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The localization of the binding site(s) on human IgG1 for the Fc receptors on homologous monocytes and heterologous mouse macrophages

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Summary. Two different methods, a rosette assay and a direct binding assay, have been employed in an examination of the binding of human IgG1 to mouse macrophages. In both cases, inhibition of IgG binding was demonstrated by Fc (C_H2+C_H3 domains) and pFc' (C_H3 domains) fragments of human IgG. In a homologous system, the binding of ^{125}I-human IgG to human peripheral-blood monocytes was inhibited by the Fc fragment whereas the pFc' fragment was inactive. Scatchard plot analysis of the binding data from both the heterologous and homologous systems allowed association constants and numbers of receptors per cell to be calculated.

A more thorough examination of the possible location of IgG Fc-receptor binding sites was made using less orthodox proteolytic cleavage fragments of IgG. The site on human IgG1 responsible for binding to mouse macrophage Fc receptors was confirmed to be within the C_H3 domains. Human IgG1 binding to homologous monocytes was shown, using a dimeric $C_\gamma2$ domain fragment, to be via the C_H2 domains, and was dependent on the integrity of the covalent interaction between the $C_\gamma2$ domains at the hinge region.

INTRODUCTION

It is now well recognized that immunoglobulins are multi-functional molecules, which not only bind to specific antigen but which also take part in a number of other biological phenomena mediated by sites located within the Fc region. Furthermore, the proposal that each domain of the immunoglobulin molecule has evolved to perform at least one specific function (Edelman *et al.*, 1969) has promoted considerable effort to associate a particular activity with an Fc effector site located within one or other domain. For instance, sites within the C_H2 domain of IgG have been implicated in C1q binding activity (Yasmeen *et al.*, 1976); whilst other studies have been directed towards establishing the structural basis of the cytophilic activity of IgG for immunocompetent cells, including macrophages (Berken & Benaceraff, 1966) and monocytes (Huber & Fundenberg, 1968).

Although it is generally agreed that the cytophilic activity of IgG for mononuclear phagocytes is located in the Fc region, there has been much controversy and confusion about the precise location of the binding sites within the $C_\gamma2$–$C_\gamma3$ domains, with both the $C_\gamma2$ domains (Alexander, Leslie & Cohen, 1976) and the $C_\gamma3$ domains (Yasmeen *et al.*, 1973, 1976; Okafor, Turner & Hay, 1974) being implicated. The present study was undertaken in an attempt to resolve some of the discordant claims about the location within the human IgG molecule of the site of binding to macrophage Fc receptors. It had been suggested previously (Alexander *et al.*, 1976) that some of the discrepancies may be attributable to the mode of assay employed; for whereas, for example, the macrophage–erythrocyte rosette techniques used by Yasmeen *et al.* (1976)

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and Okafor *et al.* (1974) relied on the binding of a particular complex, the investigations of Alexander *et al.* (1976) were based on the measurement of the direct binding of monomeric cytophilic antibody. A possible alternative explanation for the contradictory findings is the use, by some laboratories, of heterologous as opposed to homologous assay systems; and although the reactivity of IgG for heterologous macrophages has been studied more widely, there remains a substantial level of opinion that this could produce misleading results (Leslie & Niemetz, 1979; Alexander *et al.*, 1976). We have, therefore, made a direct comparison of the reactivity of proteolytic cleavage fragments of human IgG (i.e. Fc, pFc′ and Fab) with heterologous macrophages using the two major procedures in question, i.e. an erythrocyte rosette assay and a direct binding radioimmunoassay. The same IgG fragments were then employed in the direct binding assay with human monocytes, thereby allowing a heterologous/homologous comparison to be made. Moreover, in order to confirm the data thereby obtained, and to investigate in more detail the location of the Fc receptor binding site(s) on the IgG molecule, we have prepared more unusual proteolytic cleavage fragments (by methods described in earlier publications from our laboratory) and examined the cytophilic activity of these, too.

MATERIALS AND METHODS

Preparation of human IgG1 and ^{125}I-labelled IgG1

Human IgG was isolated from a myeloma IgG1 serum by batch ion-exchange chromatography using diethylaminoethyl-cellulose (Whatman DE52), as described by Stanworth (1960), and stored in freeze-dried form at 4°. The purity of the IgG preparation was assessed by immunoelectrophoresis and analytical ultracentrifugation.

The IgG was labelled with ^{125}I using chloramine T (Greenwood, Hunter & Glover, 1963). To prepare ^{125}I-IgG1 for use in the mouse macrophage direct binding assay, 1 mg of IgG was labelled with 1 mCi ^{125}I, and diluted with 5% IgG to 2 μg per 40,000 c.p.m. To prepare ^{125}I-IgG1 for use in the human monocyte direct binding assay, 25 μg of IgG was labelled with 1 mCi ^{125}I, and the final concentration adjusted to 0·0625 μg per 40,000 c.p.m. using 5% IgG.

Preparation of fragments of IgG (Fig. 1)

The Fc and Fab fragments of IgG were prepared by the digestion of IgG with papain in the presence of cysteine as described by Hunneyball & Stanworth (1976). The pFc′ fragment was produced by pepsin digestion of IgG as described by Turner & Bennich (1968), the digestion products being separated by gel filtration on a Sephadex G150 column (90 × 3·2 cm) equilibrated with 0·05 M ammonium carbonate pH 8·6, the pFc′ fraction being purified by recycling on a Sephadex G75 column (60 × 2·2 cm) equilibrated with the same buffer.

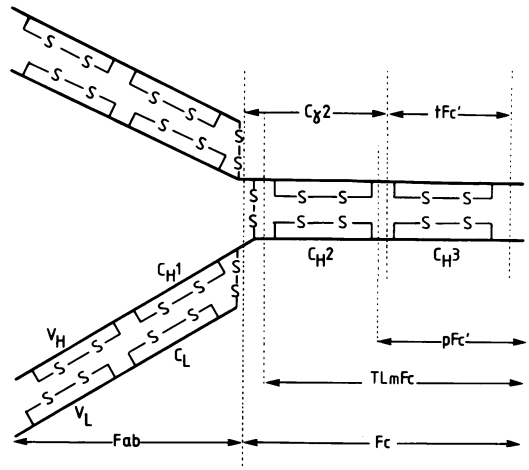

Figure 1. Schematic representation of the proteolytic cleavage fragments of IgG used in this study.

The tFc′ fragment was prepared by trypsin digestion of the Fc fragment according to the method of Matthews, Stewart & Stanworth (1971). The TLmFc fragment was produced from the Fc fragment by the method of Hunneyball & Stanworth (1976) using the enzyme thermolysin, a bacterial endopeptidase derived from *Bacillus thermoproteolyticus*. A $C_\gamma 2$ domain fragment was prepared by the method of Ellerson *et al.* (1976), employing trypsin covalently bound to Sepharose to digest acid-treated Fc fragment.

Immunological and physico-chemical characterization of the IgG fragments

Antigenic characterization of the IgG fragments was performed by immunoelectrophoresis using sheep anti-human IgG, anti-human IgG Fc fragment, anti-human light chain, and anti-human $C_\gamma 2$ domain antisera, and guinea-pig anti-human IgG pFc′ fragment antiserum. The molecular weights of the fragments were determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

using a 10% polyacrylamide gel. The standard proteins used included: myoglobin, haemoglobin, trypsin, pepsin, ovalbumin, and IgG heavy and light chains. Analytical ultracentrifugation was performed using an MSE Centriscan 75 analytical ultracentrifuge. Amino-acid analyses were performed by the Macromolecular Analysis Service, University of Birmingham.

Preparation of monomeric IgG and fragments of IgG

To ensure only monomeric IgG and fragments of IgG were present, the samples were subjected to gel filtration within 24 hr prior to the experiment. The choice of gel filtration medium was determined by the molecular size of the sample; monomeric IgG was prepared using Agarose A1.5M.

Mouse macrophage rosette assay

Ox red blood cells were actively sensitized with rabbit anti-ox red blood cell antiserum and the cells suspended at a concentration of 1×10^8 cells ml^{-1} in medium 199 buffered with 20 mM HEPES and containing 60 $\mu g\ ml^{-1}$ penicillin and 100 $\mu g\ ml^{-1}$ streptomycin. Peritoneal cells were collected from male BALB/c mice by lavage of the peritoneal cavity with 2·5 ml of buffered medium 199 containing antibiotics, and diluted to a concentration of 2×10^5 cells ml^{-1} with medium 199 containing 10% foetal calf serum. Half a millilitre of the cell suspension (1×10^5 cells) was added to 16 mm diameter wells of leucocyte migration plates, each containing a 13 mm diameter glass coverslip and incubated at 37° for 2 hr. The glass coverslips were then washed five times at 37° with medium 199, leaving a monolayer of macrophages on the surface of the glass coverslips. The cells were then incubated at 37° for 1 hr with various concentrations of inhibitor in medium 199, washed five times at room temperature with medium 199 and incubated at room temperature for 1 hr with 0·5 ml of the ox red blood cell suspension. The monolayers were then washed six times with medium 199, fixed and stained with glutaraldehyde and Giemsa and allowed to dry. Approximately 500 cells per coverslip were viewed, with four or more red blood cells per macrophage being counted as one rosette. The results were expressed graphically, plotting percentage rosettes versus concentration of inhibitor, using linear regression analysis.

Mouse macrophage direct binding assay

The method used was basically that described by Leslie & Cohen (1974), which involves the measurement of cell-bound radiolabelled IgG in the presence of various concentrations of inhibitor. Peritoneal cells from BALB/c mice were obtained 3–4 days after intraperitoneal injection with 1 ml paraffin oil. These cells, composed of 60–70% macrophages as judged by Giemsa staining, were washed twice with medium 199 containing 5 mM ethylenediamine tetracetic acid (EDTA), and finally with only medium 199, with an incubation period of 15 min with constant mixing between each wash. The washed cells were suspended in medium 199 containing 1% bovine serum albumin (BSA), at a concentration of 3×10^7 cells ml^{-1}. In the binding assay, 0·5 ml of ^{125}I-labelled IgG (2 μg), 0·5 ml of inhibitor or medium, and 0·1 ml of cell suspension (3×10^6 cells) were mixed in medium 199 containing 1% BSA at room temperature for 90 min, the cells washed three times with 0·5 ml of medium 199, and the cell-bound ^{125}I-labelled IgG counted. The results were expressed graphically as percentage binding versus concentration of inhibitor (Leslie & Niemetz, 1979). Scatchard plot analysis of the IgG inhibition data was used to estimate the equilibrium association constant (*Ka*) and the number of IgG receptors per cell (Scatchard, 1949; Leslie & Niemetz, 1979).

Human monocyte direct binding assay

A white cell preparation was made from fresh human peripheral blood (containing 5 mM EDTA) by the method of Boyum (1968), using a Ficoll–Triosil mixture of specific gravity 1·077 g ml^{-1}, after initially removing the majority of blood platelets by centrifuging the blood at 200 *g* for 10 min, removing the plasma, and making up to the orginal volume with phosphate-buffered saline (PBS). Monocytes were isolated from this white-cell preparation by exploiting their surface adherence properties; the white cells were suspended in medium 199 supplemented with 10% foetal calf serum at a concentration of 2×10^6 cells ml^{-1}, and 2 ml aliquots added to 35 mm diameter plastic tissue culture petri dishes. These were incubated at 37° for 2 hr and washed three times, leaving a monolayer consisting of approximately 90% monocytes, as determined by nuclear staining using Giemsa stain. A range of concentrations of inhibitor was prepared in 2 ml aliquots of medium 199 containing 1% BSA and 0·625 μg ^{125}I-labelled IgG. A 2 ml aliquot was added to each petri-dish and incubated at room temperature for 90 min, the monolayers washed with 2×1 ml aliquots of medium 199, and finally solubilized in 2 ml of 0·1% Triton X-100 in 0·15 M NaCl. The cell-bound ^{125}I-IgG was counted and the results expressed as percentage binding versus concentration of inhibitor. Scatchard

plot analysis of the data obtained using IgG as inhibitor was employed to determine a *Ka* value of the binding of human IgG1 to human monocytes. Accurate estimation of the number of receptors per cell was not possible since the monocytes were adherent to the petri-dish and, therefore, the total number of receptors were presumably not available for binding.

RESULTS

Immunochemical and physico-chemical analysis of the proteolytic cleavage fragments of IgG

Human IgG1, and its Fc, pFc′ and Fab fragments were shown to be pure by immunoelectrophoresis and analytical ultracentrifugation. The molecular weights of the fragments were consistent with previously published data.

Molecular weight determinations and amino-acid analyses were in agreement with published results (Matthews *et al.*, 1971; Hunneyball & Stanworth, 1976; Ellerson *et al.*, 1976). Importantly the $C_\gamma 2$ domain fragment was found to comprise a covalently linked dimeric $C_\gamma 2$ domain, each polypeptide chain corresponding to Chain A of the $C_\gamma 2$ (III) fragment described by Ellerson *et al.* (1976).

Inhibition of EA-mouse macrophage rosette formation by the Fc, pFc′, and Fab fragments of IgG

The mean slope value obtained using monomeric human IgG as inhibitor of rosette formation of immunoglobulin-sensitized red blood cells and mouse macrophages was $-20{\cdot}9 \times 10^9$. When using the Fc fragment as inhibitor, a regression slope of $-10{\cdot}4 \times 10^9$ indicated a 50% recovery of the cytophilic activity of the complete IgG; similarly, the pFc′ fragment retained 43·9% of the activity of whole IgG. The antigen-binding Fab fragment was considered to be inactive demonstrating only 2% of the binding activity of IgG (see Fig. 2).

Inhibition of ^{125}I-human IgG binding to mouse macrophages by Fc, pFc′, and Fab fragments of IgG

The inhibition of ^{125}I-labelled human IgG binding to mouse macrophages by monomeric IgG produced a linear regression slope of $-128{\cdot}6 \times 10^{11}$. The Fc and pFc′ fragments demonstrated similar capacities to inhibit ^{125}I-IgG binding, the Fc fragment retained 53%

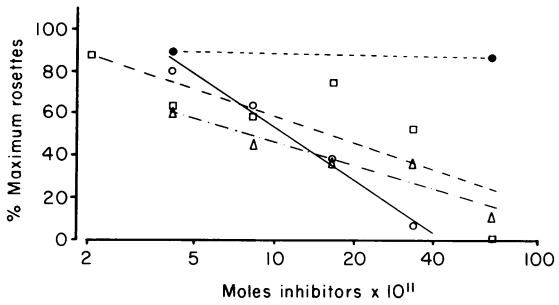

Figure 2. Summary of the inhibition of EA-mouse macrophage rosette formation. Human IgG1 (○——○), $m = 20{\cdot}9$, $P < 0{\cdot}001$; human IgG1 Fc fragment (□ - - - □), $m = 10{\cdot}4$, $P = 0{\cdot}02$–$0{\cdot}01$; human IgG1 pFc′ fragment (△ · - · - · △), $m = 9{\cdot}1$, $P < 0{\cdot}001$; human IgG1 Fab (● - - - - ●) fragment, $m = 0{\cdot}41$, $P > 0{\cdot}1$.

of the activity of IgG and the pFc′ fragment 55%; whereas no cytophilic activity was retained by the Fab fragment (Fig. 3). Scatchard plot analysis of the IgG1 inhibition data produced a linear plot, from which was derived a *Ka* value of $2{\cdot}55 \times 10^6$ M^{-1}, binding to $4{\cdot}2 \times 10^6$ receptors per cell (Fig. 4).

Inhibition of ^{125}I-human IgG binding to human monocytes by the Fc, pFc′, and Fab fragments of IgG

The data obtained from the inhibition of the binding of ^{125}I-labelled human IgG1 to human monocytes by unlabelled human IgG1, when expressed as an inhibition plot, produced a linear regression slope of $-17{\cdot}7 \times 10^{13}$. When the Fc fragment was employed as inhibitor, a slope of $-14{\cdot}0 \times 10^{13}$ revealed that the fragment had retained 79·2% of the cytophilic activity

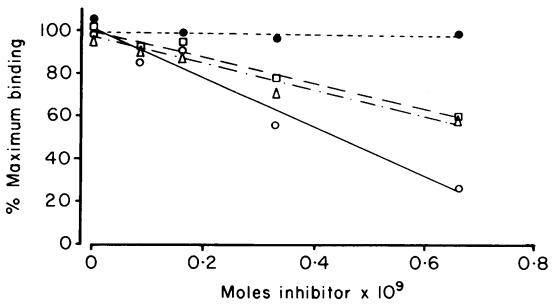

Figure 3. Summary of the inhibition of ^{125}I-IgG binding to mouse macrophages. Human IgG1 (○——○), $m = 128{\cdot}6$, $P < 0{\cdot}001$; human IgG1 Fc fragment (□ - - - □), $m = 68{\cdot}9$, $P < 0{\cdot}001$; human pFc′ fragment (△ · - · - · △), $m = 70{\cdot}7$, $P < 0{\cdot}001$; human IgG1 Fab fragment (● - - - - ●), $m = 2{\cdot}25$, $P > 0{\cdot}1$.

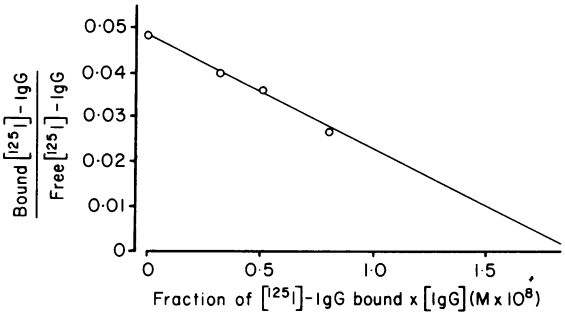

Figure 4. Scatchard plot of the binding of human IgG1 to mouse macrophages: $m = 0{\cdot}0255 \times 10^8$, $P < 0{\cdot}001$; $Ka = 2{\cdot}55 \times 10^6$ M^{-1}, $n = 4{\cdot}2 \times 10^6$ receptors.

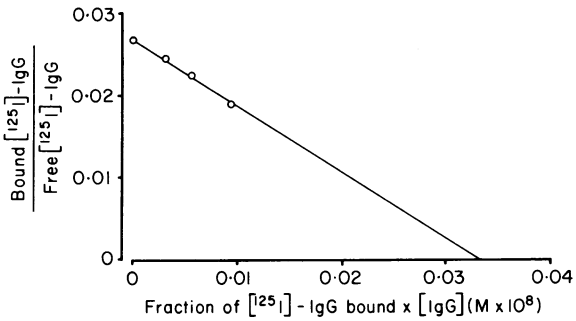

Figure 6. Scatchard plot of the binding of human IgG to human monocytes: $m = -801 \times 10^8$, $P < 0{\cdot}001$; $Ka = 8{\cdot}0 \times 10^7$ M^{-1}.

of IgG. However, using the pFc′ fragment as a potential inhibitor of IgG binding to human monocytes, only 11% of the binding activity of IgG was achieved, with the linear regression slope not statistically significant ($P > 0{\cdot}1$). Indeed, occasionally a slight stimulation in the binding of ^{125}I-IgG to human monocytes was observed in the presence of the pFc′ fragment. The Fab fragment demonstrated no cytophilic activity (see Fig. 5). Scatchard plot analysis of the IgG-binding data produced a *Ka* value of $8{\cdot}0 \times 10^7$ M^{-1} (Fig. 6).

Inhibition of ^{125}I-IgG binding to mouse macrophages by the tFc′, TLmFc, and $C_\gamma 2$ domain fragments

During this series of experiments, monomeric IgG was found to inhibit a new preparation of ^{125}I-labelled IgG to produce a linear regression slope of $-286{\cdot}9 \times 10^{11}$. The tFc′ fragment when employed as inhibitor was found to demonstrate 70% of the cytophilic activity of IgG, and similarly the thermolysin-derived TLmFc fragment retained 69% of the cytophilic activity of whole IgG. In contrast, the dimeric $C_\gamma 2$ domain fragment showed no significant inhibition, the linear regression slope of $-9{\cdot}0 \times 10^{11}$ being 3% of that produced by IgG (see Fig. 7).

Inhibition of ^{125}I-IgG binding to human monocytes by the tFc′, TLmFc, and $C_\gamma 2$ domain fragments

During these experiments unlabelled human IgG inhibited the binding of freshly labelled ^{125}I-IgG to produce an inhibition regression slope of $-12{\cdot}2 \times 10^3$. The dimeric $C_\gamma 2$ domain fragment produced a linear inhibition plot with a regression slope of $-10{\cdot}4 \times 10^{13}$, demonstrating that the fragment had successfully retained some 85% of the cytophilic activity of whole IgG. Interestingly, however, the TLmFC fragment,

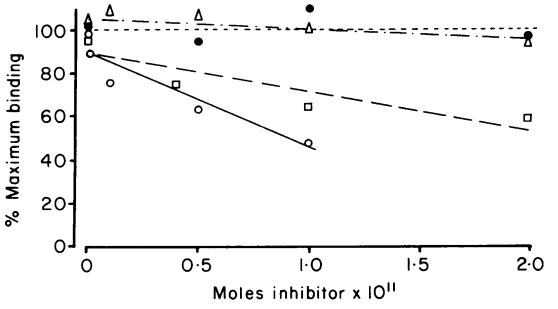

Figure 5. Summary of the inhibition of ^{125}I-IgG binding to human monocytes. Human IgG1 (O——O), $m = 17{\cdot}7$, $P < 0{\cdot}001$; human IgG1 Fc fragment (□ – – – □), $m = 14{\cdot}0$, $P < 0{\cdot}001$; human IgG1 pFc′ fragment (△ · – · – · △), $m = 2{\cdot}1$, $P = 0{\cdot}1$–$0{\cdot}05$; human IgG1 Fab fragment (● – – – – ●), $m = 0{\cdot}15$, $P > 0{\cdot}1$.

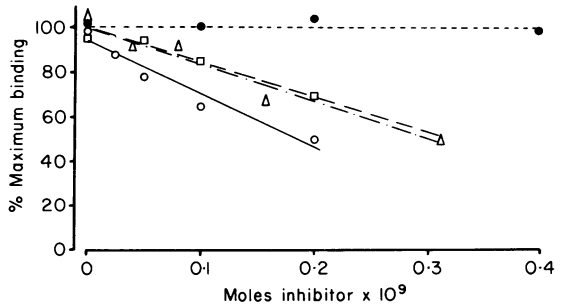

Figure 7. Summary of the inhibition of ^{125}I-IgG binding to mouse macrophages. Human IgG1 (O——O), $m = 286{\cdot}9$, $P < 0{\cdot}001$; human IgG1 TLmFc fragment (□ – – – □), $m = 197{\cdot}8$, $P < 0{\cdot}001$; human IgG1 tFc′ fragment (△ · – · – · △), $m = 202{\cdot}6$, $P < 0{\cdot}001$; human IgG1 $C_\gamma 2$ domain fragment (● – – – – ●), $m = -9{\cdot}0$, $P > 0{\cdot}1$.

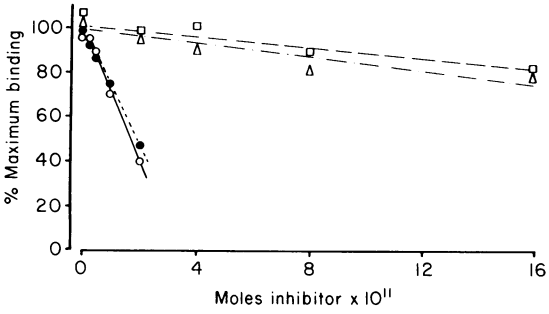

Figure 8. Summary of the inhibition of ^{125}I-IgG binding to human monocytes. Human IgG1 (○——○), $m = 12{\cdot}2$, $P < 0{\cdot}001$; human IgG1 TLmFc fragment (□ - - - □), $m = 0{\cdot}46$, $P < 0{\cdot}001$; human IgG1 tFc′ fragment (△ ·-·-· △), $m = 0{\cdot}56$, $P = 0{\cdot}05$–$0{\cdot}02$; human IgG1 $C_\gamma 2$ domain fragment (● - - - - ●), $m = 10{\cdot}4$, $P < 0{\cdot}001$.

devoid of the hinge region, showed only 4% of the binding activity of IgG: this slope deviated significantly from a zero gradient ($P < 0{\cdot}001$). The tFc′ fragment was found not to have any significant binding activity (Fig. 8).

DISCUSSION

A comparison has been made of the cytophilic activity of human IgG1 and its Fc, pFc′ and Fab fragments for mouse peritoneal macrophages using two different techniques, a rosette assay and a radiolabelled direct binding assay. Both methods showed that the Fc fragment retained approximately 50% of the binding activity of whole IgG, whereas the Fab fragment demonstrated no cytophilic activity. Furthermore, the cytophilic activity of the Fc fragment was completely retained by the smaller, dimeric $C_\gamma 3$ domain, pFc′ fragment. The rosette assay and direct binding assay have therefore provided essentially the same results, both qualitatively and quantitatively, in agreement with the findings of two other groups (Dissanayake & Hay, 1975; Alexander *et al.*, 1978). Consequently, since the direct binding assay was found to be technically easier, quicker and more quantitative (the regression slope obtained when Fc fragment was employed as inhibitor of rosette formation gave a P value of 0·02–0·01), only the direct binding assay was employed in further studies.

These results indicate that the IgG binding site involved in the heterologous assay systems is located within the $C_\gamma 3$ domains. This is in agreement with the results of Yasmeen *et al.* (1973, 1976) who employed a heterologous system, involving the binding of human IgG1 to guinea-pig macrophages; the $C_\gamma 3$ domain fragment showing activity in both direct and indirect assays. However, as mentioned previously, other investigators have reported the pFc′ fragment to be cytophilically inactive; in particular, Alexander *et al.* (1976), employing a homologous guinea-pig system in a radiolabelled direct binding assay, were unable to demonstrate binding activity in the $C_H 3$ domains of guinea-pig IgG2. Consequently, it was suggested that the use of heterologous assay systems was a possible explanation for the contradictory data. Hence the results which we obtained using a heterologous assay system were compared with those obtained by testing the same preparations in a homologous system using peripheral blood monocytes. Although the Fc fragment retained 80% of the cytophilic activity of whole IgG in this homologous binding assay, the pFc′ fragment demonstrated only 11% of the IgG activity. This value was only slightly significantly different from a zero gradient, and is remarkably consistent with the pFc′ fragment activity of 7% of the IgG value reported by Alexander *et al.* (1978) using the same homologous assay system. These results indicate that in the homologous human system, the IgG is binding to Fc receptors through a site present in the whole Fc region, but probably absent from the $C_\gamma 3$ domain. This is consistent with data obtained from the study of several other homologous systems, e.g. guinea-pig (Alexander *et al.*, 1976) and rabbit (Ganczakowski & Leslie, 1979).

However, these results are at variance with those reported by Barnett Foster, Dorrington & Painter (1980) who studied the homologous binding of IgG to human monocytes. Although $C_\gamma 3$ domain fragments were inactive over the concentration range in which IgG and the Fc fragments were active, using a 10-fold higher concentration range (not used in this study), the pFc′ and tFc′ fragments did achieve significant inhibition of IgG binding. The pFc′ fragment was estimated to retain 2% of the activity of IgG.

In order to investigate more thoroughly the results obtained using the Fc and pFc′ fragments, less orthodox proteolytic fragments were isolated and analysed for their ability to inhibit IgG binding to macrophages and monocytes. The use of these fragments in the mouse macrophage direct binding assay has produced results concordant with the data obtained using the Fc and pFc′ fragments (discussed earlier). The active tFc′ fragment indicates the binding site within the $C_\gamma 3$ domain is located between residues

341 and 439, and from the structure of the smallest $C_\gamma3$ domain fragment reported to be active (Dorrington, 1976) ie the Fc′ fragment of Turner & Bennich (1968), the binding site must be located between residues 345 and 433. This is in agreement with Ciccimarra, Rosen & Merler (1975) who isolated a cytophilic decapeptide from a Fc fragment of human IgG composed of the residues found in the sequence 407–416 of the $C_\gamma3$ domain; and in our laboratory a synthetic decapeptide representative of the same amino acid sequence was found to be cytophilic in this heterologous system (Ratcliffe & Stanworth, 1982). The TLmFc fragment also retained cytophilic activity for the mouse macrophage Fc receptors; any alteration in the quaternary structure of the $C_\gamma2$ domain by removal of the covalent linkage not affecting, therefore, the conformation of the $C_\gamma3$ domain binding site. The human dimeric $C_\gamma2$ domain fragment was shown to have no cytophilic activity for mouse macrophages, thereby qualifying the proposed hypothesis that the binding site in this heterologous system is located within the $C_\gamma3$ domain.

The use of these unconventional fragments in the homologous system provided significant data. The dimeric $C_\gamma2$ domain fragment completely retained the binding activity of the Fc fragment, demonstrating that the cytophilic site used in this system is located within the $C_\gamma2$ domain. Ovary *et al.* (1976) demonstrated homologous cytophilic activity in the Facb fragment of rabbit IgG, in contrast to the pFc′ fragment which was inactive. However, Barnett Foster *et al.* (1980) found no cytophilic activity with a monomeric $C_\gamma2$ domain fragment in a homologous human system, and other workers who have found the pFc′ fragment incapable of binding to human monocytes have found the $C_\gamma2$ domain fragments to be inactive too (Haeffner-Cavaillon, Klein & Dorrington (1979b).

The TLmFc fragment was found to have a substantially reduced cytophilic activity when employed as inhibitor in the human monocyte system. This is likely to be due to the loss of the interheavy chain disulphide bridges and indicates the necessity of a covalent interaction between the $C_\gamma2$ domains for significant cytophilic activity. Reduction and alkylation of Fc fragment has been shown to reduce markedly the cytophilic activity of the fragment in systems where the binding site has been shown to be associated with the $C_\gamma2$ domain (Alexander *et al.*, 1976; Barnett Foster *et al.* 1978; Haeffner-Cavaillon, Dorrington & Klein, (1979a). However, Barnett Foster *et al.* (1980) found this treatment significantly reduced the binding of Fc fragment in a system where the pFc′ fragment had a small but significant activity. Although the $C_\gamma2$ fragment was found to be inactive, it was monomeric, lacking $C_\gamma2$ domain covalent interaction. Unfortunately this latter investigation was performed using a rosette assay whereas the present investigation employs a radioimmunoassay. Since neither group has used both techniques, these differences remain unresolved.

In this study, two separate and distinct sites on the IgG molecule, one located within the $C_\gamma3$ domain and one within the $C_\gamma2$ domain, have been shown to bind to the Fc receptors. Further investigations now in progress in our laboratory, based on the use of synthetic peptides representative of γ chain sequences, are aimed at throwing more light on this aspect of IgG antibody–Fc receptor interaction.

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