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MAPPING AND COMPARISON OF THE INTERACTION SITES ON THE Fc REGION OF IgG RESPONSIBLE FOR TRIGGERING ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY (ADCC) THROUGH DIFFERENT TYPES OF HUMAN Fcγ RECEPTOR

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Abstract—In the present study 3-iodo-4-hydroxy-5-nitrophenacetyl (NIP)-specific antibodies were compared for induction of antibody dependent lysis of NIP-derivatised red blood cells effected by pre-stimulated U937 or HL-60 cells and by K cells. The chimaeric antibodies have heavy chains corresponding to human IgG subclasses 1–4, and include site-directed mutants of IgG3 as well as the aglycosylated form of IgG3; a mouse IgG2b antibody and a site-directed mutant IgG2b were also examined. rIFN stimulated U937 or HL-60 cells express increased levels of FcγR1 compared to unstimulated cells; PMA stimulated HL-60 and U937 cells express an increased level of FcγR11 compared to unstimulated cells; K cells express FcγR111. Using these effector cell populations and the target cells mentioned above, we have compared anti-NIP antibodies with different heavy chain constant domains for their ability to induce ADCC through human FcγR1, FcγR11 and FcγR111. The results suggest that all three human Fcγ receptors appear to recognise a binding site on IgG within the lower hinge (residues 234–237) and trigger ADCC via this site, but that each receptor sees this common site in a different way. The possibility that other amino acid residues also participate in the binding/triggering site(s) cannot be excluded.

INTRODUCTION

Three isoforms of IgG binding receptors (FcγR) have been described for murine and human leucocytes. FcγR1 has high binding affinity for monomeric IgG, while FcγR11 and FcγR111 have low affinity and interact mainly with complexed IgG (Mellman, 1988). These three types of Fcγ receptor are coded by at least five different genes, and sequence homology suggests that they originate from a common ancestor (Seki, 1989). All three of the Fcγ receptors, except a subtype of FcγR111 on neutrophils, are transmembrane glycoproteins. They belong to the immunoglobulin supergene family and consist of three (FcγR1) or two (FcγR11 and FcγR111) Ig-like extracellular domains. Though these domains show a high degree of homology in their amino acid sequence (Brooks *et al.*, 1989; Kinet, 1989), monoclonal antibodies can differentiate between them. CD64 antibodies recognise FcγR1, CD32 antibodies bind to FcγR11, while CD16 antibodies interact with FcγR111 (Anderson and Looney, 1986). Divergent evolution leading to the generation of families of molecules usually results in the retention of homologous active sites with relatively minor structural differences leading to subtle variations in molecular recognition. Thus, a common feature of the human Fcγ receptors is the recognition of the human IgG1 and IgG3 isotypes while a distinguishing feature is the recognition of IgG4 by FcγR1 and IgG2 by a polymorphic variant of FcγR11 (van de Winkel and Anderson, 1991). In the present study we have investigated the molecular specificity of the three human Fcγ receptors using as probes chimaeric human/mouse IgG3 and mouse IgG2b, and mutated variants having single amino acid interchanges.

The rationale for these experiments originates with the proposal of Woof *et al.* (1986) that residues 234–237 in the lower hinge region of the heavy chain may constitute an interaction site for human FcγR1. Possible involvement of the nearby *N*-proximal bend, Gly 336–Lys 338, was also suggested. Support for this proposal was provided by Duncan *et al.* (1988) who generated a Glu 235 → Leu mutant of the mouse IgG2b isotype that bound to human FcγR1 with high affinity. The experiment was suggested from the knowledge that human FcγR1 binds mouse IgG2a with similar affinity to human IgG1 and IgG3 but does not bind mouse IgG2b. The human and mouse isotypes binding with high affinity have the same sequence for residues 234–237 whilst IgG2b differs due to the presence of glutamic acid at residue 235.

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Abbreviations—FcγR, receptors binding the Fc fragment of IgG; ADCC, antibody dependent cellular cytotoxicity; NIP, 3-iodo-4-hydroxy-5-nitrophenacetyl; rIFN, recombinant interferon gamma; PMA, phorbol myristate acetate; K cells, killer cells; EA rosette, rosette formed with antibody sensitised erythrocytes; RBC, red blood cells; FITC, fluorescein isothiocyanate; FACS, fluorescence activated cell sorter.

The observation that FcγR11 bearing K562 cells formed rosettes with NIP derivatised red cells when sensitised with chimaeric IgG3 but not when sensitised with the aglycosylated protein (Walker *et al.*, 1989*a*) suggests that the C_H2 domain makes a major contribution to FcγR11 recognition also. By contrast studies of human FcγR111 mediated killing by K cells were interpreted to suggest that the C_H3 domain is principally involved in binding while the C_H2 domain expresses an interaction site that induces the cytotoxic signal (Sarmay *et al.*, 1984, 1985, 1986; Gergely *et al.*, 1986). All three Fcγ receptor types can mediate ADCC when expressed on cells with lytic potential (Shen *et al.*, 1989) and we have used this functional activity, together with rosette formation, to compare the recognition specificities of the human Fcγ receptors. The results support a model of overlapping, non-identical interaction sites on the lower hinge of IgG for these Fc receptors, a finding that is compatible with their suggested evolutionary origin.

MATERIALS AND METHODS

Cell lines

HL-60 (myelocytic) and U937 (monocyte-like) cells were grown in stationary cell cultures in RPMI 1640 medium containing 10% fetal calf serum, glutamine and antibiotics. Cells (5×10^6) were stimulated by 50 ng/ml PMA or 500 units/ml of recombinant interferon gamma (Cambio Ltd, U.K.) for 48 hr, washed and counted then applied as effector cells in ADCC tests.

Separation of human K cells

Human peripheral blood mononuclear cells were isolated from the blood of healthy volunteers by density gradient centrifugation on Ficoll-Hypaque (Böyum, 1968). Monocytes were depleted by plastic adherence, nonadherent cells were further fractionated using a nylon wool column to deplete B cells (Aman *et al.*, 1984). The eluted cell fraction thus enriched for T cells and null cells (including the killer cell fraction) contained less than 2% monocytes and was used as effectors in K cell mediated ADCC experiments and rosetting assays. The ADCC assay was performed as described elsewhere (Lund *et al.*, 1990). Briefly, human red blood cells were NIP-derivatised with NIP-caproate-O-succinimide (0.23 mM), labelled with 0.4 mCi $Na^{51}CrO_4$ for 4 hr at 37°C, and sensitised with 20 μg of the different anti-NIP monoclonal antibodies per 2×10^8 red cells. Effector cells (K cells and PMA or rIFN stimulated U937 and HL-60 cells) were added to 10^5 target red cells at different ratios indicated in the text, and incubated at 37°C for 12 hr. Red cell lysis was assayed by the extent of release of radiolabelled chromate, using the formula:

$$\text{Specific } {}^{51}\text{Cr release} = \frac{(\text{expt. release} - \text{spontaneous release})}{\text{total incorporated activity}} \times 100.$$

Each sample was tested in triplicate and each experiment is the mean value from four separate donors.

Flow cytometric analysis

For flow cytometric analysis one million HL-60 or U937 cells before or after stimulation by PMA or rIFN were treated with monoclonal antibodies specific for FcγR1 (32.2) or FcγR11 (IV.3 or CIKM5) for 30 min at 4°C. After washing the cells were stained with the $F(ab')_2$ fragment of FITC-labelled anti-mouse IgG (Sigma), washed again and analysed by FACS (Becton Dickinson).

EA rosetting assays

For the rosetting assays washed red cells were derivatised with NIP-caproate-O-succinimide and sensitised by the monoclonal antibodies described above. U937 cells (5×10^5), HL-60 cells or K cells were mixed with 50 μl of a 1% suspension of sensitised red blood cells, incubated for 10 min at 37°C then spun at 200 *g* for 5 mins. After gentle resuspension the percentage of rosette forming cells was determined by counting 200 cells per sample.

Production of anti-NIP antibodies

The production of chimaeric mouse human IgG monoclonal anti-NIP antibodies has been described previously (Brüggemann *et al.*, 1987). Briefly, cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, glutamine (2 mM) and the antibiotics penicillin (400 units/ml), streptomycin (74 units/ml) and gentamycin (2 units/ml). Aglycosylated IgG3 was produced by cell growth in the presence of tunicamycin at 16 μM (Brüggemann *et al.*, 1987). Anti-NIP antibodies were purified from the supernatant by affinity chromatography on a NIP-sepharose column.

The production of site-directed mutants of mouse-human chimaeric IgG3 (containing the human gamma 3 constant heavy chain gene; Huck *et al.*, 1986) has been described previously (Lund *et al.*, 1991). Briefly the mutants were constructed by subcloning a BamH1 fragment containing the human gamma 3 C_H gene into the M13mp93 vector. Each mutant was made on this template using synthetic mismatched primers of 14–21 nucleotides. The mouse IgG2b site-directed mutant was constructed as detailed in Duncan *et al.* (1988). The fragments were excised, recloned in the pSV-V_{NP} vector and transfected into the mouse light-chain carrying J558L myeloma cell line. The V domain of the endogenous light chain complements the V_{NP} domain of the transfected heavy chain to give a NIP-specific antibody.

RESULTS

FcγR1 and FcγR11 systems were generated by differential stimulation of HL-60 and U937 cells with rIFN (Shen *et al.*, 1989) or PMA (Nambu *et al.*, 1989). Flow cytometric analysis using antibodies specific for FcγR1 (32.2) and FcγR11 (IV.3 and CIKM5) expression allowed the level of Fcγ receptors, reflected in the mean fluorescence intensity, and the number of cells positive for the receptor to be evaluated (Table 1). rIFN resulted

Table 1. Flow cytometric analysis of FcγR1 and FcγR11 expression on PMA or rIFN stimulated U937 and HL-60 cells

		Mean fluorescent staining intensities			
	Antibodies	U937	U937/PMA	HL-60	HL-60/PMA
Control	—	13.6	19.6	13.5	15.6
FcγR1	32.2	42.2	45.9	29.9	27.0
FcγR11	IV.3	44.8	497.0	39.7	68.6
FcγR11	CIKM5	45.5	288.8	45.9	60.2
		U937	U937/rIFN	HL-60	HL-60/rIFN
FcγR1	32.2	36.2	118.9	34.7	75.3
FcγR11	IV.3	48.2	51.4	43.6	41.7
FcγR11	CIKM5	36.0	37.3	33.7	38.1
		Percentage positivity (% of non-stimulated samples)			
		U937/rIFN	U937/PMA	HL-60/rIFN	HL-60/PMA
FcγR1	32.2	220	106	204	73
FcγR11	IV.3	106	141	69	140
FcγR11	CIKM5	154	130	72	121

U937 or HL-60 cells before or after stimulation with 50 ng/ml PMA or 500 units/ml rIFN gamma were treated with monoclonal antibodies specific for FcγR1 (32.2), or FcγR11 (IV.3 and CIKM5). The cells were stained with the $F(ab')_2$ fragment of an FITC-conjugated anti-mouse IgG (Sigma) and analysed by FACS (Becton Dickinson). Control samples were treated with the second antibody only. The numbers are a typical data set selected from three separate experiments.

in the expected induction of FcγR1 expression on both HL-60 and U937 cells. No increase in FcγR11 expression was detected with the IV.3 antibody but there was a modest increase detected by the CIKM5 antibody. The IFN stimulated cells were consequently regarded as indicators of FcγR1 mediated activities. PMA induced FcγR11 expression only on HL-60 and U937 cells (Table 1 and Fig. 1). The increase in mean fluorescence intensity was 10-fold for U937 cells and two-fold for HL-60 cells and consequently these cells were used as indicators of FcγR11 mediated activities. K cell preparations were used to determine FcγR111 mediated activities.

Rosette formation was examined between K cells and PMA stimulated HL-60 or U937 cells and NIP-derivatised RBC sensitised with anti-NIP antibodies. The rosette profiles obtained with aglycosylated and single site mutants of the IgG3 anti-NIP antibody are shown in Fig. 2a, as a percentage of the rosettes obtained with RBC sensitised with the native chimaeric IgG3 antibody. Rosette formation between aglycosylated IgG3 sensitised RBC and PMA stimulated HL-60 and U937 cells was essentially eliminated. However, rosette formation with K cells was only reduced to half of the value obtained with wild type protein, in agreement with previous results (Lund *et al.*, 1990). Substitution of leucine 235 to either alanine or glutamate had a large effect on rosette formation with PMA stimulated HL-60 cells (< 10% of the wildtype value). Less effect was evident with PMA stimulated U937 cells (47%), perhaps reflecting the higher levels of FcγR11 expressed on these cells. These two mutations had little effect on rosette formation with K cells giving values of 80% of those

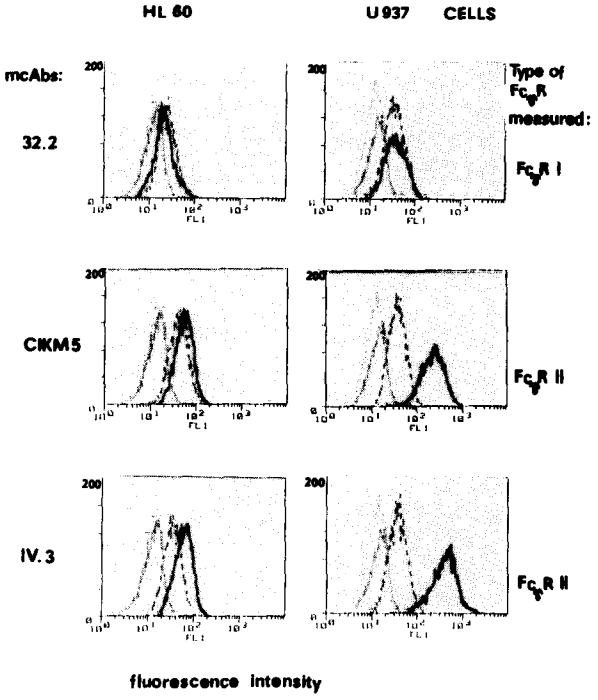

Fig. 1. Comparison of FcγR1 and FcγR11 expression on PMA stimulated HL-60 and U937 cells. One million cells were treated with the appropriate dilutions of the monoclonal antibodies recognising FcγR1 or FcγR11. Binding is detected by an indirect immunofluorescence assay using $F(ab')_2$ fragments of anti-mouse IgG-FITC antibodies. The samples were analysed by FACS. Key: Dotted lines, control samples; thin line, non-stimulated cells; thick line, PMA (50 ng/ml) stimulated cells.

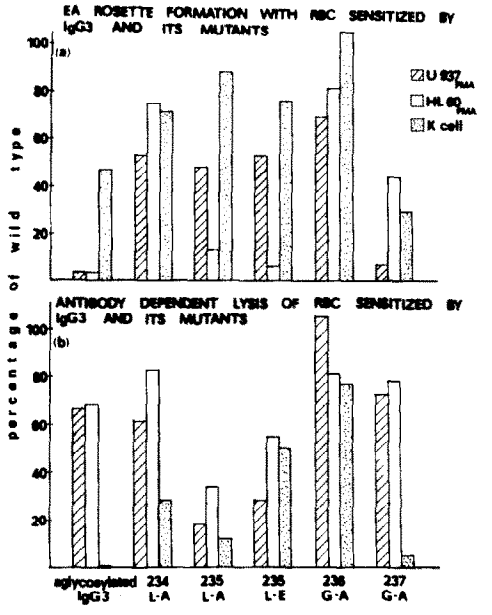

Fig. 2. EA rosetting and ADCC of PMA stimulated U937 or HL-60 cells, and K cells, using target cells sensitised with aglycosylated IgG3 or site-directed mutants of IgG3 anti-NIP mouse–human chimaeric antibody. Rh^+ RBC were derivatised with NIP hapten and sensitised by the different anti-NIP antibodies. (a) EA rosettes were counted microscopically. Key: U937 cells, cross-hatched rectangles; HL-60 cells, open rectangles; K cells, dotted rectangles. (b) For the cytotoxicity tests the sensitised RBC were labelled with ^{51}chromium. The cytotoxic assay was carried out overnight at 37°C. The counts in half the supernatant were measured.

obtained for wild type protein. Mutation at residue 237 also resulted in a reduction in the percentage of rosette formation for PMA stimulated U937 cells, PMA stimulated HL-60 cells and K cells to $<10\%$, 50% and 30% of the wild type values, respectively. Mutations at other residues had intermediate effects with mutation at residue 236 having the least influence. RBC sensitised with chimaeric human IgG2 anti-NIP failed to mediate rosette formation with any of the cell types whilst chimaeric IgG4 sensitised RBC gave 60% of the IgG3 wild type value with PMA stimulated HL-60 cells (data not shown).

ADCC activity was measured for K cells and PMA stimulated HL-60 or U937 cells with NIP-derivatised RBC sensitised with anti-NIP antibodies (Figs 2b and 3). The ADCC experiments were performed in parallel with the rosette assays and used the same IgG-sensitised RBC. The levels of cytotoxicity observed at an effector to target cell ratio of 3:1 are shown in Fig. 2b to permit comparison with the data for the rosette assays (Fig. 2a), while ADCC activities obtained at various effector:target cell ratios are shown in Fig. 3. Aglycosylated IgG3 was able to sensitise RBC for ADCC by PMA stimulated HL-60 or U937 cells (70% of wild type values), whilst ADCC mediated by K cells was abolished. Within the panel of single site mutant proteins the Leu 235 → Ala IgG3 protein exhibited the least capacity to sensitise for ADCC giving values of 10–35% for the three cell types. The Leu 235 → Glu mutation also had a marked effect on ADCC activity mediated by each of the cell types used. By contrast the Gly 237 → Ala mutant protein was unable to mediate K cell killing whilst having little effect on cytotoxicity mediated by PMA stimulated HL-60 and U937 cells. Reduced ADCC capacity was observed for the 234 mutant protein but the mutation at residue 236 was essentially without effect on ADCC mediated by any of the cells used.

Fc receptor specificity was further investigated with chimaeric IgG antibodies of each human subclass, the wildtype mouse IgG2b anti-NIP antibody and its Glu 235 → Leu mutant (Fig. 4). The pattern of ADCC lysis obtained with rIFN cells was that expected of an FcγR1 mediated response with IgG1, IgG3, IgG4 and the mutant IgG2b Glu 235 → Leu mediating efficient lysis.

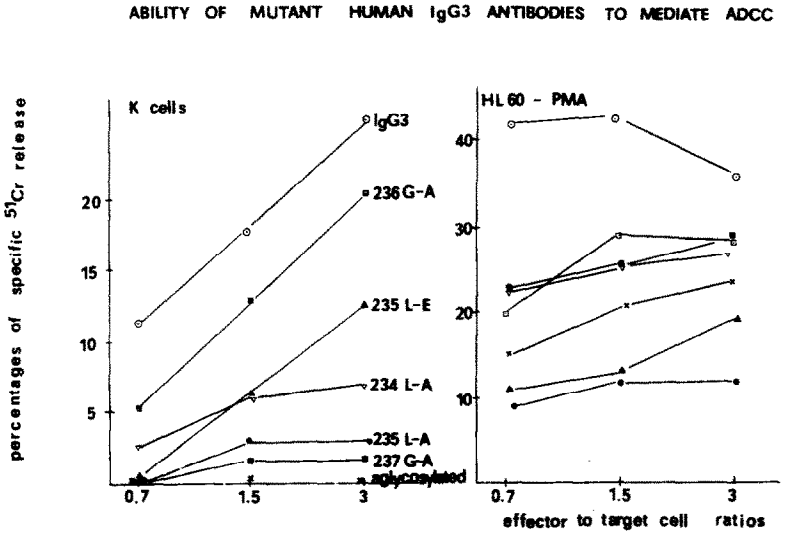

Fig. 3. Comparison of the ability of mouse–human chimaeric anti-NIP antibodies to induce antibody dependent lysis at different target:effector cell ratios. The dose-dependence of lysis on effector:target ratios with PMA-stimulated HL-60 cells is different to that of Fig. 4, probably because of the variability between different cell preparations.

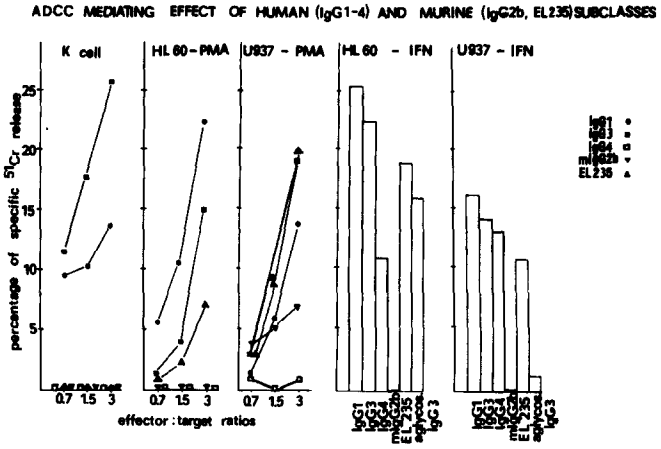

Fig. 4. ADCC mediating effect of different subclasses represented by the mouse–human chimaeric antibodies. Each data point is the mean of 2–3 experiments. The HL-60 and U937 cells were used at an effector:target ratio of 3:1. IgG2 did not induce lysis for either of these conditions.

By contrast the IgG4 isotype was unable to mediate ADCC for PMA stimulated HL-60 (mainly FcγR11) or K cells (FcγR111), whereas IgG1 and IgG3 mediated efficient lysis. However, the ability of the mouse IgG2a mimic antibody Glu 235 → Leu to trigger lysis efficiently with PMA stimulated U937 cells suggests a substantial contribution to lysis is mediated through FcγR1 in this case, although a contrary finding is that no ADCC is seen with IgG4, a characteristic expected of an FcγR11 system. The IgG2 isotype did not mediate ADCC with any of the prestimulated U937 or HL-60 cells, but occasionally mediated very low levels of ADCC with K cells.

DISCUSSION

The aim of this study was to identify amino acid residues within the Fc region of human IgG that contribute to recognition and activation of the human FcγR1, FcγR11, and FcγR111 cellular receptors. It was necessary, therefore, to establish systems which allowed interactions with essentially a single species of the Fc receptor to be investigated. Thus, stimulation of the myeloid cell lines HL-60 and U937 with interferon or phorbol myristate acetate results in the differential up-regulation of expression of FcγR1 and FcγR11 respectively and induces differentiation to more mature cell types capable of mediating ADCC. Interaction and activation through these receptors can be demonstrated by the induction of ADCC for antibody coated target cells (Shen *et al.*, 1986, 1989; Graziano and Fanger, 1987). It should be noted that up-regulation of Fcγ receptors is not synonymous with an ability to mediate ADCC (Shen *et al.*, 1989). The differential up-regulation of Fc receptors on these two cell lines is evident from Table 1. Human K cells express FcγR111 only and the function of this Fcγ receptor appears to be the induction of ADCC.

In the present study therefore, interferon stimulated U937 cells, phorbol myristate acetate stimulated HL-60 cells, and K cells, isolated from peripheral blood, were used as models for study of FcγR1, FcγR11 and FcγR111 mediated ADCC, respectively. Fc structural features essential for Fcγ receptor recognition were determined by the use of a set of chimaeric mouse/human monoclonal antibodies composed of the mouse light chain and V_H region, generating anti-NIP specificity, and the human heavy chain constant regions. This antibody set included each of the human IgG subclasses and five single site mutants of the human IgG3 molecule in which the natural residue was replaced by alanine and in one instance glutamic acid (residue 235). In addition a mouse IgG2b and a single site mutant mouse IgG2b were also employed. These reagents allow the specificity of Fcγ receptor activities to be evaluated independently of any effects due to differences in hapten specificity or affinity.

Several groups have investigated and reported on the localisation of site(s) on IgG Fc determining recognition by the Fcγ receptors. An apparently definitive study concluded that residues in the lower hinge region of the C_H2 domain (residues 234–237) are determinants of FcγR1 recognition (Woof *et al.*, 1986; Duncan *et al.*, 1988). The finding that aglycosylated IgG Fc of both IgG1 and IgG3 fail to bind FcγR11 (Walker *et al.*, 1989*a*) suggests that residues within the C_H2 domain also determine the recognition specificity of this Fcγ receptor. There is evidence that sites within both the C_H2 and C_H3 domains determine FcγR111 mediated ADCC (Sarmay *et al.*, 1985; Lund *et al.*, 1990). These findings have been interpreted to suggest that while the C_H3 domain contributes to recognition specificity the signal for generation of lytic activity results from a C_H2 domain interaction.

It is known that mouse IgG2a but not mouse IgG2b can bind to human FcγR1, whereas neither of these antibodies can bind to human FcγR11 (Lubeck *et al.*, 1985; Dougherty *et al.*, 1987; Boot *et al.*, 1989). On the basis of the dramatic ADCC inducing effect of the mouse IgG2b mutant Glu 235 → Leu, representing an antibody which mimics IgG2a, we suggest that rIFN stimulated U937 and HL-60 cells and PMA stimulated U937 cells use mainly FcγR1 to trigger ADCC (Fig. 4). This result indicates that a lower hinge site on IgG triggers ADCC through FcγR1. Conversely, PMA stimulated HL-60 cells appear to mediate lysis substantially through FcγR11, since ADCC with the mouse IgG2b mutant Glu 235 → Leu puts an upper limit on the contribution from FcγR1. Evidence consistent with this view is that IgG4 does not mediate ADCC with PMA stimulated HL-60 cells (Fig. 4), and is known not to interact with FcγR11, but can interact with FcγR1 (Walker *et al.*, 1989*b*). Additionally a concn of myeloma monomeric human IgG3 (Ga.) saturating for FcγR1 (0.13 μM) does not inhibit ADCC by PMA stimulated HL-60 cells (data not shown). However, the results for PMA stimulated U937 cells cannot be interpreted within this simple scheme, because although there is a 10-fold increase in FcγR11 expression over unstimulated cells (Table 1), and IgG4 does not mediate ADCC (Fig. 4), there is substantial triggering by the FcγR1 specific mouse mutant IgG2b

Glu 235 → Leu (Fig. 4), supportive of FcγR1 participation. Perhaps both FcγR1 and FcγR11 can mediate ADCC through these cells.

The mutant IgG3 Leu 235 → Ala mediated ADCC poorly for both PMA-stimulated U937 and HL-60 cells and for killer cells, suggesting that the lower hinge participates in forming the binding/triggering site on IgG3 for both human FcγR11 and FcγR111. The discrepancy between EA rosetting and ADCC with K cells in the case of target cells sensitised with the Leu 235 → Ala mutant is consistent with the view that binding sites on IgG are distinct from the triggering sites on IgG for ADCC mediated by FcγR111. Gly 237 has a role in forming the binding and triggering sites for ADCC mediated via FcγR111 since the mutant Gly 237 → Ala did not induce ADCC mediated by K cells (Figs 2 and 3). Thus these experiments with chimaeric IgG3 and mouse IgG2b mutants in the lower hinge implicate this region as a site on IgG for all three human Fc receptors, required both for binding (rosetting) and for triggering.

Aglycosylation at Asn 297 in the C_H2 domain of human IgG3 has a profound effect on recognition by human Fcγ receptors (Walker *et al.*, 1989*a*; Lund *et al.*, 1990). Aglycosylation of IgG3 severely reduced EA rosetting of PMA stimulated U937 and HL-60 cells, while permitting ADCC, so therefore it seems that the extent of rosetting may not correlate with the ability to trigger ADCC (Fig. 2). One possible explanation is that different sites on IgG are responsible for triggering ADCC than for rosetting through human FcγR11 and/or FcγR1. Cytotoxic activity of human rIFN stimulated U937 cells which use mainly FcγR1 to trigger ADCC (Shen *et al.*, 1986), and that of FcγR111 positive K cells was practically abolished when the target cells were sensitised by aglycosylated IgG3 (Fig. 2b and Fig. 4). These data suggest that similar sites within the C_H2 domain play an important role in triggering ADCC through FcγR1 and FcγR111. The fact that in the rosetting tests aglycosylated IgG3 gave only 50% inhibition of EA rosettes on K cells can be explained by the participation of a second binding site for FcγR111 in the C_H3 domain as suggested previously (Sarmay *et al.*, 1984, 1985). A protein structural change has been detected in the vicinity of the lower hinge of aglycosylated IgG3 at His 268, implying that the lower hinge itself may be disrupted structurally. This model explains the elimination of IgG3 recognition by human FcγR1 (Lund *et al.*, 1990), and applies equally well to the lower hinge site on IgG3 mapped here for human FcγR11 and FcγR111. Thus there is a lower hinge site on IgG for all three human Fcγ receptors, and this is consistent with the known structural effects of aglycosylation on IgG3.

Comparing the ability of the human IgG subclasses to trigger ADCC, as expected IgG1 and IgG3 were the most efficient (Fig. 4). IgG2 did not mediate lysis, and its sequence (234-Val–Ala–_–Gly-237) also deviates from the optimal motif (234-Leu–Leu–Gly–Gly-237) displayed by IgG1 and IgG3. IgG4 was able to induce a reduced level of target cell lysis when rIFNgamma stimulated effector cells were used (mainly FcγR1), but not with PMA stimulated effector cells (mainly FcγR11). The primary sequence of the lower hinge of IgG4 is 234-Phe–Leu–Gly–Gly-237, different from IgG1 or IgG3 at position 234, and suggests that residue 234 might have a role in participating in a binding site for FcγR11 recognition.

In summary, we propose that all three human Fcγ receptors appear to recognise a lower hinge binding site on IgG and can trigger ADCC via this site, but that each receptor sees this common site in a different way. The hinge here is both mobile and exposed, and so the lower hinge would be an ideal candidate for a binding site on IgG for human Fcγ receptors.

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