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Cytophilic Activity of IgG2 from Sera of Guinea-Pigs Immunized with Bovine γ-Globulin

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Summary. IgG2 from immune guinea-pig sera collected 1–2 weeks after secondary BGG challenge showed considerably enhanced cytophilic activity; the preparations contained aggregates (±10 per cent of the total immunoglobulin) shown by antigen exchange to be immune complexes with a probable composition Ag (fragments) Ab_5. After removal of aggregates the cytophilic activity of immune 7S IgG2 approximated that of IgG2 from unimmunized animals.

Artificial immune complexes from either non-immune or immune 7S IgG2 (aggregated with the $F(ab')_2$ fragments of rabbit anti-guinea-pig Fab) showed greatly enhanced cytophilic activity, maximal at antigen : antibody equivalence and comparable for both IgG2 preparations.

These data are consistent with the view that cytophilic activity for macrophages is a uniform property of the whole guinea-pig IgG2 class.

The IgG2 complexes apparently bind to glass-adherent cells and also to a population of non-glass-adherent mononuclear cells, present in peritoneal exudates but not detectable in peripheral blood.

INTRODUCTION

The cytophilic activity of guinea-pig IgG2 for macrophages has been recorded by numerous workers. The binding of ^{125}I-labelled IgG2 to macrophages in the absence of antigen is reversible (Berken and Benacerraf, 1966) with an association constant of $1{\cdot}46 \pm 0{\cdot}45 \times 10^6$ L/M (Leslie and Cohen, 1974). Berken and Benacerraf (1966) were unable to eliminate the cytophilic activity of ^{125}I-labelled IgG2 by repeated absorption with macrophages and concluded that at least a major part of the IgG2 class is cytophilic.

Other observations on antibody-related cytophilic activity have brought into question the functional homogeneity of IgG2. Gowland (1968) passively administered anti-SRBC antisera to guinea-pigs and reported that cytophilic activity showed a shorter half-life than haemagglutinating or complement-fixing activity. Nelson and Mildenhall (1968) observed poor correlation between cytophilic activity and complement fixation of IgG2 antibodies after immunization with SRBC in Freund's complete adjuvant followed by skin inoculation of antigen. Liew and Parish (1972a) reported the complete absorption of cytophilic activity from IgG2 preparations without loss of antibody activity. In an attempt to resolve these contradictions, the antigenic and physicochemical pro-

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perties of IgG2 prepared from normal and immune (anti-BGG) sera have been compared and related to observed differences in the cytophilic activities of the two preparations.

MATERIALS AND METHODS

Animals

Hartley strain guinea-pigs (300–700 g) were used.

Immunization

Anti-BGG antisera were prepared by injection of BGG (0·5 mg) in FCA (0·5 ml) intramuscularly in the hind legs on day 1, followed by subcutaneous injection of the same dose on day 8. The animals received booster injections of BGG (0·5 mg) in Al_2O_3, 0·15 M NaCl suspension (0·25 ml) intraperitoneally on day 21 and were bled by cardiac puncture on days 28 and 35 and the recovered sera were pooled.

Rabbit anti-guinea-pig Fab antiserum was raised by immunization with Fab fragments from IgG2 as described above. Blood was collected from the ear vein on days 28 and 35 and subsequently at 1–2-month intervals, 7 days after re-challenge.

Preparation of IgG2 and $F(ab')_2$

IgG2 and $F(ab')_2$ were prepared from a single pool of anti-BGG antiserum and from normal sera as described previously (Leslie and Cohen, 1970; Leslie, Melamed and Cohen, 1971).

Radioiodination of IgG2 and $F(ab')_2$

This was performed as described by Leslie and Cohen (1974).

Assay of ^{125}I-labelled IgG2 binding by peritoneal exudate cells

Isolation of cells and assay of binding were performed as described by Leslie and Cohen (1974).

Preparation of $F(ab')_2$ fragments of IgG from rabbit anti-guinea-pig Fab sera

Rabbit anti-guinea-pig Fab serum (28 ml, 4·8 mg of antibody/ml) was precipitated with 50 per cent saturated ammonium sulphate (SAS); the precipitate was dissolved in 10 ml of phosphate-buffered saline (PBS) and reprecipitated with SAS (10 ml). The precipitate, dissolved in water was dialysed against 0·021 M Na_2HPO_4, 0·05 M KH_2PO_4, pH 7·5 at 20° for 4 days, and fractionated on DEAE–cellulose (DE22, Whatman) using the same buffer for elution. The eluted IgG was concentrated, dialysed against 0·1 M acetate buffer, pH 4·5 and digested with 1 per cent w/w pepsin (Worthington) for 6 hr at 37°. Digestion was stopped by neutralization with 2·5 M Tris. Filtration of the digest on Sephadex G-200 using 50 mM phosphate buffer, pH 7·5 yielded three peaks corresponding to $F(ab')_2$, pFc′ and small peptides, respectively. The pooled, concentrated $F(ab')_2$ peak (54 mg) was titrated for antibody by precipitation with IgG2 as antigen. The antibody content of the $F(ab')_2$ preparation was 21·3 per cent and the molar combining ratio was 3·7 : 1 (Ab : Ag).

RESULTS

PROPERTIES OF IgG2 PREPARATIONS

IgG2, prepared from anti-BGG sera, developed as a single arc on immunoelectrophoresis against rabbit anti-guinea-pig serum (Fig. 1). The antibody content was 22 per cent of the total IgG2 (0·44 mg of antibody/2·0 mg of IgG2) representing a recovery of 0·9 mg of anti-BGG IgG2 antibody per ml of antiserum. The molar ratio of antigen : antibody at equivalence was 1 : 3·3.

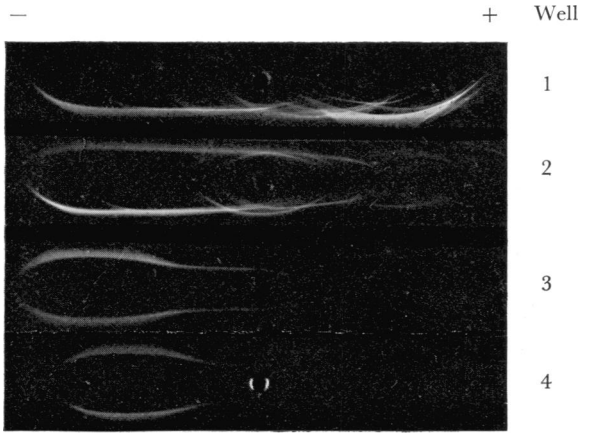

FIG. 1. Immunoelectrophoresis of IgG2 fractions from normal and immune (anti-BGG) antisera prepared by salt precipitation and ion-exchange chromatography. Wells: (1) GPS (anti-BGG); (2) 40 per cent SAS precipitate; (3) IgG2 (anti-BGG); (4) normal IgG2. All troughs contained rabbit anti-GPS.

GEL FILTRATION OF IgG2

The elution pattern on Sephadex G-200 of IgG2 from anti-BGG antisera was compared with that of IgG2 prepared from normal sera (Fig. 2). The latter eluted as a single symmetrical peak, while the immune fraction showed a major peak in the 7S monomer position together with a pronounced leading shoulder which, assuming symmetry in the 7S peak, contained 9–10 per cent of the total protein. The peak tube of the shoulder lay within the included volume of the column, indicating a molecular weight of less than 800,000, consistent with the presence of oligomers of IgG2 containing between two and five subunits.

NATURE OF THE AGGREGATE

The presence of antigen, or antigenic fragments, in the aggregated IgG2 material was tested for as follows. IgG2 (anti-BGG) was radiolabelled with ^{125}I and passed through Sephadex G-200 giving the two peaks described above. Aliquots of ^{125}I-labelled IgG2 (5×10^5 cpm, 10 μg), taken from the tube at the apex of the 7S peak, were incubated for 1 hour at 37° with (i) unlabelled IgG2 aggregate (2·0 OD_{280} units) recovered from the previously described Sephadex G-200 separation (Fig. 2b) together with normal IgG2

(1·5 mg) and (ii) with normal IgG2 only. Both mixtures were rerun on Sephadex G-200. A separate ^{125}I-labelled IgG2 monomer preparation with anti-OA specificity was incubated as a control with the same unlabelled aggregate used in (i) above together with normal IgG2.

The aggregate material after concentration, in the absence of 7S IgG2, eluted with the

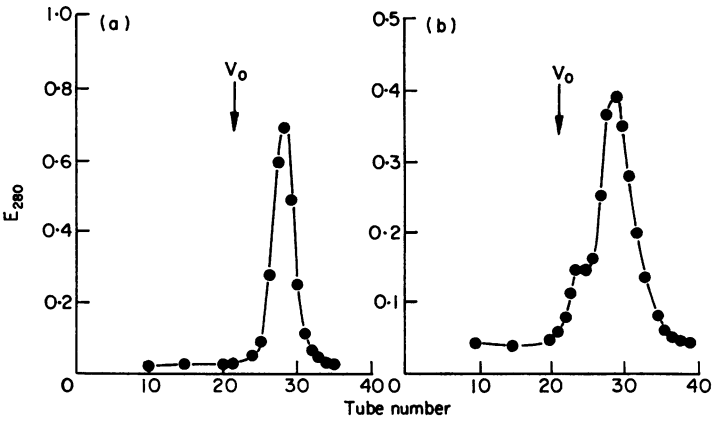

FIG. 2. Filtration through Sephadex G-200 of (a) IgG2 prepared from normal serum and (b) IgG2 prepared from anti-BGG antiserum. The eluting buffer was 50 mM PO_4, pH 7·5. Bed volume: (a) 550 ml; (b) 580 ml.

TABLE 1

DISTRIBUTION OF ^{125}I-LABELLED IgG2 (ANTI-BGG) BETWEEN AGGREGATE AND MONOMER ON FILTRATION THROUGH SEPHADEX G 200

Mixture analysed		$E^{280\ nm}_{1\ cm}$ at:		Percentage of ^{125}I in:	
^{125}I-labelled	Unlabelled	V_0	V IgG2	Aggregate	Monomer
IgG2 (anti-BGG) monomer	IgG2 monomer	0	0·057	3·1	96·9
IgG2 (anti-BGG) monomer	IgG2 monomer + IgG2 (anti-BGG) aggregate	0·052	0·058	18·0	82·0
IgG2 (anti-OA) monomer	IgG2 monomer + IgG2 (anti-BGG) aggregate	0·108	0·053	3·7	96·3

excluded volume of the column, facilitating quantification. The data in Table 1 show that 7S IgG2 (anti-BGG) does not reaggregate; however, the same labelled monomer binds to the admixed aggregate and this binding is dependent upon antibody specificity (compare anti-OA, Table 1). This indicates that the aggregate in immune IgG2 is a soluble complex between circulating antigen, or antigenic fragments and excess antibody, since reversible specific exchange of unlabelled with labelled antibody occurred.

BINDING OF NORMAL IgG2 AND IgG2 (ANTI-BGG) WITH PERITONEAL EXUDATE CELLS

After ultracentrifugation, the binding of anti-BGG ^{125}I-labelled IgG2 (2 μg) to peri-

toneal exudate cells (containing 2×10^6 macrophages) was measured. The inhibition of ^{125}I-labelled IgG2 binding by unlabelled IgG2 (20 μg and 80 μg) from the same source and by six unlabelled IgG2 preparations from individual normal sera was measured (Fig. 3). The binding of ^{125}I-labelled IgG2 (anti-BGG) in the absence of excess IgG2 (4·5 per cent) is considerably greater than the level observed with normal IgG2 preparations (1 per cent, Leslie and Cohen, 1974). In addition, IgG2 (anti-BGG) shows enhanced ability to inhibit binding.

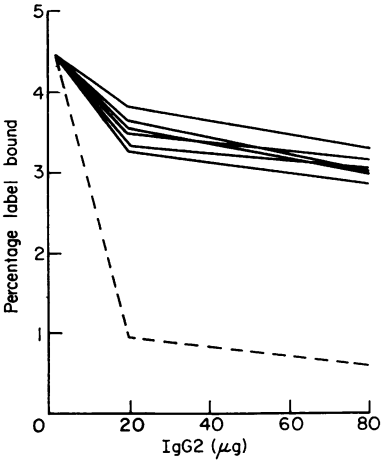

FIG. 3. Binding of ^{125}I-labelled IgG2 (anti-BGG) (2 μg) to PEC (2×10^6 macrophages) in the presence of (– – –) unlabelled IgG2 (anti-BGG) and (——) six IgG2 preparations from individual sera of non-immunized guinea-pigs.

INTERACTION BETWEEN PERITONEAL EXUDATE CELLS AND IgG2 (ANTI-BGG) AFTER AGGREGATE REMOVAL

The monomeric forms of ^{125}I-labelled IgG2 (anti-BGG), unlabelled IgG2 (anti-BGG) and unlabelled IgG2 (normal) were isolated from the apical tubes of 7S peaks after gel filtration. The labelled 7S IgG2 (2 μg, 0·5 ml) was incubated with peritoneal exudate cells (5×10^6 macrophages, 0·1 ml) in the presence of medium alone (0·5 ml) or the unlabelled monomeric IgG2 preparations (5, 20 and 50 μg in 0·5 ml) (Fig. 4). The binding observed with monomeric ^{125}I-labelled IgG2 in the absence of unlabelled inhibitor (1·9 per cent) corresponds to an uptake of 7·6 ng of IgG2/10^6 macrophages; this compares with 45 ng of IgG2/10^6 macrophage for the ^{125}I-labelled IgG2 preparation containing aggregate and 6–10 ng of IgG2/10^6 macrophages for normal IgG2 monomer. These values indicate that complexes are largely responsible for the enhanced cytophilic activity of immune IgG2. The same conclusion derives from the observation that monomers from IgG2 (anti-BGG) and normal IgG2 show similar inhibition of labelled IgG2 binding (compare Figs 3 and 4).

In a second experiment (Table 2) the macrophage binding of ^{125}I-labelled IgG2 (anti-BGG) and normal ^{125}I-labelled IgG2 were compared before and after various procedures designed to remove aggregates. These methods abrogated almost entirely the difference in binding between normal and immune IgG2 preparations at a single concentration

($1{\cdot}2 \times 10^{-8}$ M). However, complete identity in binding only occurred when the physically purified 7S IgG2 (anti-BGG) was preincubated with macrophages under conditions comparable to those employed in the binding assay. The data infer that either the physical

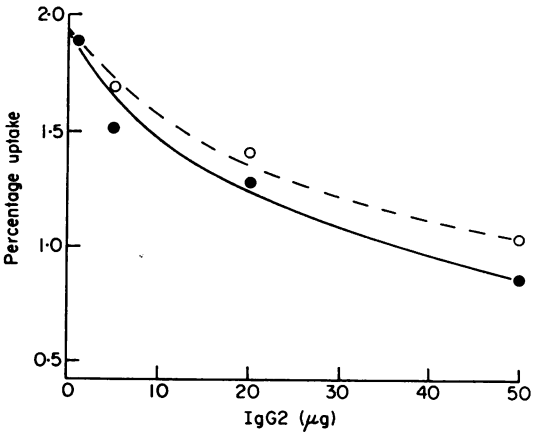

FIG. 4. Binding of 7S ^{125}I-labelled IgG2 (anti-BGG) (2 μg) to PEC (5×10^6 macrophages) in the presence of (● — ●) unlabelled 7S IgG2 (anti-BGG) and (○ – – ○) unlabelled 7S IgG2 (normal).

TABLE 2

CYTOPHILIC ACTIVITY OF IgG2 PREPARATIONS DE-AGGREGATED BY VARIOUS PROCEDURES

IgG2 preparation*	De-aggregation steps: Gel filtration	Ultracentrifugation†	Preincubation with macrophages	Percentage uptake‡
Anti-BGG	–	–	–	11·4 ±3·9
	–	+	–	6·3 ±0·1
	–	–	+§	5·9 ±0·1
	+	+	–	0·87±0·12
	+	+	+¶	0·66±0·03
Normal	–	–	–	1·44±0·18
	+	+	–	0·63±0·17

* Two micrograms of ^{125}I-labelled IgG2.

† Ultracentrifugation at 40,000 rev/min for 90 minutes in 3 × 3 ml swing-out head on MSE SS50.

‡ Uptake observed on incubation with PEC containing 2×10^6 macrophages.

§ Preincubation of ^{125}I-labelled IgG2 (10 μg) with $2{\cdot}5 \times 10^6$ macrophages at 20° for 1 hour.

¶ Preincubation of ^{125}I-labelled IgG2 (2 μg) with 2×10^6 macrophages at 20° for 90 minutes.

procedures employed fail to remove all the immune complexes or that immune IgG2 monomer contains a subpopulation with somewhat enhanced binding affinity for peritoneal exudate cells.

ASSOCIATION CONSTANT FOR IgG2 (ANTI-BGG) MONOMER

The percentage uptake of 2 μg of monomeric ^{125}I-labelled IgG2 (anti-BGG) in the presence of varying amounts of unlabelled homologous IgG2 (0–200 μg) was determined. The association constant and the number of receptor sites/macrophage were derived from a Scatchard plot of the data (Table 3). The K_a values derived from two experiments lie within the range determined for normal IgG2. The number of sites/cell ($1{\cdot}15 \pm 0{\cdot}36 \times 10^6$) is somewhat lower than that determined for the normal IgG2 ($1{\cdot}7 \pm 0{\cdot}24 \times 10^6$) but the significance of this difference has only a 50 per cent confidence limit.

TABLE 3

K_a AND BINDING SITES PER MACROPHAGE FOR MONOMER IgG2 (ANTI-BGG)

Experiment number	Number of macrophages ($\times 10^{-6}$)	K_a ($\times 10^{-6}$ L/M)	Sites/cell ($\times 10^{-6}$)	r
L99	5	1·92	0·89	−0·958
L100	2·7	1·30	1·40	−0·970
	Mean	1·61 ± 0·44	1·15 ± 0·36	
	Normal IgG2*	1·5 ± 0·45	1·7 ± 0·24	

* See Leslie and Cohen (1974).

INTERACTION BETWEEN PERITONEAL EXUDATE CELLS AND ARTIFICIALLY PREPARED IMMUNE COMPLEXES

If normal and immune 7S IgG2 preparations contain immunoglobulin subclasses differing in cytophilic activity, then their complexes might be expected to amplify this difference. To investigate this possibility ^{125}I-labelled IgG2 (10·8 μg) from normal and anti-BGG sera were prepared as monomers by gel filtration and ultracentrifugation. These were aggregated by incubation at 37° for 1 hour with $F(ab')_2$ fragments of IgG from rabbit anti-guinea-pig Fab sera. The proportions used were 3, 10, 25 and 50 ml of antibody/10·8 μg of IgG2. The 7S and complexed ^{125}I-labelled IgG2 (2 μg) were incubated with peritoneal exudate cells (2×10^6 macrophages) and the percentage uptake of label determined. ^{125}I-labelled $F(ab')_2$ from IgG2 was complexed with the same rabbit $F(ab')_2$ antiserum in identical molar proportions in a control experiment.

Both the normal and immune IgG2 preparations displayed considerable enhancement of binding after complex formation (Fig. 5), reaching maximum specific binding at about equivalence (Ab : Ag = 3·7 : 1). IgG2 (anti-BGG) did give persistently slightly higher binding than normal IgG2 with complexes formed at equivalence and in antigen excess, but the difference was no greater porportionally than that observed with the monomers, and approached statistical significance only at equivalence.

CELLULAR SPECIFICITY IN BINDING OF IgG2–ANTIBODY COMPLEXES

The binding of ^{125}I-labelled IgG2, complexed as described above, was investigated using whole peritoneal exudate cells, a non-glass-adherent subpopulation of PE cells and peripheral white blood cells. ^{125}I-labelled $F(ab')_2$ from IgG2 in equimolar complex

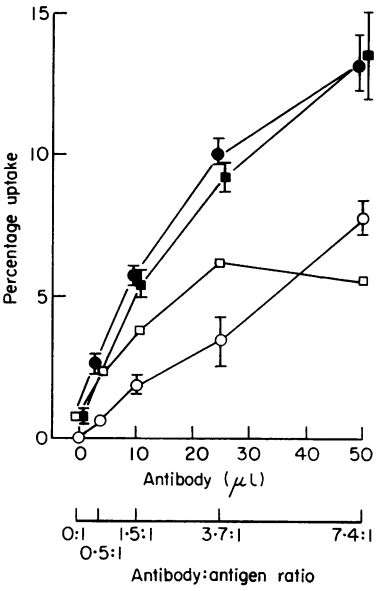

FIG. 5. Macrophage binding of normal and immune ^{125}I-labelled IgG2 and ^{125}I-labelled F(ab')$_2$ in their monomer forms and after complexing with rabbit anti-Fab serum (see text).
(●) ^{125}I-labelled IgG2 (anti-BGG) : anti-Fab complexes; (■) ^{125}I-labelled IgG2 (normal) : anti-Fab complexes; (○) ^{125}I-labelled F(ab')$_2$: anti-Fab complexes; (□) net IgG2 binding = mean of percentage binding of IgG2 (anti-BGG) and IgG2 (normal) − percentage binding of F(ab')$_2$.

TABLE 4

CELLULAR SPECIFICITY IN THE BINDING OF IgG2–ANTIBODY COMPLEXES

Experiment number	Protein*	Cell preparation	Number of cells ($\times 10^{-6}$)	M (%)	L (%)	PMN (%)	Uptake (%)	Net IgG2 uptake (%)	Fraction bound in NA	Fraction of M in NA preparation
L97	IgG2–Ab	PEC	2·8	58	25	19	4·72	3·09		
L97	F(ab')$_2$–Ab	PEC					1·63		0·265	0·155
L97	IgG2–Ab	NA	2·8	9	90	1	1·50	0·82		
L97	F(ab')$_2$–Ab	NA					0·68			
L94	IgG2–Ab	PEC	2·6	64	22	14	7·40	3·96		
L94	F(ab')$_2$–Ab	PEC					3·44		0·42	0·062
L94	IgG2–Ab	NA	2·6	4	91	5	3·10	1·67		
L94	F(ab')$_2$–Ab	NA					1·43			
L92	IgG2–Ab	WBC	2·0	5	27	68	0·14	−0·07		
L92	F(ab')$_2$–Ab	WBC					0·21			
L92										

PEC = peritoneal exudate cells; NA = non-glass-adherent PEC; WBC = white blood cells; M = macrophages/monocytes; L = lymphocytes; PMN = polymorphonuclear leucocytes.

* IgG2–Ab = ^{125}I-labelled IgG2 (2 μg) + rabbit anti-guinea-pig-Fab F(ab')$_2$ fragments (11 μl); molar ratio = 1·65 : 1; incubated for 1 hour at 37°. F(ab')$_2$–Ab = ^{125}I-labelled F(ab')$_2$ (2 μg) + rabbit anti-guinea-pig Fab-F(ab')$_2$ fragments (16 μl); molar ratio = 1·65 : 1; incubated for 1 hour at 37°.

with the rabbit anti-Fab was used as a control (Table 4). Substantial uptake of immune complexes of IgG2 and $F(ab')_2$ is observed with both the whole peritoneal exudate cell population and with the non-adherent cell preparations. The last two columns in Table 4 show that the binding activity of non-adherent cells (expressed as a fraction of binding observed with an equal number of unfractionated peritoneal exudate cells) exceeds the expected contribution to binding by residual macrophages present in the non-adherent preparations.

DISCUSSION

The IgG2 fraction from guinea-pigs immunized with BGG in FCA 4 and 3 weeks previously and boosted with alumina-absorbed antigen 1–2 weeks before bleeding, showed considerably enhanced cytophilic activity for macrophages as compared with IgG2 from normal animals. The immune fraction displayed heterogeneity on gel filtration not observed with normal IgG2. The oligomeric form of IgG2 (anti-BGG) was shown to contain determinants from the immunizing antigen available for antibody exchange with ^{125}I-labelled monomeric IgG2 possessing anti-BGG specificity. These immune aggregates eluted after the void volume of a Sephadex G-200 column, indicating a molecular weight of less than 8×10^5 which is consistent with dimers–pentameters of IgG. If the bound antigen were intact, then the composition of the immune complexes would be Ab_{1-4}:Ag and the antigen would represent 20–50 per cent of the total protein in the complexes. The recovery of aggregate (14·5 mg/50 ml serum) is equivalent to approximately 8 mg of circulating complex in a guinea-pig, and this probably includes only those immune complexes containing IgG2 type antibodies. This would imply the presence of at least 1·5–4 mg of intact antigen in the circulation which exceeds the total antigen (1·5 mg) employed for immunization. One must conclude, therefore, that the complexes are formed with fragments of antigen released into the circulation after degradation.

A rough estimate of the average association constant of the complexes with macrophages may be derived by assuming: (i) the binding contribution by aggregate to be the uptake observed with unfractionated IgG2 (anti-BGG) (6·3 per cent, Table 2) less the uptake observed with monomeric IgG2 (0·66 per cent); (ii) the concentration of aggregate to be one tenth the total IgG2 concentration ($1{\cdot}2 \times 10^{-8}$ M); (iii) the number of receptor sites per macrophage to be $2{\cdot}5 \times 10^6$ (Leslie and Cohen, 1974). Using these assumptions a K_a value of $1{\cdot}8 \times 10^8$ L/M is obtained—about 100-fold greater than that of monomeric IgG2. Complexes with such high affinity for phagocytic cells are unlikely to remain free in the circulation for long periods. It seems more probable that a dynamic situation exists involving gradual release of antigenic fragments into the circulation to form relatively short-lived complexes.

The enhanced binding activity observed with the heterogeneous IgG2 (anti-BGG) preparation can be virtually abolished by removal of immune aggregate. Conversely the formation of artificial immune complexes between monomeric IgG2 and the $F(ab')_2$ fragments of rabbit anti-guinea-pig Fab leads to marked enhancement in the binding of both normal and anti-BGG IgG2 preparations. Recent studies on the binding activities of IgG2 fragments (Alexander, Leslie and Cohen, 1974) have revealed low affinity cytophilic activity in guinea-pig $F(ab')_2$ fragments (K_a 3×10^5 L/M). The use of the $F(ab')_2$ fragment of rabbit anti-Fab to form the control immune complexes probably therefore overestimates non-specific binding. The specific uptake observed with normal IgG2–anti-Fab complexes (Fig. 5) can therefore be regarded as a minimum estimate. Slight

differences between the two IgG2 preparations are recorded both in monomer binding at low immunoglobulin concentrations (2 μg/ml, Table 2) and in the binding of complexes formed in antigen (IgG2) excess or at equivalence (Fig. 5). On the other hand, two determinations of the association constant of IgG2 (anti-BGG) monomer gave values indistinguishable from the K_a determined for normal IgG2. The strictly comparable binding activity of the normal and immune monomeric IgG2 preparations and the correlation of enhanced activity with the presence of immune aggregates, support the view (Berken and Benacerraf, 1966) that macrophage cytophilic activity is a uniform property of the guinea-pig IgG2 class. Furthermore, the relatively low binding affinity of 7S IgG2 and its readily reversible nature is consistent with failure of normal immunoglobulin to block the cytophilic activity of macrophages despite its high concentration in the circulation.

The evidence presented here does not accord with reports of a separate subpopulation of IgG2 possessing high cytophilic activity. Gowland (1968) and Nelson and Mildenhall (1968) showed that the cytophilic activity of guinea-pig anti-SRBC antisera did not correlate with haemolytic activity. Liew and Parish (1972a) found that cytophilic activity could be absorbed from IgG2 anti-SRBC and anti-flagellin preparations, without loss of antibody activity. In all these experiments cytophilic activity was measured by rosette formation (Boyden, 1964; Jonas, Gurner, Nelson and Coombs, 1965) which involved an initial interaction of antibody with peritoneal exudate cells, followed by washing of the cells and presentation of the antigen. Berken and Benacerraf (1966) demonstrated that initial absorption of anti-SRBC antisera (0·5 ml) with PE cells was accompanied by a perceptible drop in cytophilic activity measured by direct passive sensitization; this was not observed when antigen and antibody were complexed before incubation with macrophages (indirect passive sensitization). Subsequent absorptions of the antisera did not produce any further loss of activity as determined by either technique. These findings imply that direct passive sensitization is particularly responsive to oligomeric IgG2.

In two reports (Liew and Parish, 1972; Gowland, 1968) loss of cytophilic activity was recorded under circumstances that would ensure removal of immune complexes, i.e. a preincubation with macrophages and passage through a guinea-pig. Although their methods of immunization (FCA primary and skin challenge after 2 weeks) differ somewhat from that used in this report, it is reasonable to propose that the cytophilic subpopulation of IgG2 described by these workers comprises immune aggregates formed with soluble antigenic fragments from the inoculated sheep erythrocytes. Regulatory effects upon the immune response (Liew and Parish, 1972), achieved by intraperitoneal injection of the IgG2 antibody 1 hour before antigen challenge by the same route, may be similarly interpreted. Thus, injected immune complexes would be more effectively bound at the site of injection than monomeric antibody and so confer enhanced antigen binding capacity (by antigen exchange) on the macrophages at the immunization site. This explanation is consistent with other observations (Liew and Parish, 1972b; Unanue and Cerottini, 1970; Seeger and Oppenheim, 1972) on immune response regulation after administration of macrophage-bound antigen.

The cells chiefly involved in binding of IgG2 aggregates appear to be macrophages, but binding to non-glass-adherent cells also occurs. This observation might be explained in the case of monomer IgG2 binding (Leslie and Cohen, 1974) if limited numbers of macrophages in the non-adherent population bound a small amount of remaining aggregate. However, the binding studies on immune complexes (Table 4) indicate the

presence of a non-adherent cell type binding IgG2. The virtual absence of polymorphonuclear leucocytes from the non-adherent cells and the lack of immune complex binding by peripheral white blood cells which contain a high proportion of polymorphs, suggests that the second IgG2-binding cell is mononuclear. Antigen-independent binding of aggregated IgG by B cells (Basten, Miller, Sprent and Pye, 1972; Dickler and Kunkel, 1972) and the binding of immune complexes to a mononuclear cell of disputed origin which mediates antigen-specific cytotoxicity (Greenberg, Hudson, Shen and Roitt, 1973; Greenberg, Shen and Roitt, 1973; Perlmann and Perlmann, 1970) have been recorded.

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REFERENCES

ALEXANDER, M. D., LESLIE, R. G. Q. and COHEN, S. (1974). 'Cytophilic activity of enzymatically derived fragments of guinea-pig IgG_2.' (In preparation.)

BASTEN, A., MILLER, J. F. A. P., SPRENT, J. and PYE, J. (1972). 'A receptor for antibody on B lymphocytes. I. Method of detection and functional significance.' *J. exp. Med.*, **135,** 610.

BERKEN, A. and BENACERRAF, B. (1966). 'Properties of antibodies cytophilic for macrophages.' *J. exp. Med.*, **123,** 119.

BOYDEN, S. V. (1964). 'Cytophilic antibody in guinea-pigs with delayed-type hypersensitivity.' *Immunology*, **7,** 474.

DICKLER, H. B. and KUNKEL, H. G. (1972). 'Interaction of aggregated γ-globulin with B lymphocytes.' *J. exp. Med.*, **136,** 191.

GOWLAND, E. (1968). 'The physico-chemical properties of cytophilic antibody.' *Aust. J. exp. Biol. med. Sci.*, **46,** 73.

GREENBERG, A. H., HUDSON, L., SHEN, L. and ROITT, I. M. (1973). 'Antibody-dependent cell-mediated cytotoxicity due to a "null" lymphoid cell.' *Nature: New Biology*, **242,** 111.

GREENBERG, A. H., SHEN, L. and ROITT, I. M. (1973). 'Characterisation of the antibody-dependent cytotoxic cell. A non-phagocytic monocyte?' *Clin. exp. Immunol.*, **15,** 251.

JONAS, W. E., GURNER, B. W., NELSON, D. S. and COOMBS, R. R. A. (1965). 'Passive sensitization of tissue cells. I. Passive sensitization of macrophages by guinea-pig cytophilic antibody.' *Int. Arch. Allergy*, **28,** 86.

LESLIE, R. G. Q. and COHEN, S. (1970). 'Chemical properties of guinea-pig immunoglobulins γ_1G, γ_2G and γM.' *Biochem. J.*, **120,** 787.

LESLIE, R. G. Q. and COHEN, S. (1974). 'Cytophilic activity of IgG_2 from sera of unimmunized guinea-pigs.' *Immunology*, **27,** 577.

LESLIE, R. G. Q., MELAMED, M. D. and COHEN, S. (1971). 'The products from papain and pepsin hydrolyses of guinea-pig immunoglobulins γ_1G and γ_2G.' *Biochem. J.*, **121,** 829.

LIEW, F. Y. and PARISH, C. R. (1972a). 'Regulation of the immune response by antibody. II. The effects of different classes of passive guinea-pig antibody on humoral and cell-mediated immunity in rats.' *Cell. Immunol.*, **5,** 499.

LIEW, F. Y. and PARISH, C. R. (1972b). 'Regulation of the immune response by antibody. III. The effect of macrophage cytophilic antibody on humoral and cell-mediated immunity.' *Cell. Immunol.* **5,** 520.

NELSON, D. S. and MILDENHALL, P. (1968). 'Studies on cytophilic antibodies. II. The production by guinea-pigs of macrophage cytophilic antibodies to sheep erythrocytes and human serum albumin: relationship to the production of other antibodies and the development of delayed-type hypersensitivity.' *Aust. J. exp. Biol. med. Sci.*, **46,** 33.

PERLMANN, P. and PERLMANN, H. (1970). 'Contactual lysis of antibody-coated chicken erythrocytes by purified lymphocytes.' *Cell. Immunol.*, **1,** 300.

SEEGER, R. C. and OPPENHEIM, J. J. (1972). 'Macrophage-bound antigens. I. Induction of delayed hypersensitivity and priming for production of serum antibodies in guinea-pigs.' *J. Immunol.*, **109,** 244.

UNANUE, E. R. and CEROTTINI, J.-C. (1970). 'The immunogenicity of antigen bound to the plasma membrane of macrophages.' *J. exp. Med.*, **131,** 711.