.

As can be seen in Fig. 3, neutrophils bound not only IgG1 and IgG3 proteins, but also IgA myeloma proteins and S-IgA. However, except for one IgA1 protein, Pu, IgG1 and IgG3 proteins bound to a greater extent than IgA1, IgA2, and S-IgA proteins. IgG4 proteins bound slightly better than IgG2 proteins. As with IgG1 and IgG3 proteins, the amount of each IgA myeloma protein bound within the IgA class differed. Protein Pu bound exceptionally well to neutrophils, but did not bind to lymphocytes or monocytes. Binding of the nonimmunoglobulin proteins, BSA and human serum albumin, indicated that nonspecific cytophilic binding was less than 10 pg/10^6 cells.

Effects of temperature, azide, and EDTA on binding of unaggregated immunoglobulins. The cytophilic activity assay was usually performed at 4°C, with a homologous system of human cells and human myeloma proteins being used to minimize phagocytosis or pinocytosis. When cytophilia was tested at 37°C, greater amounts of Ig bound to all cell types. However, though the amount of all IgG proteins bound to neutrophils increased at 37°C, only proteins which bound well at 4°C increased significantly. Binding of IgG1 and IgG3 proteins at 37°C increased 4–6-fold, whereas the amount of IgG4 bound increased approximately threefold, and IgG2 binding did not significantly increase. The binding of IgM, IgD, and IgE proteins also did not significantly increase at 37°C. To determine if phagocytosis was contributing to higher uptake at 37°C, sodium azide was added to the incubation medium. The amount of Ig bound at 37°C was not significantly altered by the presence of 0.1% azide. In addition, concentrations of sodium EDTA ranging from 0.7 to 20 mM also had no influence on Ig binding to any type of cells tested which indicated that Ig binding did not require Ca^{++} and Mg^{++} ions.

Autoradiographic studies. Autoradiographic studies with lymphocytes, monocytes, and neutrophils were in-

TABLE II

*Inhibition of Binding of ^{125}I-Labeled Myeloma Proteins to Human Cells**

Cell type	[^{125}I]Ig	Inhibitor‡	% Inhibition
Lymphocyte	Ma (IgC1)	Ma§	95
		Ni	80
	Ni (IgG3)	Ma	81
		Ni	83
Monocyte	Ma (IgG1)	Ma	89
		Ca	85
	Ni (IgG3)	Ma	92
		Ni	90
	Ca (IgG3)	Ma	86
		Ca	81
	Fe (IgG4)	Ma	75
		Ca	80
Neutrophil	Ma (IgG1)	Ma	76
		Ca	52
		Pu	18
		Wu	13
	Ni (IgG3)	Ma	82
		Ni	79
		Pu	17
		Wu	12
	Ca (IgG3)	Ma	71
		Ca	80
	Pu (IgA1)	Ma	43
		Ni	16
		Pu	79
		Wu	42

* Inhibition determinations were done by incubating 5 × 10^6 cells with inhibitor for 30 min at 4°C, adding 40 ng of [^{125}I]Ig, and then incubating the mixture 30 min at 4°C. The figures represent the mean of duplicate determinations and the results are representative of a number of similar experiments.

‡ 4 μg of inhibitor was used.

§ Proteins are identified by abbreviation of patient's name.

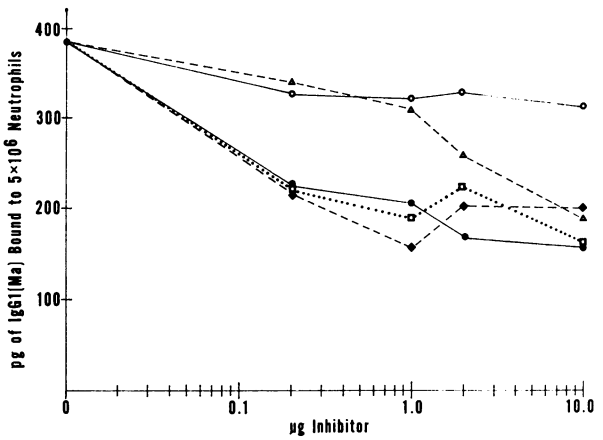

FIGURE 4 Quantitative inhibition of IgG1 protein (Ma) binding to human neutrophils. Inhibition study performed by simultaneous addition of 40 ng [^{125}I]Ma and various quantities of IgA1 (Pu), ○——○; IgG1 (Ma), ●——●; IgG2 (Do), △---△; IgG3 (Ni), □ · · · □; or IgG4 (Fe), ◆---◆, to a suspension of 5×10^6 neutrophils followed by an incubation at 4°C for 30 min. Each point represents the mean of duplicate determinations. Note the more effective inhibition by the IgG4 protein as compared to the IgG2 protein.

conclusive. Three to five grains were seen on each cell and, occasionally, there were cells with ten or more grains on them. A high nonspecific background was present which probably was due to a dissociation of cytophilic immunoglobulins from the cells as a results of their low affinities; this high background made it very difficult to interpret the findings.

Inhibition of immunoglobulin binding. Binding of ^{125}I-labeled Ig was inhibited by prior addition of 100-fold excess of unlabeled protein in order to characterize cell receptor specificity for different Ig and to learn more about the submolecular Ig site involved in the binding. In addition, the inhibitory studies confirmed the direct binding data (Table I). Proteins of the IgG subclasses inhibited HGG binding to neutrophils in direct correlation with their binding ability, IgG1 and IgG3 proteins inhibited as well as HGG, producing 42–68% and 48–53% inhibition, respectively. IgG4 proteins were less effective giving 25–27% inhibition; IgG2 proteins gave no significant inhibition. Since there was a correlation between ability to bind and to inhibit, only proteins exhibiting significant binding were usually used as inhibitors for further studies.

The specificity of lymphocyte, monocyte, and neutrophil receptors for the Ig that they could bind is demonstrated in Table II. Interestingly, the cell receptors did not express any subclass specificity for the IgG proteins which could bind since the IgG proteins used did not significantly differ in their inhibition of each other. Neutrophils have the ability to bind proteins of the IgA as well as IgG class, and cross-inhibition with proteins of these two classes was studied. As shown in Table II, IgG1 protein Ma partially inhibited the binding of IgA1 protein Pu. Other IgG proteins which bound to a lesser degree than Ma did not show cross-inhibition and Pu did not inhibit IgG binding. Without preincubation with inhibitors, the protein Ma (IgG1) did not inhibit the binding of protein Pu (IgA1) though both proteins still blocked their own binding. Fig. 4 shows the quantitative inhibition of Ma binding to neutrophils by a myeloma protein of each IgG subclass and an IgA protein. The IgG4 protein inhibition was equal to the IgG1 and IgG3 protein inhibition,

TABLE III

*Inhibition of Binding of ^{125}I-Labeled Myeloma Proteins to Human Cells by Ig Fragments**

Cell type	[^{125}I]Ig	Inhibitor‡	% Inhibition
Lymphocyte	Ma (IgG1)	Ma-Fc§	90
		Ma-Fab	11
Monocyte	Ma (IgG1)	Ma-Fc	87
		Ma-Fab	20
		Ma-F(ab')$_2$	34
		Ca-Fc	85
		Ca-Fab	15
	Ca (IgG3)	Ma-Fc	83
		Ma-Fab	8
		Ma-F(ab')$_2$	28
		Ca-Fc	83
Neutrophil	Ma (IgG1)	Ma-Fc	64
		Ma-Fab	2
		Ma-F(ab')$_2$	28
		Ca-Fc	44
		Ca-Fab	18
		Ca-F(ab')$_2$	23
	Ca (IgG3)	Ma-Fc	62
		Ma-Fab	6
		Ma-F(ab')$_2$	18
		Ca-Fc	57
		Ca-Fab	16
		Ca-F(ab')$_2$	20

* Inhibition determinations were done by incubating 5×10^6 cells with inhibitor for 30 min at 4°C, adding of 40 ng [^{125}I]Ig, and then incubating the mixture for 30 min at 4°C. The figures represent the mean of duplicate determinations and the results are representative of a number of similar experiments.

‡ 4 μg of inhibitor was used.

§ Enzymatic fragments of proteins are identified by abbreviation of patient's name and type of fragment.

TABLE IV

*Percent Binding Activity of ^{125}I-Labeled Enzymatic Fragments of Ig to Human Cells**

Cell type	Protein	% Activity‡
Lymphocyte	Ma (IgG1)	100
	Fc	45
	Fab	7
	F(ab')$_2$	26
	Ca (IgG3)	100
	Fc	71
	Fab	30
	F(ab')$_2$	36
	R&A	24
Monocyte	Ma (IgG1)	100
	Fc	77
	Fab	22
	F(ab')$_2$	13
	Ca (IgG3)	100
	Fc	72
	Fab	9
	F(ab')$_2$	7
	R&A	9
Neutrophil	Ma (IgG1)	100
	Fc	53
	Fab	21
	F(ab')$_2$	20
	Ca (IgG3)	100
	Fc	42
	Fab	14
	F(ab')$_2$	16
	R&A	19

* 40 ng of [^{125}I]Ig, native, mildly reduced and alkylated (R&A), or enzymatic digests, were incubated with 5×10^6 cells for 30 min at 4°C. The figures represent the mean of duplicate determinations and the results are representative of a number of similar experiments.

‡ Binding activity expressed as percent of the amount of native molecule bound.

whereas the IgG2 protein inhibited weakly and the IgA protein not at all.

The inhibitory activity of Fc, Fab, and F(ab')$_2$ fragments was examined to determine the location of the binding site in the IgG molecule (Table III). On the whole, the results were the same for all cell types. Fc fragments were inhibitory, but not to the same extent as the intact molecules. F(ab')$_2$ fragments exhibited a small but greater degree of inhibition (20–34%) than the Fab fragments (2–18%), which was considered insignificant.

Binding of immunoglobulin fragments and mildly reduced and alkylated immunoglobulins. Since the inhibition experiments indicated that there may be differences between Fab and F(ab')$_2$ binding, direct binding studies with ^{125}I-labeled Ig fragments were performed. The percent of cytophilic activity remaining in the various fragments is shown in Table IV. Fc fragments retained the most activity, but even these fragments had 23–58% less activity than the intact molecules. Fab and F(ab')$_2$ fragments usually had only low ability to bind, and there was no significant difference between their binding capabilities. When calculated on a picogram per 10^6 cell basis the binding of the different Fab fragment preparations varied from 4 to 15 pg/10^6 cells. This is probably insignificant since it is in the range of the IgG2 proteins and of BSA. IgG proteins lost approximately 90% of their original cytophilic activity when mildly reduced and alkylated.

The study of mildly reduced and alkylated Ig is of interest because recent studies have indicated that there is a receptor for monomeric IgM (8S subunits) on guinea pig macrophages (18). In order to find out if monomeric IgM can bind to human lymphocytes or monocytes, direct binding studies were undertaken with ^{125}I-labeled normal or reduced and alkylated Ig (Table V). Reduction and alkylation of IgM did not affect its ability to bind to lymphocytes or monocytes, i.e., neither the 8S nor 19S IgM could bind to these cells. Mild reduction and alkylation of IgM, IgA1, and IgG3 protein did not enhance their ability to bind; in fact, as reported above, this treatment caused a significant reduction in cytophilia with the IgG3 protein Ca and some reduction with IgA1 protein Kr.

Binding of aggregated immunoglobulins by lymphocytes and neutrophils. Since myeloma proteins with

TABLE V

Ability of Mildly Reduced and Alkylated Ig to Bind to Human Lymphocytes and Monocytes

Cell type	[^{125}I]Ig	Immunoglobulin
		pg/10^6 cells
Lymphocyte	Og (IgM)	9
	R&A Og	16
Monocyte	Og (IgM)	12
	R&A Og	5
	Kr (IgA2)	39
	R&A Kr	14
	Ca (IgG3)	160
	R&A Ca	26

* 40 ng of [^{125}I]Ig, native or mildly reduced and alkylated (R&A), were incubated with 5×10^6 cells for 30 min at 4°C. The figures represent the mean of duplicate determinations and the results are representative of a number of similar experiments.

‡ Ig are identified by abbreviation of patient's name.

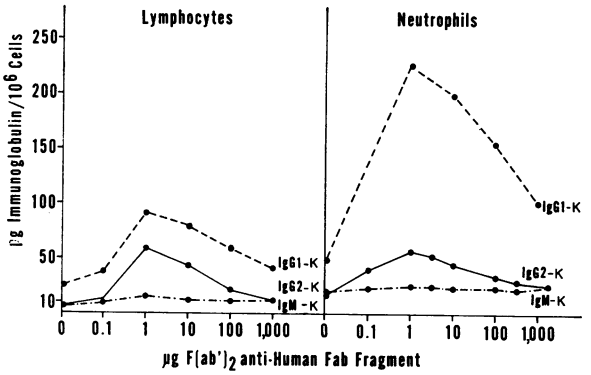

FIGURE 5 Binding of Ig after aggregation with $F(ab')_2$ fragments of rabbit anti-human light chain antibodies. Various quantities of anti-Fab fragment antibodies were added after a 30-min incubation of an ^{125}I-labeled IgG1, IgG2, or IgM protein with either a lymphocyte or neutrophil preparation in order to determine the optimal concentration of antibody for maximum binding. Each point represents the mean of duplicate determinations.

no known antibody activities were used, aggregation could not be achieved by addition of antigen. Instead, cross-linking of Fab regions was achieved with rabbit $F(ab')_2$ fragments of anti-human Fab fragments. Rabbit $F(ab')_2$ fragments were used so that they would not compete with the myeloma proteins for cell receptors. To determine the optimal antibody-Ig ratio for binding, the amount of antibody added to 40 ng of [^{125}I]Ig was varied over a five log range (Fig. 5). The amount of IgG1 and IgG2 proteins bound by lymphocytes and neutrophils was greatest with 1 μg of antibody; the amount of IgM bound was not significantly affected by any antibody concentration. 1 μg of antibody was found to give optimal binding with the IgG1-K and IgG2-K proteins. Therefore, all proteins with κ chains were examined after addition of 1 μg. Proteins with λ chains were optimally aggregated with 5 μg of antibody. The increased binding due to aggregation is shown in Table VI. Due to the large increase in binding of IgG2 and IgG4 proteins and the small increase of the IgG1 and IgG3 protein binding, after aggregation, proteins of all IgG subclasses bound to lymphocytes to a more similar extent. In addition, all four IgE myeloma proteins tested bound significantly to lymphocytes after aggregation, whereas proteins of the other classes did not. On neutrophils, myeloma proteins of all IgG subclasses increased in binding after aggregation. Relative amounts bound, however, remained similar to those of the unaggregated proteins. Proteins of the other classes did not show this specific increase in binding.

TABLE VI

Binding Ratio of Aggregated to Unaggregated [^{125}I]Ig to Human Lymphocytes and Neutrophils

[^{125}I]Ig*	Binding ratio‡	
	Lymphocyte	Neutrophil
Ma (IgG1)	2.9	4.6
Do (IgG2)	7.6	3.3
Ni (IgG3)	3.7	3.7
Fe (IgG4)	8.0	2.6
Vi (IgM)	2.0	1.3
Er (IgM)	—	1.8
Pu (IgA1)	1.1	2.2
Wu (IgA2)	2.2	1.7
Di (IgD)	1.6	1.3
He (IgE)	8.3	2.2
Sh (IgE)	5.2	2.4
Be (IgE)	4.6	1.7
Nd (IgE)	5.7	2.7
HGG	—	3.6
S-IgA	—	2.3

* Ig identified by abbreviation of patient's name. Ig was aggregated or unaggregated as described in Methods. The figures represent the mean of duplicate determinations and the results are an average of four experiments.

‡ Binding ratio is equal to amount of aggregated [^{125}I]Ig per 10^6 cells/amount of unaggregated [^{125}I]Ig per 10^6 cells.

DISCUSSION

Binding of myeloma proteins to lymphocytes, monocytes, and neutrophils indicated that unaggregated IgG1 and IgG3 immunoglobulins (Ig) were cytophilic to all three cell types, whereas IgG4 was cytophilic only to monocytes and IgA only to neutrophils. Unaggregated IgG2, IgM, IgD, and IgE proteins did not significantly bind to these cell types. In contrast to the binding of unaggregated IgG proteins, aggregated IgG proteins of all four subclasses bound significantly to the cell types tested. These findings suggest that although all IgG subclasses are cytophilic, only IgG1 and IgG3 proteins have an affinity for the cell receptors sufficiently high to be detectable by binding assays for unaggregated proteins. This could explain why in previous studies of inhibition of rosette formation by unaggregated Ig, cytophilia could only be indirectly demonstrated with IgG1 and IgG3 proteins (5, 6). Lymphocytes bound aggregated IgE proteins as well as the aggregated IgG proteins. Aggregated IgE proteins did not bind to neutrophils. In addition, aggregated IgM and IgD proteins did not bind significantly to the cell types tested. Neutrophils were the only cell type that bound IgA, a finding which is in agreement with previous data on the release of lysosomal enzymes from neutrophils by aggregated IgA (14) and preliminary findings on binding of IgA to neutrophils

(27). The receptors for IgG and IgA on neutrophils appeared to be different since no significant cross-inhibition was observed. In contrast, IgG of different subclasses bound to the same receptor as indicated by the extensive cross-inhibition. Fc fragments retained most of the cytophilic activity, though less than the intact molecules, the $F(ab')_2$ fragment which contains the amino-terminal portion of the Fc fragment retained some activity whereas the Fab fragment neither bound to the cells nor inhibited the binding of Ig significantly. Reduction and alkylation of the interchain disulfide bonds greatly abolished the binding, indicating that the native conformation of the Ig molecule is important for the cytophilic activity.

The amounts of unaggregated Ig bound to lymphocytes, monocytes, and neutrophils were expressed in picograms per 10^6 cells; however, the numbers most likely do not represent the absolute amount of Ig that can be bound on these cells, and, therefore, only indicate the relative cytophilic capabilities of Ig classes and subclasses. The maximum quantity of Ig that could be bound to the cells was difficult to determine because various amounts of cytophilic Ig probably remained bound to the cells after isolation and were not replaced by the [^{125}I]Ig added. In addition, the binding affinities of cytophilic Ig are probably low and washing after incubation undoubtedly removed some [^{125}I]Ig. Moreover, because myeloma proteins within a subclass differed in their degree of binding, the binding of one protein does not necessarily represent the average binding of the corresponding normal Ig class or subclass. This variation has been reported for most biological systems in which myeloma proteins were used as an Ig source, and it is presently not fully understood (3, 14). The tertiary structures of intact Ig molecules may influence the binding sites by a modulation of the Fc fragments as a result of the variability of the Fab fragments. An alternate or additional mechanism for this binding variance could be due to a differential ability of the myeloma proteins to spontaneously aggregate, because it has been shown that aggregated Ig bound better than unaggregated Ig and presence of small amounts of aggregates could not be excluded even after ultracentrifugation of the labeled proteins.

Binding of aggregated Ig differed markedly from that of unaggregated Ig in that generally greater amounts of aggregated Ig were bound and cytophilia of IgG2, IgG4, and IgE proteins could only be demonstrated by this technique. The ability of IgG2 and IgG4 proteins to bind is in agreement with previous reports demonstrating that aggregated proteins of all four IgG subclasses release granule constituents from neutrophils (14), and aggregated HGG, IgG1, and IgG2 proteins bound equally well to lymphocytes (9). The increased binding of aggregates is probably due to their ability to bind to multiple receptors on the cells resulting in a higher affinity.

IgG subclasses apparently share the same receptor on all cell types tested, as evidence by the equal abilities of IgG1, IgG3, and, in the case of monocytes, IgG4 proteins to inhibit each other's binding. The specificity of lymphocyte receptors for IgG and IgE proteins could not be tested because unaggregated IgE proteins did not bind and inhibition studies with aggregated proteins were technically not possible. The neutrophil receptors for the two classes of Ig (IgG and IgA) are probably different because there was only weak, if any, cross-inhibition with most proteins analyzed. There may, however, be some structural similarities since one IgG1 protein (Ma), which had a particularly high affinity to cells, partially inhibited IgA binding. It could be, therefore, that some Ig have affinities for both receptors because the binding site on the Ig molecule appears to differ slightly from molecule to molecule as evidenced by the intrasubclass variation mentioned above.

It has been reported that 8S subunits but not intact IgM are cytophilic to guinea pig macrophages (18) and that chicken IgM is cytophilic for chicken T lymphocytes (11). In our study, human IgM macroglobulins did not bind to lymphocytes and monocytes either as intact proteins or as reduced and alkylated proteins. The reason for this difference might be a species difference, or may be because monocytes and not macrophages were used. Lay and Nussenzweig (28) reported that IgM can bind to mouse macrophages but not to monocytes.

The biologic role of cytophilic antibody to monocytes and neutrophils is probably promotion of phagocytosis, but their role on lymphocytes is less clear. IgG bound to lymphocytes probably plays a role in the lymphocyte cytotoxicity described by Perlmann and Holm (29), and it has been postulated that cytophilic Ig is involved in delayed hypersensitivity (11, 13). IgE has previously been reported to bind to T lymphocytes and inhibit the sheep red blood cell rosetting (30), the biological significance of IgE binding to lymphocytes, however, remains to be shown. Lymphocytes can be divided into T and bone marrow-derived (B) cells and possibly a third type referred to as "null" or A cells (31). The autoradiographic procedure employed in this study failed to show binding to a subpopulation of lymphocytes. Preliminary experiments of binding of IgG to nylon fiber purified T cell population and B lymphocytes obtained from a patient with chronic lymphatic leukemia suggest that both cell types have a receptor for IgG, which is in agreement with recent reports on binding of IgG to human lymphocytes (9, 32) as well as murine T cells (33). How-

ever, more extensive and particularly quantitative studies have to be performed in order to clarify this point.

ACKNOWLEDGMENTS

We thank Dr. Ben G. Fishkin for providing the sera of most of the myeloma patients, Patricia Thickstun for her excellent technical assistance, and Mrs. Linda Norwood for her secretarial assistance.

This work was supported by U. S. Public Health Service Grants AI-07007 and AI-10734-03, American Heart Association Grant 73-753, American Cancer Society IM42D, and Atomic Energy Commission Contract AT (04-3) 410.

REFERENCES

1. Boyden, S. V., and E. Sorkin. 1960. The adsorption of antigen by spleen cells previously treated with antiserum *in vitro*. *Immunology*. **3**: 272–283.
2. Berken, A., and B. Benacerraf. 1966. Properties of antibodies cytophilic for macrophages. *J. Exp. Med.* **123**: 119–144.
3. Inchley, C., H. M. Grey, and J. W. Uhr. 1970. The cytophilic activity of human immunoglobulins. *J. Immunol.* **105**: 362–369.
4. LoBuglio, A. F., R. S. Cotran, and J. H. Jandl. 1967. Red cells coated with immunoglobulin G: binding and sphering by mononuclear cells in man. *Science (Wash. D. C.)*. **158**: 1582–1585.
5. Huber, H., and H. H. Fudenberg. 1968. Receptor sites of human monocytes for IgG. *Int. Arch. Allergy Appl. Immunol.* **34**: 18–31.
6. Messner, R. P., and J. Jelinek. 1970. Receptors for human γG globulin on human neutrophils. *J. Clin. Invest.* **49**: 2165–2171.
7. Ishizaka, K., H. Tomioka, and T. Ishizaka. 1970. Mechanisms of passive sensitization. I. Presence of IgE and IgG molecules on human leukocytes. *J. Immunol.* **105**: 1459–1467.
8. Ishizaka, K. 1970. Human reaginic antibodies. *Annu. Rev. Med.* **21**: 187–200.
9. Dickler, H. B., and H. G. Kunkel. 1972. Interaction of aggregated γ-globulin with B lymphocytes. *J. Exp. Med.* **136**: 191–196.
10. Basten, A., and M. Feldmann. 1973. The role of soluble factors in T-B cell collaboration. *In* Microenvironmental Aspects of Immunity. B. D. Jankovic and K. Isakovic, editors. Plenum Press, New York. 171.
11. Webb, S. R., and M. D. Cooper. 1973. T cells can bind antigen via cytophilic IgM antibody made by B cells. *J. Immunol.* **111**: 275–277.
12. Nelson, D. S., and S. V. Boyden. 1967. Macrophage cytophilic antibodies and delayed hypersensitivity. *Br. Med. Bull.* **23**: 15–20.
13. Zembala, M., W. Ptak, and M. Hanczakowska. 1974. The induction of delayed hypersensitivity by macrophage-associated antigen. The role of macrophage cytophilic antibody. *Immunology*. **26**: 465–476.
14. Henson, P. M., H. B. Johnson, and H. L. Spiegelberg. 1972. The release of granule enzymes from human neutrophils stimulated by aggregated immunoglobulins of different classes and subclasses. *J. Immunol.* **109**: 1182–1192.
15. Spiegelberg, H. L., J. W. Prahl, and H. M. Grey. 1970. Structural studies of human γD myeloma proteins. *Biochemistry*. **9**: 2115–2122.
16. Spiegelberg, H. L., and W. O. Weigle. 1968. The production of antisera to human γG subclasses in rabbits using immunological unresponsiveness. *J. Immunol.* **101**: 377–380.
17. Spiegelberg, H. L., and W. O. Weigle. 1965. The catabolism of homologous and heterologous 7S gamma globulin fragments. *J. Exp. Med.* **121**: 323–338.
18. Rhodes, J. 1973. Receptor for monomeric IgM on guinea pig splenic macrophages. *Nature (Lond.)*. **243**: 527–528.
19. Spiegelberg, H. L., and W. O. Weigle. 1967. The immune response to human γG-immunoglobulin in rabbits unresponsive to Fc fragment and H-chain protein. *J. Immunol.* **98**: 1020–1027.
20. Nisonoff, A., F. C. Wissler, L. N. Lipman, and D. L. Woernley. 1960. Separation of univalent fragments from the bivalent rabbit antibody molecule by reduction of disulfide bonds. *Arch. Biochem. Biophys.* **89**: 230–244.
21. Mendelsohn, J., A. Skinner, and S. Kornfeld. 1971. The rapid induction by phytohemagglutinin of increased γ-aminoisobutyric acid uptake by lymphocytes. *J. Clin. Invest.* **50**: 818–826.
22. Henson, P. M. 1971. The immunologic release of constituents from neutrophil leukocytes. I. The role of antibody and complement on nonphagocytosable surfaces or phagocytosable particles. *J. Immunol.* **107**: 1535–1546.
23. Tai, C., and J. E. McGuigan. 1971. Detection of cell-absorbed protein antigen by an equilibrium diffusion method. *J. Immunol.* **106**: 1540–1544.
24. Unanue, E. R. 1971. Antigen-binding cells. I. Their identification and role in the immune response. *J. Immunol.* **107**: 1168–1174.
25. Lawrence, D. A., H. L. Spiegelberg, and W. O. Weigle. 1973. 2,4-Dinitrophenyl receptors on mouse thymus and spleen cells. *J. Exp. Med.* **137**: 470–482.
26. Davie, J. M., and W. E. Paul. 1971. Receptors on immunocompetent cells. II. Specificity and nature of receptors on dinitrophenylated guinea pig albumin-^{125}I-binding lymphocytes of normal guinea pigs. *J. Exp. Med.* **134**: 495–516.
27. Spiegelberg, H. L., D. A. Lawrence, and P. A. Henson. 1974. Cytophilic properties of IgA to human neutrophils. *Adv. Exp. Med. Biol.* **45**: 67–74.
28. Lay, W. H., and V. Nussenzweig. 1969. Ca^{++}-dependent binding of antigen-19 S antibody complexes to macrophages. *J. Immunol.* **102**: 1172–1178.
29. Perlmann, P., and G. Holm. 1969. Cytotoxic effects of lymphoid cells *in vitro*. *Adv. Immunol.* **11**: 117–193.
30. Hanifin, J. M., and B. R. Gottlieb. 1974. IgE inhibits T-cell rosette formation. *Clin. Res.* **22**: 328A.
31. Greenberg, A. H., L. Hudson, L. Shen, and I. M. Roitt. 1973. Antibody-dependent cell-mediated cytotoxicity due to a null lymphoid cell. *Nature (Lond.)*. **242**: 111–113.
32. 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**: 610–626.
33. Anderson, C. L., and H. M. Grey. 1974. Receptors for aggregated IgG on mouse lymphocytes. Their presence on thymocytes, thymus-derived, and bone marrow-derived lymphocytes. *J. Exp. Med.* **139**: 1175–1188.