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The binding of human and guinea-pig IgG subclasses to homologous macrophage and monocyte Fc receptors

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Summary. Guinea-pig IgG_2 and IgG_1 bind to contiguous Fc receptors on homologous peritoneal macrophages. Equilibrium association constants determined for the binding of human IgG subclasses to homologous peripheral blood monocytes show that the order of binding is $IgG_1 > IgG_3 > IgG_4 > IgG_2$. Direct binding and rosette assay techniques independently established that both guinea-pig IgG_2 and human IgG bind to homologous macrophage-monocyte Fc receptors through a site present in whole $Fc(C_H2 . C_H3)_2$, but absent in pFc' subfragments $(C_H3)_2$.

INTRODUCTION

Macrophage and monoctye Fc receptors are capable of binding immunoglobulin in monomeric or complexed form (Berken & Benacerraf, 1966; Arend & Mannik, 1973; Leslie, Alexander & Cohen, 1977; Wood, 1977). Such receptors can be detected by rosette formation of antibody coated particles around the cells or by the uptake of radiolabelled immunoglobulin. There is conflicting evidence concerning the domain of IgG that carries the binding site and both C_H3 (Yasmeen, Ellerson, Dorrington & Painter, 1973; 1976) and C_H2 (Alexander, Leslie & Cohen, 1976) have been implicated.

The present report shows that human IgG binds to homologous peripheral blood monocytes with a higher affinity than that observed between guinea-pig IgG_2 and homologous peritoneal macrophages. Both rosette assays and direct binding assays indicate that the interaction of human IgG and guinea-pig IgG_2 with homologous macrophage-monocyte Fc receptors involves sites in the C_H2 domain.

Abbreviations used in this paper: TCM 199; Tissue culture medium 199; OA; Ovalbumin; EA; Antibody senistized erythrocytes; DTT; dithiothreitol; hPBld, human peripheral blood; PEC; peritoneal exudate cells.

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MATERIALS AND METHODS

Isolation of guinea-pig IgG_2 and IgG_1 with anti-sheep erythrocyte specificity

Outbred guinea-pigs (250–500 g/either sex) were immunized with 0·25 ml 30% sheep erythrocyte suspension in Freund's Complete Adjuvant by simultaneous intramuscular and subcutaneous injection followed by an intradermal injection of 0·1 ml 30% erythrocytes in saline 14 days later. The animals were bled by cardiac puncture 4 to 5 weeks after the first injection (Nelson, 1969).

Decomplemented serum was fractionated to

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isolate pure IgG_2 and IgG_1 as described by Leslie & Cohen (1970); Alexander (1975).

Haemagglutination tests were performed by adding 0·2 ml of 1% sheep erythrocytes to 0·2 ml of serially diluted immunoglobulin preparations. The titre, determined after 2 h incubation at room temperature, was taken as the dilution where visible agglutination ceased.

The numbers of immunoglobulin molecules specifically bound to erythrocytes were measured using ^{125}Iodine labelled IgG_2 or IgG_1 (McFarlane, 1958) anti-erythrocyte preparations. IgG_2 and IgG_1 of unrelated specificity were used as controls. Erythrocyte numbers were enumerated using a model ZF Coulter Counter.

Isolation of guinea-pig and human IgG papain or pepsin hydrolysis products

Guinea-pig IgG_2 Fc pool material was isolated by immunoadsorption as described by Alexander *et al.* (1976). IgG_2pFc′ subfragment was separated from $F(ab')_2$ following peptic hydrolysis by G200 gel filtration as described by Leslie, Melamed & Cohen (1971).

Human IgG was prepared from human serum by salt precipitation and ion exchange chromatography using the procedure described for the isolation of guinea-pig IgG_2 (Leslie & Cohen, 1970).

Human IgG Fc was isolated from a 4 h papain hydrolysate by anion exchange chromatography using a 0 to 0·4 M sodium chloride gradient to elute the Fc peptides. The IgG pFc′ subfragment was prepared from human IgG by a 10 h peptic hydrolysis and the pFc′ was separated from $F(ab')_2$ by G200 gel filtration in 0·05 M Tris-Cl pH 8·2 100 mM NaCl.

Antigenic characterization of human Fc fragments was carried out by immunoelectrophoresis and double immunodiffusion using a rabbit anti-human IgG Fc specific antiserum.

Reduction and alkylation of human IgG

[^{131}I-]IgG Fc fragment was incubated with dithiothreitol (final concentration 10 mM) in 0·5 M Tris-Cl pH 8·2 for 30 min at 37° under nitrogen. The reaction was stopped by adding a 1·2 molar excess of iodoacetamide at 2°.

Human IgG subclasses

Human monoclonal IgG proteins were kindly donated by Dr D. Catty and Mr R. Drew, Department of Experimental Pathology, University of Birmingham Medical School (IgG1 and IgG3) and Dr D. Rowe, W.H.O. Immunol. Lab., Switzerland (IgG_2 and IgG_4). The purified, lyophilized proteins were analysed by double immunodiffusion. 7S fractions were isolated by gel filtration.

Human IgG with anti-D specificity

High titre anti-D plasma was kindly donated by Dr Andre Fleer, Central Laboratory of the Netherlands Red Cross Transfusion Service. The IgG fraction was isolated as described previously.

Sensitization of human erythrocytes

Erythrocytes from a single donor (O, R_1R_1 phenotype) were used throughout this work. One volume of 20% erythrocyte suspension was incubated with four volumes of human IgG anti-D (5 mg/ml) for 1 h at 37°. The cells were washed with normal saline three times before measuring the extent of antibody binding.

Guinea-pig macrophage and human monocyte preparation

Guinea-pig oil stimulated peritoneal macrophage suspensions were produced as described by Leslie & Cohen (1974).

Human peripheral blood white cells were isolated from buffy coat preparations provided by the Blood Transfusion Unit, Guy's Hospital. Plasma was defibrinated by addition of $CaCl_2$ to a final concentration of 20 mM followed by gentle shaking of the suspension at room temperature. The cells were then layered on a Ficoll-Hypaque solution (24 : 10) and centrifuged at 1150 rev/min for 40 min at room temperature (Boyum, 1968). Cells at the interface were removed and counted; between 10% and 20% were identified as monocytes, the remaining cells being mainly lymphocytes with trace polymorphonuclear leucocyte contamination.

Rosette assay

0·1 ml containing 5×10^6 blood or peritoneal white cells was incubated with 0·1 ml containing 1×10^7 sensitized erythrocytes in the presence of 0·5 ml of immunoglobulin or Ig-fragment solution. All dilutions were made with TCM 199 and some initial experiments with TCM 199 1% OA. The suspensions were incubated for approximately 20 min at room temperature, 1 h in an ice bath and finally 16 h at 4°. The pellets were gently resuspended and

rosetting cells counted in a modified Neubauer chamber. The ability of immunoglobulin or Ig fragments to inhibit rosette formation was assessed by comparing rosettes formed in the presence and absence of inhibiting ligand. White cells binding four or more erythrocytes were scored as rosettes.

Direct binding assay

The binding of ^{125}Iodine labelled proteins to white cells was assayed by the method of Leslie & Cohen (1974); and Alexander *et al.* (1976). In a series of tubes 2 μg of 7s [^{125}I-]immunoglobulin was incubated at 23° or 37° with $2{\cdot}5-5 \times 10^6$ monocytes in the presence of increasing concentrations of either immunoglobulin or Ig fragments. After 90 min the cells were washed with cold TCM 199 and the retained radioactivity measured. Cell bound counts were expressed as a percentage of the total counts in the incubation mixture.

Presentation of results

Rosette assay data are expressed as follows: The $\log_{10}$ of the molar concentration of inhibitory ligand $\times 10^9$ for guinea-pig and 10^{11} for human is plotted against the percentage rosette value using linear regression analysis.

Direct binding assay results are expressed using a Scatchard Plot (Scatchard, 1949; Leslie & Cohen, 1974; Alexander *et al.*, 1976) to derive relative equilibrium association constants and number of receptor sites per cell.

RESULTS

(i) Guinea-pig IgG

Inhibition of EA-macrophage rosette formation by IgG_1 and IgG_2

Rosette formation by immunoglobulin sensitized sheep erythrocytes around guinea-pig peritoneal macrophages was dependent upon the number of immunoglobulin molecules bound. It was difficult to enumerate rosetting cells sensitized with agglutinating concentrations of immunoglobulin, even though dispersion of erythrocytes was attempted by vortex mixing (Parish & Hayward, 1974). Experiments were therefore conducted using erythrocytes sensitized at subagglutinating concentrations of IgG_2 or IgG_1: Such erythrocytes were coated with 10^4–10^5 molecules of immunoglobulin per cell.

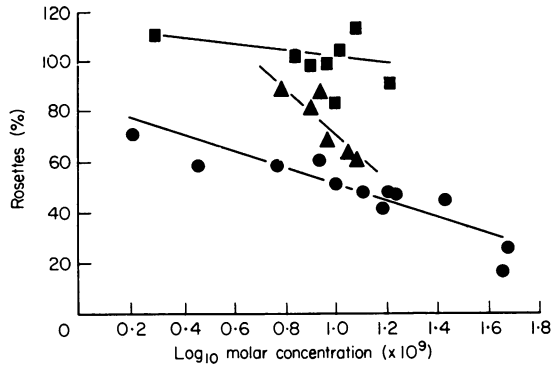

Figure 1. Inhibition of EA: Macrophage rosette formation by guinea-pig 7s IgG_2 and Fc Fragments. (●), 7s IgG_2 r* = −0·820; m = −29·9; b = 79; (▲), IgG_2 Fc pool material r = −0·799, m = −90·4, b = 163; (■), IgG_2 pFc′ r = −0·396, m = −13·2, b = 113.

* Results from linear regression analysis; r refers to correlation coefficient; m the slope value and b the y axis intercept.

The ability of 7S guinea-pig IgG_2 to inhibit rosette formation by IgG_2 sensitized erythrocytes is shown in Fig. 1. Up to 6000 μg/ml of IgG_2 of different specificity from that sensitizing the erythrocytes, was

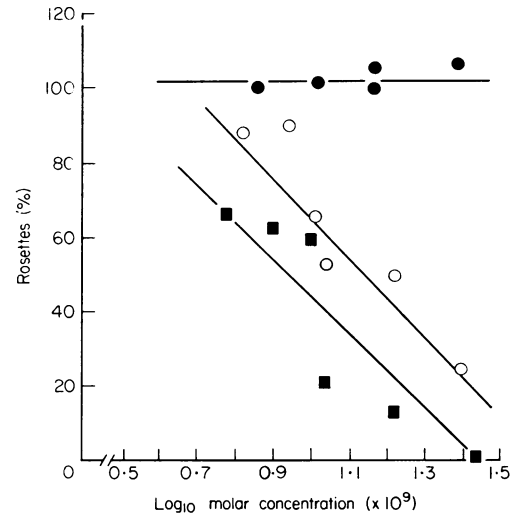

Figure 2. Cross inhibition by guinea-pig IgG_1 and IgG_2 of EA: Macrophage rosette formation. (●), Inhibition of E. IgG_2: Macrophage rosette formation by 7s IgG_1 r = 0·778; m = 11; b = 89. (○), Inhibition of E. IgG_1: Macrophage rosette formation by 7s IgG_1 r = −0·939; m = −116; b = 185. (■), Inhibition of E. IgG_1: Macrophage rosette formation by 7s IgG_2 r = −0·908; m = −109; b = 153.

required to reduce rosette formation to 0–10% of the value obtained in the absence of inhibitory ligand.

Fig. 2 shows the macrophage Fc receptor specificity for guinea-pig IgG_1 and IgG_2. 7S IgG_1 mediates rosette formation and both IgG_1 and IgG_2 inhibit such rosettes. In contrast, 7S IgG_1 was unable to inhibit IgG_2 mediated rosette formation.

Inhibition of EA-macrophage rosette formation by IgG Fc fragments

We have compared the ability of IgG_2 Fc pool material and IgG_2 pFc′ to inhibit IgG_2 mediated rosette formation. Fig. 1 shows that IgG_2 Fc pool material is considerably more active than IgG_2 pFc′. The calculated slope value for Fc was greater than that for whole immunoglobulin and good correlation coefficients were obtained for both. The inhibition by pFc′ of IgG_2 mediated rosette formation was not quantitative and yielded a poor correlation coefficient (−0·396).

(ii) Human IgG

The binding of human IgG subclasses to homologous peripheral blood monocytes

Direct binding assays using radiolabelled human monomeric IgG subclasses and peripheral blood white cell suspensions were performed. Table 1 shows that IgG_1 and IgG_3 have the highest binding affinities with equilibrium Ka values of $10{\cdot}7 \times 10^7 \pm 3{\cdot}9$ and $7{\cdot}8 \times 10^7 \pm 4{\cdot}6$ L/M respectively. IgG_2 and IgG_4 bind with lower Ka values, $0{\cdot}2 \times 10^7$ and $4{\cdot}4 \times 10^7 \pm 0{\cdot}21$ L/M respectively. Normal human IgG binds with a Ka value of $7{\cdot}3 \times 10^7$ L/M. The percentage of ^{125}I-labelled human IgG subclass proteins bound to 5×10^6 peripheral blood monocytes is shown in Fig. 3. Removal of glass adherent cells from human peripheral blood white cell preparations reduced the percentage binding of human IgG_1 by 80%, suggesting that the monocyte population of cells was primarily responsible for the cytophilic activity.

Table 1. Comparison of the equilibrium association constants and numbers of binding sites per cell for whole human IgG and IgG subclass proteins in the direct binding assay

Protein	Equilibrium association constant	Number of sites per monocyte
IgG_1(8)*	$10{\cdot}7 \times 10^7 \pm 3{\cdot}9$	$3{\cdot}1 \times 10^4 \pm 1{\cdot}6$
IgG_2 (2)	$0{\cdot}2 \times 10^7$	$10{\cdot}0 \times 10^4$
IgG_3 (7)	$7{\cdot}8 \times 10^7 \pm 4{\cdot}6$	$3{\cdot}4 \times 10^4 \pm 1{\cdot}1$
IgG_4 (3)	$4{\cdot}4 \times 10^7 \pm 0{\cdot}21$	$2{\cdot}1 \times 10^4 \pm 0{\cdot}67$
Normal IgG (2)	$7{\cdot}3 \times 10^7$	$5{\cdot}3 \times 10^4$

* Figures in parenthesis indicate the number of experiments performed.

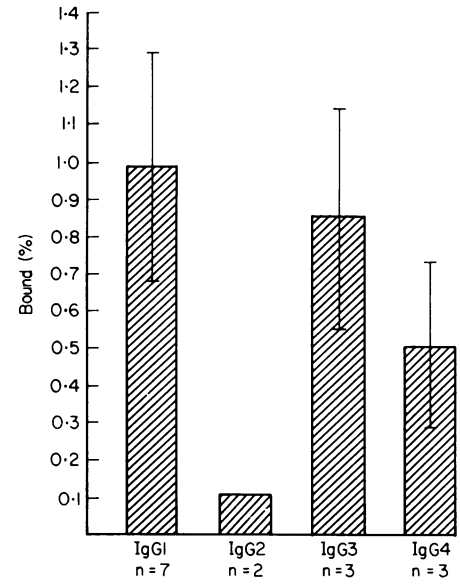

Figure 3. Comparison of the percentage binding of human IgG subclasses to human peripheral blood monocytes 2 μg of 7s [^{125}I]-Ig incubated with ~5×10^6 monocytes.

Analysis of human IgG Fc fragments

Double immunodiffusion analysis demonstrated that the human IgG pFc′ fragment was antigenically deficient to both whole immunoglobulin and the IgG Fc fragment. The latter showed complete identity with whole immunoglobulin.

IgG Fc eluted from a G100 column equilibrated with 20% acetic acid with a Kd 0·116 and molecular weight 60,000 (approx.). Partial reduction and alkylation of the Fc fragment with 10 mM DTT and 12 mM iodoacetamide shifted the elution profile to give a Kd of 0·194 and molecular weight 36,000 (approx.).

IgG pFc′ eluted from a G200 column in 50 mM

Tris-Cl pH 8·2 100 mM NaCl with an approximate molecular weight of 26,000.

Inhibitory activity of human IgG, IgG Fc and IgG pFc′ in the direct binding assay

2 μg of 7s ^{125}I-human IgG was incubated with 6×10^7 human peripheral blood white cells. The inhibition of IgG uptake by 7s IgG, IgG Fc and IgG pFc′ is shown in Fig. 4. Only whole IgG and Fc fragment have significant binding activity, the Fc fragment manifesting 30% of that of whole IgG. The IgG pFc′ fragment retained only 7% of the activity of whole immunoglobulin.

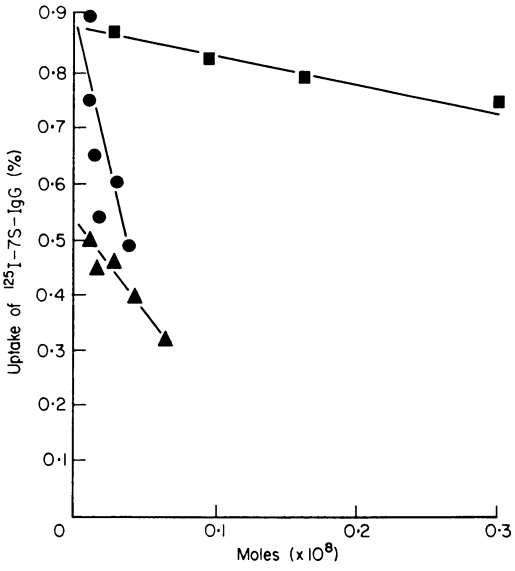

Figure 4. Inhibition of the binding of 7s ^{125}I-human IgG to homologous peripheral blood monocytes. (●), 7S IgG, r = −0·706, m = −10·1; (▲), human IgG Fc, r = −0·964, m = −3·08; (■), human IgG pFc′, r = −0·708, m = −0·727.

Inhibition of EA-monocyte rosette formation by human IgG and IgG Fc fragments

Human erythrocytes (D+) sensitized with human anti-D IgG, formed rosettes around human peripheral white blood cells. The rosetting cells were primarily monocytes. Rosette formation could be inhibited almost completely by the addition of 100 μg of human IgG. Fig. 5 shows that IgG Fc was able to inhibit rosette formation, apparently retaining about 80% of the activity of whole immunoglobulin. The IgG pFc′ did not reduce the percentage rosette value significantly below 60% although from the slope value it appears to retain 37% of the activity of whole IgG.

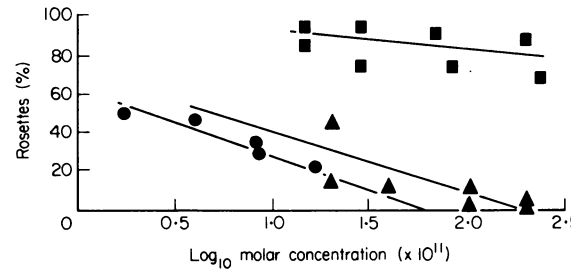

Figure 5. Inhibition of EA: Monocyte rosette formation by human IgG and Fc fragments. (●), 7s IgG, r = −0·971 m = −35·4; (▲), IgG Fc, r = −0·733, m = −31·3; (■), IgG pFc′, r = −0·508, m = −11·8.

DISCUSSION

The guinea-pig peritoneal macrophage manifests Fc receptors with specificities for guinea-pig IgG_2 and IgG_1 (Shinomiya & Koyama, 1976; Leslie & Cohen, 1977). In the present work we have shown, using a rosette test, that such receptors will bind particulate immune complexes, i.e. immunoglobulin sensitized erythrocytes. IgG_2 inhibits both IgG_2 and IgG_1 mediated rosette formation, IgG_1, on the other hand inhibits only IgG_1 mediated rosettes. These results accord with those of Leslie & Cohen (1977) who used a direct binding assay and concluded that receptors for guinea-pig IgG_1 and IgG_2 occupy closely contiguous regions of the macrophage membrane. Although both guinea-pig IgG classes are cytophilic their differing reactivities towards complement components may lead to different biological consequences following macrophage attachment (Eichmann, 1974; Portis & Coe, 1975).

Direct binding assays performed with 7s radiolabelled human IgG subclasses have confirmed earlier findings (Abramson, Gelfand Jandl, & Rosen, 1970; Inchley, Grey & Uhr, 1970; Okafor, Hay & Turner, 1974) that human IgG_1 and IgG_3 are primarily responsible for the cytophilic activity of human IgG. These subclasses bind to homologous monocyte Fc receptors with relatively high affinity,

Table 2. Summary of reported findings concerning the location of the Fc domain of IgG responsible for Fc receptor interaction

Author(s)	Fc receptor/ cell type	Source	State of cells	Cytophilic immunoglobulin				Assay technique			**Active site location In cytophilic Ig for Fc receptor			Active IgG subclasses
			adherent cells or suspension	*monomeric direct	*monomeric indirect	Soluble complexes	Particulate complexes	DBA	Particulate complex: chemical coupling†	Particulate complex: antibody coating§	Fc (C_H2 C_H3)$_2$	C_H2	pFc′ (C_H3)$_2$	
Abramson *et al.*, 1970	monocyte	hPBld	adherent	nd¶	+	nd	+	nd	nd	+	+	nd	–	IgG_1/IgG_3
Yasmeen *et al.*, 1973	macrophage	G. pig PEC	adherent	nd	+	nd	+	nd	+	nd	+	–	+	IgG_1
Okafor *et al.*, 1974	monocyte	hPBld	adherent	nd	+	nd	+	nd	nd	+	+	nd	+	IgG_1/IgG_3
Dissanayake & Hay, 1975	macrophages	murine PEC	adherent	+	+	nd	+	÷	nd	+	+	nd	+	$IgG_{2b}/_{2a}$
Ramasamy *et al.*, 1975	lymphocytes	murine	suspension	nd	+	nd	+	nd	nd	+	+	–	+	IgG_1
Ciccimarra *et al.*, 1975	monocyte	hPBld	adherent	nd	+	nd	+	nd	nd	+	+	+	+	IgG_1/IgG_3
Alexander *et al.*, 1976	macrophages	G. pig PEC	suspension	+	+	nd	nd	+	nd	nd	+	nd	–	IgG_2
Guyer *et al.*, 1976	intestinal epithelial cell *in vivo*	mouse	*in vivo*	+	+	nd	nd	+	nd	nd	+	–	–	IgG_{2a}
McNabb *et al.*, 1976	placental membrane preparations	human	suspension	+	+	nd	nd	+	nd	nd	+	–	–	IgG_1
Ovary *et al.*, 1976	macrophages	G. pig lung	suspension	nd	+	nd	+	nd	nd	+	+	–	–	IgG (rabbit)
Leslie *et al.*, 1977	macrophages	G. pig PEC	suspension	+	+	+	nd	+	nd	nd	+	nd	–	IgG_2
Present work:	macrophages	G. pig PEC	suspension	nd	+	nd	+	nd	nd	+	+	nd	–	IgG_2
	monocytes	hPBld	suspension	+	+	nd	nd	+	nd	nd	+	nd	–	$IgG_1/_3$
			suspension	nd	+	nd	+	nd	nd	+	+	nd	–	IgG

* Indirect refers to the ability of monomeric Ig to inhibit either rosette formation or uptake of radiolabelled Ig. Direct refers to sensitization of either macrophages/ monocytes with monomer before measuring cytophilic activity

† DBA = Direct Binding Assay (see Materials and Methods).

‡ Tannic acid, gluteraldehyde or cyanogen bromide treatment of particles in order to bind ligand (Ig/fragment).

§ Subagglutinating sensitizing concentration of immunoglobulin, i.e. anti-erythrocyte specificity coated on to particles.

¶nd, not done.

** +/– refers to demonstrable activity or not.

comparable to the binding of mouse IgG_{2a} to homologous macrophages (Unkeless & Eisen, 1975). As well as similarity in equilibrium association constants, both systems show binding to a relatively small number of Fc receptor sites per cell. This high affinity binding is in contrast to that reported for guinea-pig IgG_2 (Ka $1{\cdot}5 \times 10^6$ L/M; 2×10^6 sites per cell) (Leslie & Cohen, 1974), with homologous peritoneal macrophages and rabbit IgG with homologous alveolar macrophages ($\sim 1 \times 10^6$L/M; 2×10^6 sites per cell) (Arend & Mannik, 1973).

We have compared the cytophilic activity for homologous monocytes of human and guinea-pig IgG Fc and pFc′ fragments by direct binding and rosette assay techniques. The guinea-pig IgG_2 Fc fragment contains C_H2 and C_H3 domains, while pFc′ fragments consist of C_H3 domains alone (Leslie *et al.*, 1971; Alexander *et al.*, 1976). In the present work antigenic analysis showed the human pFc′ fragment to be antigenically deficient to both Fc and IgG. Reduction and alkylation of the human IgG Fc produced a single peptide of approximately half the molecular weight of the native Fc peptide. We conclude that the human IgG Fc contains the C_H2 and C_H3 domains and IgG pFc′, the C_H3 domain alone. No significant cytophilic activity could be demonstrated in either human IgG pFc′ or guinea-pig IgG_2 pFc′ fragments, whereas activity was retained by the Fc fragments of both species. The homologous human rosette test was inhibited by much lower inhibitory ligand concentrations than were required in the homologous guinea-pig system. This is explained by the finding that human cytophilic immunoglobulins bind with higher affinity than guinea-pig IgG to homologous monocyte Fc receptors. Our results with the human rosette test agree with those of Abramson *et al.* (1970) who demonstrated that low concentrations of human IgG would inhibit rosette formation around homologous monocytes. Similar results were obtained by Huber, Douglas & Fudenberg (1969) who compared Fc receptors on splenic and hepatic macrophages with peripheral blood monocytes.

Rosette tests and direct binding assays gave similar results in both the guinea-pig and human studies so that differences in assay technique cannot account for the discrepant results reported for C_H3 cytophilic activity (Table 2), and other explanations must be sought for the observed differences. Unkeless (1977) has demonstrated heterogeneity with regard to trypsin sensitivity of mouse macrophage Fc receptors. The receptors for homologous IgG_{2a} are trypsin sensitive, irrespective of whether the IgG_{2a} is presented as monomer or artificially attached to particles treated with either glutaraldehyde or cyanogen bromide. Mouse macrophages also manifest Fc receptors with heterologous specificity which are trypsin resistant for the binding of some particles coated with heterologous immunoglobulin. The use of a heterologous assay system may explain the cytophilic activity of C_H3 fragments observed by Yasmeen *et al.* (1973; 1976) who used erythrocytes treated with tannic acid and coated with human IgG_1 and studied their interaction with guinea-pig peritoneal macrophages, although Unkeless (1977) has shown that particles artificially coated with heterologous immunoglobulin interact with the receptors that bind homologous monomeric immunoglobulin. Okafor *et al.* (1974) used a homologous human rosette assay system of Rh positive erythrocytes sensitized with anti-Rh IgG and human monocytes. The phenotype of the erythrocytes used was not recorded (Rochna & Hughes-Jones, 1965). It is possible that erythrocyte phenotype and immunoglobulin distribution may influence the interaction with the Fc receptor (Unkeless, 1977).

The non-covalent interactions between C_H3 domains (Charlwood & Utsumi, 1969) contribute to the structural integrity of the Fc region of IgG molecules (Alexander *et al.*, 1976). Mutant mouse myeloma proteins lacking C_H3 domains were shown to be inactive in inhibiting rosette formation around homologous lymphoid cells (Ramasamy, Secher & Adetugbo, 1975). Although this was presented as evidence for the direct involvement of the C_H3 domain, the loss of activity through dissociation of the C_H2 domains cannot be discounted. However, the findings of Colomb & Porter (1974) and Ovary *et al.* (1976) that rabbit IgG Facb fragment mediates complement fixation and macrophage Fc receptor interaction respectively, suggest that effector functions are retained by the C_H2 domains in the absence of C_H3.

There is conflicting evidence as to whether biological effector sites are generated by polypeptide chain conformation or by linear sequences of amino acids. From a comparison of sequences of complement fixing immunoglobulins, Putnam (1974) concluded that conformation rather than linear sequence generated the site for complement fixation. In contrast, a ten residue peptide from human IgG

C_H3 domain has been shown to retain the ability to interact with homologous monocyte Fc receptors (Ciccimarra, Rosen & Merler, 1975). The significance of these data is in doubt, since Dorrington (1976) has concluded that such a sequence is most probably buried within the C_H3 domain. Interestingly, Ciccimarra *et al.* (1975) did show reduced cytophilic activity in an IgG_3 myeloma protein with deletions in the C_H2 domain.

Following the discovery that $\beta 2$ microglobulin can mediate both complement fixation and macrophage Fc receptor interaction (Painter, Yasmeen, Assimeh & Poulik, 1974), it has recently been established that structural and conformational modifications to C_H3 fragments of human IgG_1 generate complement fixing activity in these fragments (Isenman, Ellerson, Painter & Dorrington, 1977). These findings suggest that each domain may be potentially capable of mediating several biological activities.

No definite explanation can therefore be offered for contradictory observations regarding the cytophilic activity of C_H3 fragments (Table 2). Recent studies concerning the immunoglobulin domain responsible for Fc receptor interaction in human, rat, mouse and rabbit IgG have shown little or no cytophilic activity in isolated C_H3 fragments (McNabb, Koh & Painter, 1976; Guyer, Koshland & Knopf, 1976; Ovary, Saluk, Quijada & Lamm, 1976; Arend & Webster, 1977). This has been the case for receptors on macrophages as well as on placental and gut epithelial tissues (Table 2). These studies all indicate that IgG cytophilic activity for a variety of cells may involve a site located in the C_H2 domain of the Fc fragment.

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