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Aglycosylation of human IgG1 and IgG3 monoclonal antibodies can eliminate recognition by human cells expressing FcγRI and/or FcγRII receptors

Matthew R. WALKER,*‡ John LUND,* Keith M. THOMPSON† and Royston JEFFERIS*

* Department of Immunology, The Medical School, Edgbaston, Birmingham B15 2TJ, U.K., and †MRC Centre, Hills Road, Cambridge CB2 2QH, U.K.

Aglycosylated human IgG1 and IgG3 monoclonal anti-D (Rh) and human IgG1 and IgG3 chimaeric anti-5-iodo-4-hydroxy-3-nitrophenacetyl (anti-NIP) monoclonal antibodies produced in the presence of tunicamycin have been compared with the native glycosylated proteins with respect to recognition by human FcγRI and/or FcγRII receptors on U937, Daudi or K562 cells. Human red cells sensitized with glycosylated IgG3 form rosettes via FcγRI with 60% of U937 cells. Inhibition of rosette formation required > 35-fold concentrated more aglycosylated than glycosylated human monoclonal anti-D (Rh) antibody. Unlabelled polyclonal human IgG and glycosylated monoclonal IgG1 and anti-D (Rh) antibody inhibited the binding of ^{125}I-labelled monomeric human IgG binding by U937 FcγRI at concentrations > 50-fold lower than the aglycoslyated monoclonal IgG1 anti-D (Rh) ($K_{50} \sim 3 \times 10^{-9}$ M and $\sim 6 \times 10^{-7}$ M respectively). Similar results were obtained using glycosylated and aglycosylated monoclonal human IgG1 or IgG3 chimaeric anti-NIP antibody-sensitized red cells rosetting with FcγRI$^-$/FcγRII$^+$ Daudi and K562 cells. Rosette formation could be inhibited by the glycosylated form (at > 10^{-6} M) but not by the aglycosylated form. Haemagglutination analysis using a panel of murine monoclonal antibodies specific for epitopes located on Cγ2, Cγ3 or Cγ2/Cγ3 interface regions did not demonstrate differences in Fc conformation between the glycosylated or aglycosylated human monoclonal antibodies. These data suggest that the FcγRI and FcγRII sites on human IgG are highly conformation-dependent and that the carbohydrate moiety serves to stabilize the Fc structure rather than interacting directly with Fc receptors.

INTRODUCTION

Interaction of IgG with cell-bound receptors for the Fc region of immunoglobulins (FcγR) is an initiating event for a wide range of biological effector functions including immune regulation, antigen clearance or elimination (Silverstein *et al.*, 1977; Cerrotini & Bruner, 1974), and hypersensitivy reactions (Rouzer *et al.*, 1980). Several criteria, primarily reactivity with specific monoclonal antibodies, have identified three Fc receptors (FcγRI, FcγRII and FcγRIII; previously FcR_{lo}) and resulted in their partial characterization (Anderson & Looney, 1986). Further heterogeneity within these classes is now becoming evident (Anderson *et al.*, 1987) particularly as a result of gene cloning studies. The three classes of FcγR may be expressed on distinctive and overlapping populations of cells and their expression has been determined for several human cell lines, namely U937, HL60, Daudi, Jurkat, and K562 (Looney *et al.*, 1986; Jones *et al.*, 1985; Rosenfeld *et al.*, 1987).

The 73 kDa human FcγRI receptor binds monomeric human IgG with high affinity ($K_a \sim 5.5 \times 10^8$ M^{-1}) whilst the 40 kDa FcγRII and 50–70 kDa FcγRIII bind monomer IgG weakly ($K_a < 10^6$ M^{-1}). The specificity of human FcγRI for the human IgG subclasses has been demonstrated to be IgG1 = IgG3 ⩾ IgG4 with IgG2 essentially non-binding (Woof *et al.*, 1986; Walker *et al.*, 1988*a*). FcγRII expressed by platelets has been demonstrated to bind all four human IgG subclasses (Martin *et al.*, 1978; Karas *et al.*, 1982), whilst FcγRII expressed on Daudi and K562 cells has been demonstrated to bind only the IgG1 and IgG3 subclasses (Walker *et al.*, 1989). The IgG subclass specificity of human FcγRIII remains unresolved.

The FcγRI interaction site on IgG has been proposed to be located within the 'hinge-link' region, probably involving residues 234–238, within the Cγ2 domain (Woof *et al.*, 1984, 1986; Partridge *et al.*, 1986). Indeed, site-directed mutagenesis within this region of a mouse IgG2b monoclonal antibody permitted binding to FcγRI (Duncan *et al.*, 1988) whereas the unmutated IgG2b antibody did not. The interaction site on IgG for human FcγRII has yet to be determined, whilst both Cγ2 and Cγ3 domains are implicated in the recognition of human IgG by FcγRIII (Sarmay *et al.*, 1986).

The role of Fc *N*-linked carbohydrate in interaction of IgG with effector molecules remains to be conclusively demonstrated, although several studies have shown that aglycosylation of IgG prevents its interaction with several important biological effector molecules (Nose & Wigzell, 1983; Leatherbarrow *et al.*, 1985). Differences in the patterns of IgG *N*-linked Fc glycosylation have

Abbreviations used: IgG1f, IgG1.G1m(f) allotype; IgG3b, IgG3.G3m(b) allotype; NIP, 5-iodo-4-hydroxy-3-nitrophenacetyl; FcγRI, human Fc receptor type I; FcγRII, human Fc receptor type II; Cγ2, Cγ3, heavy chain constant domains 2 and 3 of IgG; PBS, phosphate-buffered saline; BSA, bovine serum albumin; MAb, monoclonal antibody; ADCC, antibody-dependent cellular cytoxicity.

‡ To whom correspondence should be addressed at present address: Department of Clinical Chemistry, Wolfson Research Laboratories, Queen Elizabeth Medical Centre, Birmingham B15 2TH, U.K.

been implicated in rheumatoid arthritis and primary osteoarthritis (Parekh *et al.*, 1985).

In this paper we report studies of the interaction of FcγRI expressed on U937 cells and human IgG1f and IgG3b monoclonal anti-D(Rh) antibodies produced under standard culture conditions in the absence or presence of tunicamycin. Parallel studies have been performed using IgG1f and IgG3b human chimaeric monoclonal antibodies (Brüggemann *et al.*, 1987) specific for the hapten NIP (5-iodo-4-hydroxy-3-nitrophenacetyl), allowing study of the interaction of antigen-bound glycosylated or aglycosylated IgG with the FcγRII receptor expressed on Daudi or K562 cells. This system avoids problems of interpretation associated with previous studies employing murine antibodies reacting with human FcRs.

MATERIALS AND METHODS

Monoclonal antibodies

The hetero-hybridoma cell lines REG-A and FOG-3 were produced by the fusion of human EBV-lymphocytes with the mouse myeloma cell line X63-Ag8.653 (Thompson *et al.*, 1986). These cell lines produce human monoclonal anti-D(Rh) antibody of IgG1.G1m(f) and IgG3.G3m(b) subclasses respectively, although both express human κ light chains. Cells were maintained in RPMI 1640 (Gibco) medium containing 5% foetal calf serum (FCS). Antibody was isolated from culture supernatant by affinity chromatography using an in-house murine anti-κ constant region monoclonal antibody (72/2) coupled to glutaraldehyde-activated silicate (Boehringer-Mannheim). The production of chimaeric human IgG subclass monoclonal anti-NIP antibodies has been previously described (Brüggemann *et al.*, 1987). Purified anti-NIP antibody was produced from culture supernatant by affinity chromatography using a NIP-hexanoate–Sepharose column (Brüggemann *et al.*, 1987).

Tunicamycin treatment

Aglycosylated IgG1 REG-A or IgG3b FOG-3 was produced by maintaining hybridoma cells in medium containing the glycosylation inhibitor tunicamycin (Sigma) as follows. Hybridoma cells at 1×10^6/ml were incubated in 500 ml of medium containing 1.25 μg of tunicamycin/ml on a rotary shaker at 37 °C for 3 h. Cells were spun down and resuspended in 500 ml of fresh medium containing 1.25 μg of tunicamycin/ml and incubated for 24 h at 37 °C after which time the supernatant was harvested. Following $(NH_4)_2SO_4$ precipitation of the supernatant, the precipitate was resolubilized in saline and the IgG1 or IgG3 anti-D(Rh) purified by affinity chromatography as described above.

Aglycosylated human IgG1f or IgG3b anti-NIP was similarly generated using tunicamycin at 10 μg/ml.

Cell lines

The human monocytic cell line U937, human erythroblastic line K562 and Burkitt lymphoma line Daudi were maintained in RPMI 1640 medium (Gibco) supplemented with 10% FCS at 37 °C. Cells were washed three times in sterile phosphate-buffered saline (PBS) prior to use in the assays. In all cases the percentage viability of the cells assessed by their ability to exclude the dye Trypan Blue was consistently > 95%.

Inhibition of monomeric IgG–FcγRI interaction assay

Quantitative microassay of inhibition of human IgG–FcγRI binding was performed as previously described (Woof *et al.*, 1984; Partridge *et al.*, 1986). Briefly, monomeric polyclonal ^{125}I-labelled human IgG (^{125}I-IgG) was incubated to equilibrium with a U937 cell suspension (2×10^6 cells/tube) in a balanced salt solution containing 0.2% BSA and 0.1% NaN_3 at 37 °C for 1 h. Bound and free ^{125}I-IgG was determined following separation of supernatant and pellet by overlaying with water-immiscible oil (Versilube F.50; a gift from Dr. D. R. Burton, Sheffield, U.K.) followed by rapid centrifugation. In each assay duplicate tubes containing an excess of unlabelled polyclonal IgG (1×10^{-6} M) gave values for non-specific binding. The inhibitor concentration generally varied in the range 5×10^{-5}–10^{-10} M while the concentration of cells and ^{125}I-IgG remained constant. Assays were performed in duplicate and a control inhibition curve for normal polyclonal IgG was included in each case.

SDS/PAGE

SDS/PAGE was performed in 10% crosslinked gels under reducing conditions according to the method of Weber & Osborn (1969). Samples were prepared in 0.1 M-Tris/HCl, pH 7.0, containing 2% SDS, 2% mercaptoethanol and 10% sucrose and heated to 100 °C for 10 min prior to electrophoresis.

Red cell sensitizations

Human O+ (R1R2) red blood cells, kindly provided by Dr. D. McDonald (Blood Transfusion Service, Edgbaston, Birmingham, U.K.) were washed five times in sterile PBS and packed by centrifugation at 2600 rev./min for 5 min.

Washed red blood cells were sensitized with human monoclonal anti-D(Rh) antibody as described previously (Walker *et al.*, 1989). Sensitized cells were then washed three times in sterile PBS and resuspended to 1% suspension in PBS.

For sensitization with anti-NIP MAb, washed cells were washed twice with iso-osmotic borate buffer, pH 8.3, and packed in the same buffer. Packed cells (1 ml) were incubated for 1 h at room temperature on a rotator with 9 ml of borate buffer, pH 8.3, containing 100 μg of NIP-hexanoate-*O*-succinimide (Cambridge Research Biochemicals, Harston, Cambridge, U.K.) dissolved in a minimum volume of dimethylformamide (Sigma). The NIP-derivatized cells were then washed four times with PBS and 200 μl of a 10% suspension of cells was incubated with 100 μl of purified anti-NIP MAb at 200, 100, 50, 25, 10 and 5 μg/ml in PBS for 1 h at 37 °C. Sensitized red cells were further washed three times with PBS and resuspended to give a 1% suspension.

Rosette assays

Rosetting of sensitized red blood cells to effector cells was performed according to the protocol described by Anderson *et al.* (1986) using a ratio of 100 red blood cells: 1 effector cell as previously described (Walker *et al.*, 1988). Inhibition of rosette formation was performed by incubating the reaction mixture in the presence of 50 μl of purified IgG diluted in PBS to the concentrations indicated in the text.

Haemagglutination assay

Haemagglutination was performed in U-shaped microtitre trays (Titertek) as previously described (Lowe *et al.*, 1982). Ascitic fluid containing murine MAb specific for epitopes on human IgG (Lowe *et al.*, 1982; Nik Jaafar *et al.*, 1983) (see Fig. 2) were doubly diluted from a 1:10 external dilution in Hepes buffer containing 2% FCS.

RESULTS

Preliminary experiments for each of the anti-Rh(D) secreting hetero-hybridoma cell lines (REG-A and FOG-3) established that a tunicamycin concentration of 1.25 μg/ml was optimal for secretion of aglycosylated IgG with acceptable cell mortality (results not shown). Similar experiments established that for the IgG1 and IgG3 anti-NIP secreting hybridoma cell lines the optimal concentration of tunicamycin was 10 μg/ml (results not shown). Fig. 1 shows SDS/PAGE analyses under reducing condition of the IgG affinity-purified from culture supernatant derived from each hybridoma cell line grown in the absence or presence of tunicamycin. The aglycosylated heavy chains are evident from their increased relative mobility through the gel compared to the glycosylated heavy chains (Cushley *et al.*, 1982). Staining of an immunoblot of the heavy chains of glycosylated REG-A but not aglycosylated REG-A by concanavalin A bound to horseradish peroxidase (Walker *et al.*, 1987) was also consistent with aglycosylation of the heavy chain produced in the presence of tunicamycin. Differences in the mobilities of the light chains of anti-D(Rh) and anti-NIP clones probably reflects usage of different variable region genes and/or rearrangements. However, significant ($\sim 40\%$) of the tunicamycin-treated FOG-3 can be seen to be degraded yielding a fragment equivalent to IgG3Fc; this was accounted for in calculating antibody concentrations used for red blood cell sensitizations. Contaminating albumin and other medium components were also evident in some tracks, but were omitted from such calculations.

Analysis of red blood cells sensitized with maximal levels of anti-D(Rh) antibody or 20 μg of anti-NIP antibody in a haemagglutination assay employing a panel of murine monoclonal antibodies specific for epitopes on human Fcγ (Fig. 2) demonstrated no significant differences in the reactivity observed using glycosylated or aglycosylated human IgG1 or IgG3 antibodies. Equivalent levels of sensitization of the red blood cells with glycosylated or aglycosylated IgG were evident in the haemagglutination assay using IgG subclass-specific murine MAb (results not shown).

Significant differences were however evident when sensitized red blood cells were applied to rosette assays using U937, Daudi or K562 cell lines. Red blood cells sensitized by glycosylated FOG-3 IgG3k anti-D(Rh) formed significant ($> 60\%$) rosettes with the FcγRI$^+$/FcγRII$^+$ U937 cells whilst no rosetting was observed with aglycoslyated FOG-3 or glycosylated or aglycosylated IgG1 anti-D(Rh) REG-A (Table 1). No anti-D(Rh) sensitization of red blood cells was capable of mediating rosette formation with the FcγRI$^-$/FcγRII$^+$ Daudi or K562 cell lines (results not shown). Rosettes formed between U937 cells and red blood cells sensitized with IgG3 anti-D(Rh) monoclonal antibodies CB6 or PhT1A3 (see Walker *et al.*, 1989) could be inhibited by monomeric glycoslyated IgG1 REG-A (Fig. 3*a*, open symbols) whilst >35-fold more aglycosylated REG-A was required to produce equivalent inhibition (Fig. 3*a*, closed symbols). Similar results were obtained using tunicamycin-treated IgG3b FOG-3 to inhibit FcγRI-mediated rosette formation with glycosylated FOG-3-sensitized red blood cells (Fig. 3*b*). The binding of REG-A by human FcγRI on U937 cells was further demonstrated in an inhibition assay measuring inhibition

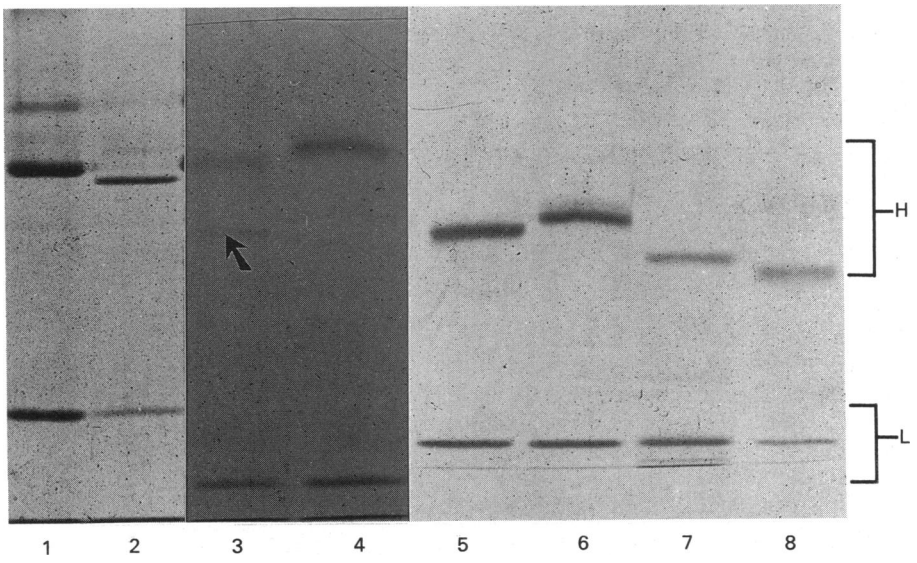

Fig. 1. 10% cross-linked SDS/PAGE gel (under reducing conditions) stained with Coomassie Blue

Tracks: 1, purified native IgG1f anti-D(Rh) REG-A; 2, purified IgG1f tunicamycin-treated REG-A; 3, purified IgG3b tunicamycin-treated anti-D(Rh) FOG-3; 4, purified IgG3b anti-D(Rh) FOG-3; 5, purified tunicamycin-treated IgG3b anti-NIP; 6, purified native IgG3b anti-NIP; 7, purified native IgG1 anti-NIP; 8, purified tunicamycin-treated IgG1 anti-NIP. H, heavy chains; L, light chains; the arrow indicates a breakdown product ($\sim 40\%$ of total by laser densitometry).

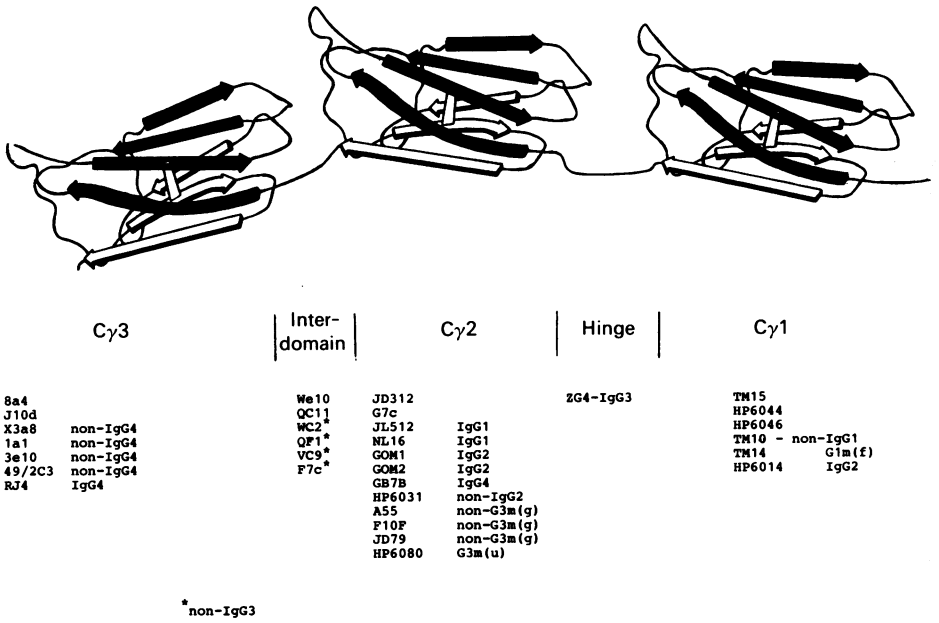

Fig. 2. Diagramatic representation of the location of epitopes recognized by the panel of murine MAb specific for IgG Fc epitopes

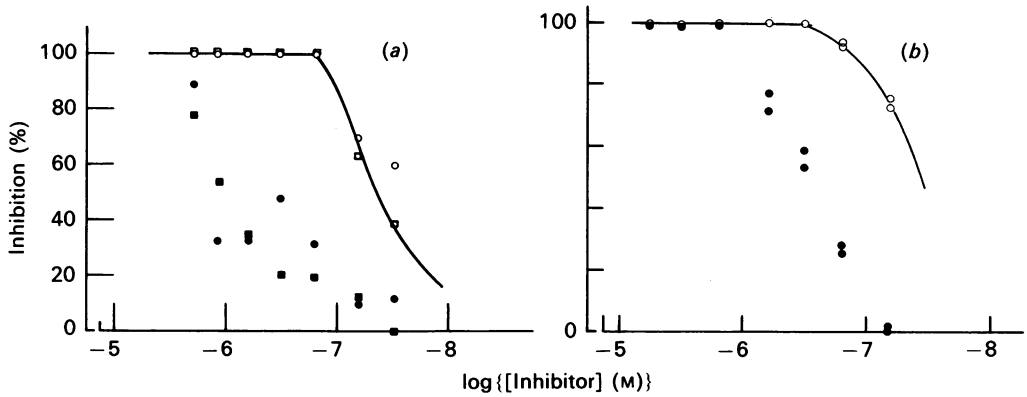

Fig. 3. Inhibition of rosette formation between red blood cells sensitized with glycosylated anti-D(Rh) antibody and U937 cells

(*a*) Inhibition of rosetting of red blood cells sensitized with monoclonal IgG3b PhT1A3 (○) or IgG3b CB6 (□) anti-D(Rh) antibodies by native glycosylated IgG1k REG-A (open symbols) and tunicamycin-treated REG-A (closed symbols). (*b*) Inhibition of rosetting of IgG3b CB6-sensitized red blood cells by native glycosylated IgG3b FOG-3 (open symbols) and tunicamycin-treated FOG-3 (closed symbols).

of ^{125}I-labelled monomeric polyclonal human IgG binding (Woof *et al.*, 1984, 1986). Glycosylated REG-A gave an almost identical inhibition curve to unlabelled polyclonal human IgG ($K_{50} = 6 \times 10^{-9}$ M) binding inhibition activity (Fig. 4).

NIP-derivatized red blood cells sensitized with varying amounts of glycosylated IgG1 or IgG3b anti-NIP monoclonal antibodies gave significant rosettes with both the FcγRI$^-$/FcγRII$^+$ Daudi (Figs. 5*a* and 6*a*) and K562 (Figs. 5*b* and 6*b*) cell lines. However, red blood cells sensitized with aglycosylated IgG1 and IgG3b anti-NIP monoclonal antibodies produced in the presence of tunicamycin gave no rosetting at any level of sensitization of the cells with either the K562 or Daudi cell lines (Figs. 5 and 6). Similar results were obtained for IgG3b anti-NIP monoclonal-antibody-sensitized red blood cells using the FcγRI$^+$/FcγRII$^+$ U937 cells (Fig. 5*c*).

Significant inhibition of rosette formation between Daudi cells and glycosylated IgG3b anti-NIP-sensitized red blood cells could be achieved using glycosylated IgG3b at concentrations $> 10^{-6}$ M whilst no significant inhibition was evident using the aglycosylated IgG3b at concentrations $> 10^{-5}$ M (Fig. 7).

Table 1. Rosetting between U937 and red blood cells sensitized with normal or tunicamycin-treated human anti-D(Rh) monoclonal antibodies

Sensitization	Rosette formation (% ± S.E.M., $n = 6$)
REG-A (IgG1f)	0
REG-A$_t$* (IgG1f)	0
FOG-3 (IgG3b)	62 ± 3
FOG-3$_t$* (IgG3b)	1 ± 1

* FOG-3$_t$ and REG-A$_t$ are antibodies produced in the presence of tunicamycin.

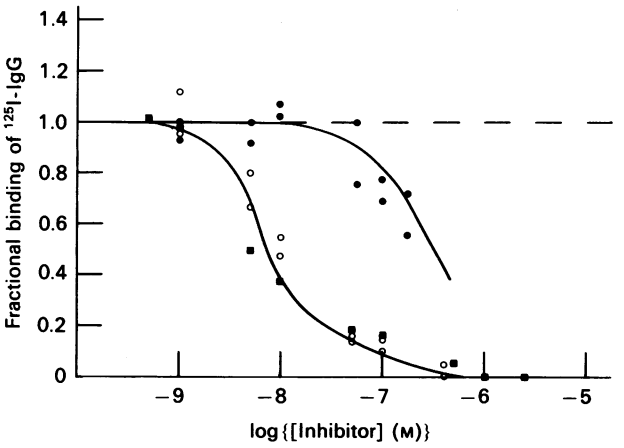

Fig. 4. Inhibition of ^{125}I-IgG binding to FcγRI on U937 cells by unlabelled polyclonal IgG (■), native REG-A (○) and tunicamycin-treated REG-A (●)

DISCUSSION

Several workers have investigated the ability of aglycosylated IgG, from several animal species, to interact with biologically important effector molecules and subsequent effector function activation. Two approaches have been used; removal of carbohydrate by glycosidase treatment (Williams *et al.*, 1973; Koide *et al.*, 1977; Winkelhake *et al.*, 1980) or culturing IgG-secreting hybridoma cells in the presence of tunicamycin which prevents *N*-linked carbohydrate attachment at Asn-297 in the Cγ2 domain (Nose & Wigzell, 1983; Leatherbarrow *et al.*, 1985). Using the latter approach, aglycosylated murine monoclonal IgG2a and IgG2b anti-hapten monoclonal antibodies have been demonstrated to exhibit impaired complement activation, loss of binding to murine macrophage Fc receptors and ability to activate ADCC (Nose & Wigzell, 1983), a lowered rate of C1 activation and a >50-fold decrease in the apparent binding constant of aglycosylated IgG2a to the human FcγRI receptor on U937 cells (Leatherbarrow *et al.*, 1985).

In this paper we describe the interaction of aglycosylated human IgG1 and IgG3b anti-D(Rh) or anti-NIP monoclonal antibodies with human FcγRI and FcγRII receptors expressed on U937, Daudi and K562 cell lines. Aglycosylated human monoclonal antibodies were produced by culturing hybridoma cells in the presence of 1.25 or 10 μg of tunicamycin/ml (see the Materials and methods section).

Analysis of red blood cells sensitized with glycosylated or aglycosylated human IgG1 or IgG3b monoclonal antibodies in a haemagglutination assay using a panel of murine MAbs specific for epitopes within the Cγ2, Cγ3 or Cγ2/Cγ3 interface region of IgG Fc (Fig. 2) demonstrated no differences in reactivity between the glycosylated or aglycosylated IgG. This suggests that tunicamycin treatment has not resulted in significant conformational changes within the Fc region. In contrast, red blood cells sensitized with aglycosylated FOG-3 IgG3b anti-D(Rh) antibody were unable to form rosettes with the FcγRI$^+$/FcγRII$^+$ U937 cells whilst cells sensitized with glycosylated FOG-3 antibody formed significant (>60%) rosettes (Table 1). We have previously demonstrated that rosetting of human IgG-anti-D(Rh)-sensitized red blood cells is an essentially FcγRI-mediated event and, without γ-interferon stimulation or bromelain treatment, U937 cells only form rosettes with IgG3-sensitized red blood cells; this is

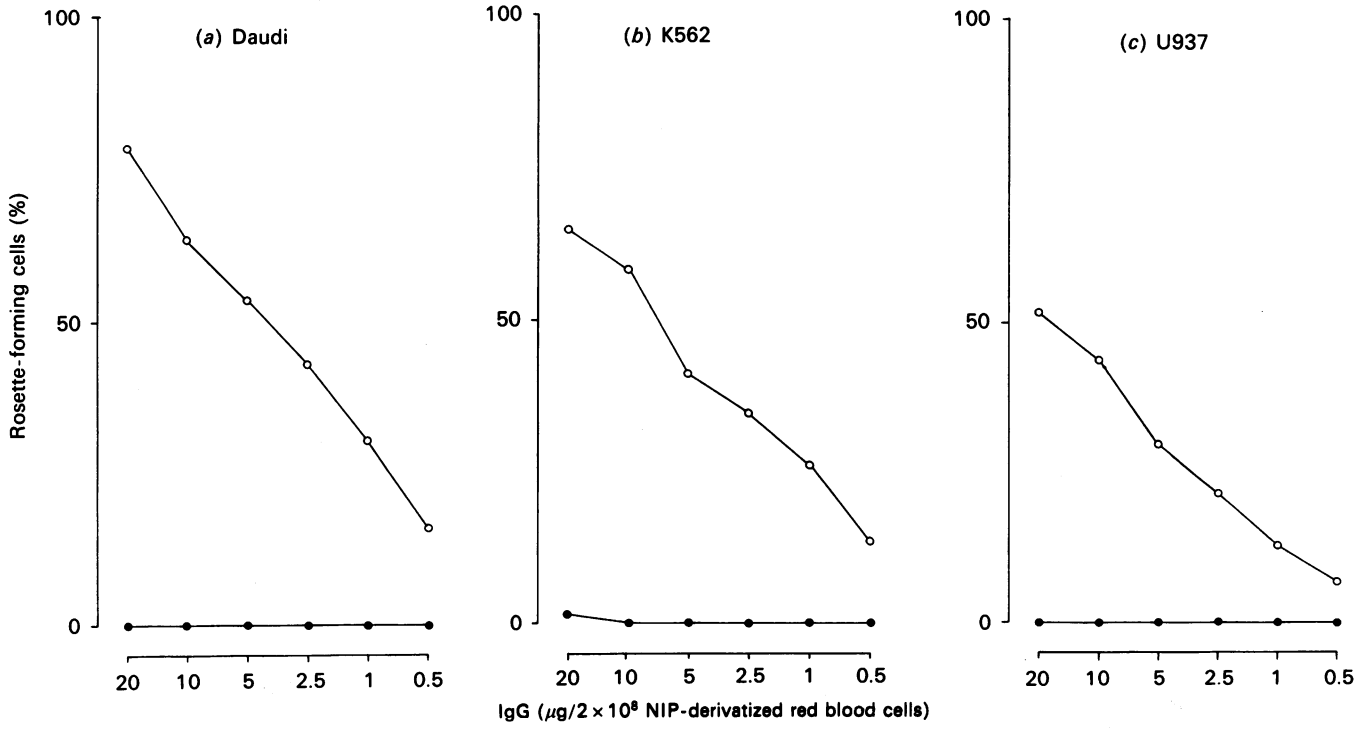

Fig. 5. Rosette formation between red blood cells sensitized with glycosylated human IgG3b anti-NIP (open symbols) or tunicamycin-treated IgG3b anti-NIP (closed symbols) with (*a*) Daudi cells, (*b*) K562 cells, and (*c*) U937 cells

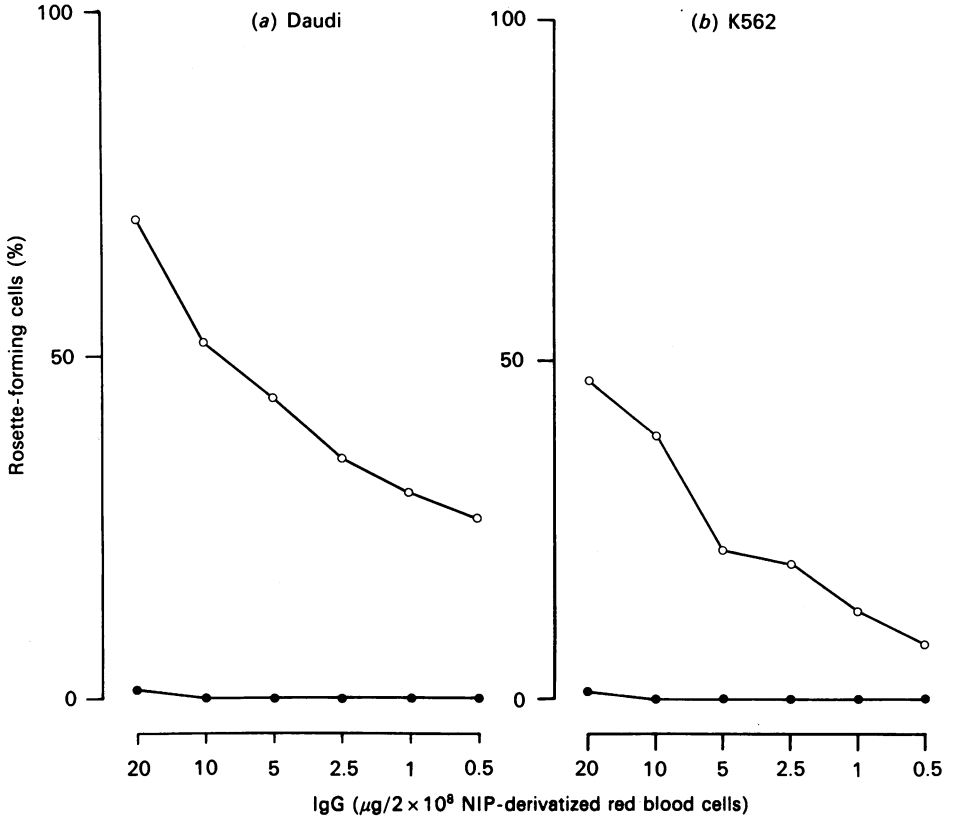

Fig. 6. Rosette formation between red blood cells sensitized with glycosylated IgG1 anti-NIP (open symbols) or tunicamycin-treated IgG1 anti-NIP (closed symbols) with (*a*) Daudi cells and (*b*) K562 cells

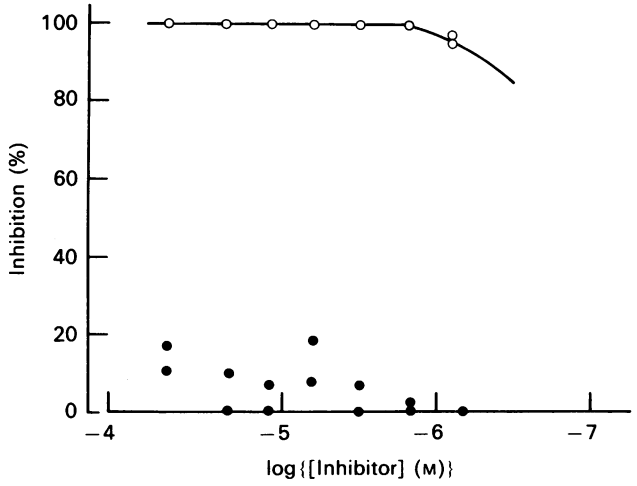

Fig. 7. Inhibition of rosette formation between red blood cells sensitized with native IgG3b anti-NIP antibody and Daudi cells by native glycosylated IgG3b anti-NIP (open symbols) and tunicamycin-treated IgG3b anti-NIP (closed symbols)

postulated to be due to its extended hinge region (Walker *et al.*, 1988). Monomeric IgG1 or IgG3 can however be demonstrated to bind to human FcγRI due to its high affinity for monomer either by inhibition of IgG3 anti-D(Rh) mediated rosetting or by competition of ^{125}I-labelled monomeric IgG binding (Woof *et al.*, 1984, 1986). Inhibition of rosette formation by both the aglycosylated IgG1 (REG-A) and IgG3b (FOG-3) anti-D(Rh) antibodies exhibited a > 35-fold lower inhibitory capacity than by the glycosylated forms (Fig. 3). Inhibition of binding of monomeric ^{125}I-labelled polyclonal human IgG to U937 FcγRI was reduced by > 50-fold for the aglycosylated form of IgG1 REG-A ($K_{50} \sim 3 \times 10^{-7}$ M) (Fig. 4) compared with unlabelled polyclonal IgG or glycosylated REG-A ($K_{50} = 6 \times 10^{-9}$ M). These values compare favourably with those obtained by Leatherbarrow *et al.* (1985) for aglycosylated murine IgG2a monoclonal antibody. Residual binding of the aglycosylated form of murine IgG2a was suggested to result from contamination of the preparation (by < 3%) with native glycosylated murine IgG2a. Within our study, significant contamination of the aglycosylated REG-A by glycosylated REG-A is evident (Fig 1, lanes 1 and 2), but no similar contamination is observed for the aglycosylated forms of FOG-3 or IgG1 or IgG3b anti-NIP monoclonal antibodies (lanes 3–8). It is therefore possible that aglycosylation of human IgG1 or IgG3 results in a lowered binding affinity (rather than abolition) of these forms to human FcγRI similar to the observation of a reduction in the affinity of C1q binding to aglycosylated murine IgG2a (Leatherbarrow *et al.*, 1985). The possibility that Fc carbohydrate residues directly interact with the human FcγRI receptor has been raised by several workers; however, as yet there remains no definitive evidence (for review see Burton, 1985). Recently, the involvement of residues Leu-234–Pro-238 in the human FcγRI interaction site on IgG (Woof *et al.*, 1986) has been confirmed by site-directed mutagenesis. A monoclonal murine IgG2b anti-NIP

protein, normally not recognized by human FcγRI, has been engineered to give a Glu/Leu interchange at position 235 (giving the same sequence as murine IgG2a bound with high affinity by human FcγRI), producing a product recognized with high affinity by human FcγRI (Duncan *et al.*, 1988).

It is of interest that whilst the site recognized by FcγRI is substantially altered in aglycosylated IgG we could not detect loss of epitope expression using a large panel of murine monoclonal antibodies, including antibodies previously shown to inhibit Fcγ–FcγRI interactions (Woof *et al.*, 1986; Partridge *et al.*, 1986). This suggests that aglycosylation results in subtle conformational changes within the Cγ2 domain which can have an important effect on local sites. The direct involvement of the carbohydrate moiety in FcγRI recognition is unlikely because the sugar residues are essentially 'buried' in the quaternary structure (Burton, 1985); this is evidenced by the resistance to removal by endoglycosidases.

The site of interaction of human FcγRII on IgG has as yet not been delineated; however, the data presented herein suggest that similar conformational restraints are imposed upon FcγRII interaction with IgG. Both glycosylated human IgG1 and IgG3b anti-NIP-sensitized red blood cells form rosettes with the $Fc\gamma RI^-/Fc\gamma RII^+$ Daudi and K562 cells, the degree of rosette formation being related to the IgG concentration on the cell surface (Walker *et al.*, 1989; the present paper). Examination of the rosetting of red blood cells sensitized with equivalent amounts of aglycosylated IgG1 and IgG3 reveals that removal of Fc carbohydrate abolished FcγRII-mediated rosette formation (Fig. 5). Similar results were obtained if glycosylated and aglycosylated IgG3b were applied to rosette inhibition (Fig. 7), i.e. inhibition was observed with glycosylated but not aglycosylated IgG3b. Since aglycosylation of murine IgG2b has been demonstrated not to affect binding of staphylococcal protein A, which requires integrity of the Fc Cγ2/Cγ3 interface region (Nose & Wigzell, 1983), it would suggest that FcγRII recognizes a site within the Cγ2 domain.

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