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EFFECTOR FUNCTIONS OF A MONOCLONAL AGLYCOSYLATED MOUSE IgG2a: BINDING AND ACTIVATION OF COMPLEMENT COMPONENT C1 AND INTERACTION WITH HUMAN MONOCYTE Fc RECEPTOR

ROBIN J. LEATHERBARROW,* THOMAS W. RADEMACHER and RAYMOND A. DWEK†
Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

JENNIFER M. WOOF, ANTHONY CLARK and DENNIS R. BURTON
Departments of Biochemistry and Medical Microbiology, University of Sheffield, Sheffield S10 2TN, U.K.

and

NEIL RICHARDSON and ARNOLD FEINSTEIN
Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, U.K.

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Abstract—Aglycosylated monoclonal anti-DNP mouse IgG2a produced in the presence of tunicamycin was compared with the native monoclonal IgG2a with respect to its ability to interact with the first component of complement, C1, and to compete with human IgG for binding to human monocyte Fc receptors. The aglycosylated IgG2a was found to bind subcomponent C1q with an equivalent capacity to the native IgG2a, but the dissociation constant was found to be increased three-fold. When activation of C1 by the glycosylated and aglycosylated IgG2a was compared, the rate of C1 activation by the aglycosylated IgG2a was reduced approximately three-fold. In contrast aglycosylation was accompanied by a large decrease ($\geq$50-fold) in the apparent binding constant of monomeric IgG2a to human monocytes. The data suggest that the aglycosylated IgG2a has a structure which differs in the C_H2 domain from the native IgG2a, and that the heterogeneous *N*-linked oligosaccharides of this monoclonal IgG2a which occur at a conserved position in the C_H2 domain play a role in maintaining the integrity of its monocyte-binding site. This lack of monocyte binding may result either from a localized conformational change occurring in a single C_H2 domain or from an alteration in the C_H2–C_H2 cross-domain architecture which is normally structured by a pair of opposing and interacting oligosaccharides. The minimal changes in C1q binding and C1 activation suggest that the oligosaccharides are, at most, indirectly involved in these events.

INTRODUCTION

An integral feature of all normal IgG class antibodies is the *N*-linked oligosaccharides in the C_H2 domain. Analyses of the structural and functional aspects of the *N*-linked oligosaccharides are of biological interest for four main reasons: (1) the glycosylation of the C_H2 domain has been conserved throughout evolution, suggesting an important role for the oligosaccharides, (2) the combinatorial association of two heavy chains places two oligosaccharide units in direct contact with each other from which it follows that (3) the immunoglobulin molecule is a model for both specific protein–carbohydrate and carbohydrate–carbohydrate interactions, and (4) the immunoglobulin molecule serves as a model system for the analysis of oligosaccharide heterogeneity (Rademacher and Dwek, 1984; Rademacher *et al.*, 1982).

Crystallographic studies on immunoglobulin Fc fragments (Diesenhofer, 1981; Sutton and Phillips, 1983) have shown that, unlike other immunoglobulin domains, the two C_H2 domains do not form extensive lateral associations. The resultant interstitial region is filled by the inclusion of the oligosaccharide side chains attached to Asn-297 on each heavy chain such that the carbohydrates form a bridge across the domains. The $\alpha(1-6)$ arms of the biantennary complex oligosaccharides interact with the protein and the $\alpha(1-3)$ arms of the two oligosaccharides form the bridge.

Possible roles for the oligosaccharides could include both a structural and/or functional one. In the former the oligosaccharides would exist as spacers between the two C_H2 domains, thereby imparting

*Current address: Department of Chemistry, Imperial College of Science and Technology, London SW7 2AY, U.K.
†To whom correspondence should be addressed.

stability or protection (e.g. from proteolysis). However, the presence of oligosaccharide heterogeneity could also increase the functional diversity of the immunoglobulin molecule with the oligosaccharides acting as specific ligands for recognition by receptors.

This paper describes the production of a monoclonal aglycosylated IgG2a and the purification of this IgG2a to homogeneity. We then compare the interaction of native and aglycosylated IgG2a with the first component of complement C1. This is achieved by quantitative comparison of the binding affinity and capacity for the subcomponent C1q alone, and by comparison of the rates of activation of the entire C1 complex. The results allow a quantitative description of the effect of aglycosylation on the binding and activation of C1. We also compare the interaction of native and aglycosylated mouse IgG2a with human monocyte Fc receptor and discuss the role that the oligosaccharide may be playing in immunoglobulin structure and function.

MATERIALS AND METHODS

Materials

All culture media were obtained from Gibco. Ficoll 400 and CNBr-activated Sepharose 4B were purchased from Pharmacia Fine Chemicals. Metrizoic acid [sodium salt, 32.8% (w/v) aq. solution], tunicamycin and rabbit anti-bovine IgG were obtained from Sigma Chemical Co. L-[4,5-^{3}H]leucine (197 Ci/mmole) and D-[1-^{14}C]glucosamine HCl (57.9 mCi/mmole) were obtained from Amersham International. [^{125}I]-C1 was generously provided by B. Gorick and N. R. Hughes-Jones (ARC, Cambridge). The hybridoma cell line K3 was made available by Dr B. A. Askonas (N.I.M.R., Mill Hill, U.K.).

Immunoglobulin

Pooled human IgG was isolated using ammonium sulphate precipitation, ion-exchange and gel-filtration chromatography (Hudson and Hay, 1980) and labelled with ^{125}I using immobilised lactoperoxidase/glucose oxidase (Bio-Rad) to a level of $\sim$0.02 I/mole.

The IgG2a-producing hybridoma (K3) was grown as an ascitic tumour in pristane-primed (BALB/c $\times$ CBA) F_1 mice. Viable hybridoma cells were purified from ascitic fluid on a discontinuous Ficoll/Metrizoate density gradient (Davidson and Parish, 1975) by recovery from the interface. The purified hybridoma cells were seeded at a density of 2×10^5 cells/ml, and subcultured every 3 days as necessary in Falcon 3028 tissue culture flasks containing RPMI 1640/10% heat-inactivated foetal calf serum (FCS)/penicillin (100 units/ml)/streptomycin (100 μg/ml)/2-mercaptoethanol (25 μM)/glutamine (2 mM).

Aglycosylated IgG2a was produced by suspending cells from the above cultures at 2×10^6 cells/ml in medium containing 2 μg/ml tunicamycin. The cells were incubated in this medium at 37°C/5% CO_2 for 4 hr, which control experiments had determined to be sufficient time for all remaining glycosylated IgG2a to be secreted. The cells were then centrifuged at 160 g for 10 min, the supernatants removed, and the cells washed twice with fresh tunicamycin-containing medium, and resuspended at 2×10^6 cells/ml. They were then re-cultured for 3 days before removing the aglycosylated IgG2a-containing supernatant. The supernatants were concentrated 10-fold by ultrafiltration using an Amicon PM30 membrane, dialysed vs PBS/EDTA, and any precipitated material removed by centrifugation. The supernatant, with [^{3}H]leucine IgG2a added to act as a tracer, was then applied to a DNP–lysine–Sepharose column (5×1.4 cm) in PBS/EDTA, and unbound material washed through with the same buffer. Bound protein was eluted with 50 mM DNP–glycine, pH 7.2. After concentration, this protein was applied to a Sephacryl S-200 column (140×1.6 cm) in PBS/EDTA to purify the product further and remove excess DNP–glycine. Removal of bovine IgG derived from the foetal calf serum was by passage of the IgG fraction through an anti-bovine IgG–Sepharose column. Remaining traces of bound DNP–glycine were removed by exchange dialysis against dinitrophenol in PBS at pH 7.2 followed by ion-exchange chromatography on Dowex 1×8 (-400).

Tracer [^{3}H]leucine IgG was produced in culture using leucine-deficient RPMI 1640 (Gibco, select amine kit). Cells were incubated in the modified medium containing tunicamycin for 4 hr at 37°C/5% CO_2, washed and resuspended in fresh modified medium followed by the addition of radiolabelled amino acid (10 μCi/ml) and incubated for a further 30 hr prior to isolation of the IgG as above.

In order to determine the optimal amount of tunicamycin, cells (2×10^6 cells/ml, 0.5 ml) with varying amounts of tunicamycin (added from a stock solution in 5 mM NaOH/165 mM NaCl) were incubated for 3.5 hr at 37°C/5% CO_2 in Falcon 3033 tissue culture tubes. 2 μCi/ml L-[4,5-^{3}H]leucine and 1 μCi/ml D-[1-^{14}C]glucosamine hydrochloride were then added and incubation continued for 36 hr. After centrifugation the supernatants were removed and the labelled IgG isolated on columns of DNP–lysine–Sepharose. After washing with PBS the IgG was eluted with 50 mM DNP–glycine, pH 7.2, which was subsequently removed on a Dowex 1×8 (-400) column equilibrated in PBS.

IgG samples were analysed on a discontinuous SDS–PAGE. Fluorography was performed using ENHANCE (New England Nuclear) and Kodak X-Omat AR film. Proteins were visualized by staining with Coomassie blue.

Binding of IgG2a to human monocyte Fc receptors

Human mononuclear cells were isolated from freshly-drawn heparinized blood by use of lymphocyte separation medium (Flow). They were then

washed with RPMI 1640/10% FCS and incubated in plastic petri dishes at 37°C/5% O_2 for 45 min. The adherent monocytes were harvested by washing in 1:1 BSS [−Ca, −Mg]/EDTA (10 m*M*):RPMI 1640/10% FCS. Cells were then suspended at 10^8/ml in BSS [−Ca, −Mg]/BSA (0.2%)/NaN_3 (0.1%) and 2 vols of suspension was added to 1 vol of [^{125}I]-human IgG (4 n*M*), BSS [−Ca, −Mg]/BSA (0.2%)/NaN_3 (0.1%) and 1 vol of test IgG2a solution (various dilutions) and incubated for 2 hr at 37°C. Both the iodinated IgG and the test IgG2a stock solutions were centrifuged (100,000 *g* for 30 min) before use to remove aggregates. The monocytes were resuspended and duplicate aliquots were layered over 1 vol Versiluble F50 (Alfa) which had in turn been layered over 1 vol of 1:2 lymphocyte separation medium:BSS in siliconized tubes. The monocytes with bound IgG were separated from unbound IgG by centrifuging them through the Versiluble phase. The tubes were then frozen and snapped to separate the pellet and supernatant fractions, and the amount of [^{125}I]-IgG in each was determined. As well as varying amounts of test IgG, the following controls were also included: (1) [^{125}I]-IgG alone, to give the initial binding; and (2) a large excess of unlabelled IgG, to give the non-specific background binding.

Protein in the stock IgG samples after ultracentrifugation was determined by the Bio-Rad dye-binding protein assay (micro version) using dilutions of an IgG sample of known concn to construct the standard curve.

C1q binding

This was performed as described previously (Leatherbarrow and Dwek, 1984) except that the final concn of IgG2a/DNP-Affigel 701 was 0.6% (v/v) and the total assay vol was 100 μl. The data were analysed using a FORTRAN non-linear regression program.

C1 activation

This assay followed essentially the C1 activation assay of Folkerd *et al.* (1980) but used IgG/DNP-Affigel 701 to activate the C1. The supernatant and pellet fractions were analysed by SDS-PAGE using 10% acrylamide gels. Positions of the labelled protein bands were determined by autoradiography. The intensity of each band was estimated by densitometry of the developed film and the percentage activation expressed as:

$$\frac{(\overline{C1r}[H] + \overline{C1s}[H] + (\overline{C1r}[L])}{(C1r + C1s) + (\overline{C1r}[H] + \overline{C1s}[H] + (\overline{C1r}[L])} \times 100\%,$$

where [H] and [L] denote the heavy and light chains of C1r or C1s produced after activation (denoted by a bar) which is due to the proteolytic cleavage.

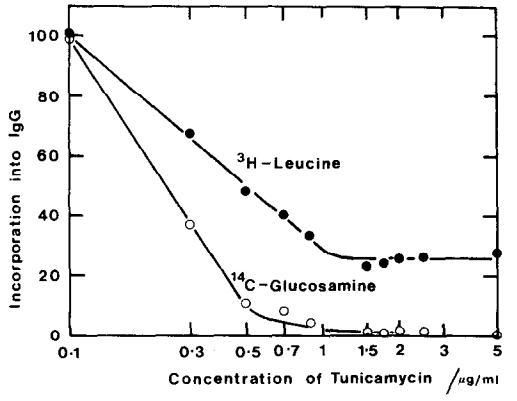

Fig. 1. Effect of tunicamycin on the incorporation of [^{3}H]leucine (●) and [^{14}C]glucosamine (○) into IgG2a isolated from culture supernatants. The results are expressed as the percentage incorporated in the absence of tunicamycin.

RESULTS

Effect of tunicamycin on IgG2a secretion and glycosylation

The effect of increasing tunicamycin concn on the incorporation of [^{3}H]leucine and [^{14}C]glucosamine into IgG2a produced by the hybridoma cell line K3 is shown in Fig. 1. Tunicamycin inhibits the glycosylation of the immunoglobulin in a dose-dependent manner, with complete inhibition occurring at >1.5 μg/ml tunicamycin. These results are in accordance with previous findings where it has been found that, in contrast to other types of immunoglobulin, aglycosylated IgG is still secreted from the cells, albeit at a reduced rate (Hickman *et al.*, 1977; Hickman and Kornfeld, 1978; Blatt and Haimovich, 1981; Sidman, 1981). It is interesting to note here (see Fig. 1) that when the protein is completely aglycosylated no further inhibition of the IgG2a secretion is observed. Consequently a slight excess of tunicamycin (to ensure complete aglycosylation) was used (2 μg/ml) in the experiments with the K3 cell line.

Purification of aglycosylated IgG2a from culture supernatants

A total of 2.5 l of aglycosylated IgG2a-containing culture supernatant was produced as described above. The concn of mouse IgG2a in this supernatant was estimated to be 1–2 μg/ml by radial immunodiffusion. Glycosylated IgG2a was also produced as a control from parallel cell cultures in the absence of tunicamycin. Gel filtration of the eluate from the DNP–lysine–Sepharose column showed a single radioactive peak at $M_r \sim 150$ kilodaltons. Monitoring at 280 nm revealed two peaks, however, one eluting with the void vol, and the second at the position of the radioactive peak and corresponding to that of authentic IgG2a ($M_r \sim 150$ kilodaltons). The material eluting in the void vol did not contain IgG, and consisted of components of extremely high apparent mol. wt which did not enter a 5% acrylamide stacking gel. By criteria of reaction with anti-bovine IgG antisera and from the SDS gel profiles it was found that in addition to the mouse IgG2a the

150 kilodalton fraction also contained ~20% bovine IgG, present from the FCS. Removal of the bovine IgG was achieved by passage of the 150 kilodalton fractions through an anti-bovine IgG–Sepharose column [any anti-mouse IgG activity in this antiserum had been removed prior to coupling to the Sepharose by passage down a mouse IgG (K3)–Sepharose column]. This was verified by examination of the SDS–PAGE profiles of the 150 kilodalton fraction before and after the anti-bovine IgG column and secondly by the lack of precipitin reaction against anti-bovine IgG (data not shown).

The SDS gel profiles of glycosylated and aglycosylated IgG show two distinct positions for the migration of their respective H-chains (see Figs 2 and 3), with the latter having the expected lower apparent M_r for an aglycosylated polypeptide. This difference is perhaps most apparent in the fluorograph of Fig. 2, where the better resolution also shows that the more diffuse glycosylated heavy chain band actually runs as a "doublet". This has also been observed for IgG synthesized by a human lymphoblastoid cell line (Owen and Kissonerghis, 1982). Since the doublet is not apparent after tunicamycin treatment oligosaccharide heterogeneity could cause these effects. The other possibility of a second glycosylation site which was partially glycosylated was discounted, as

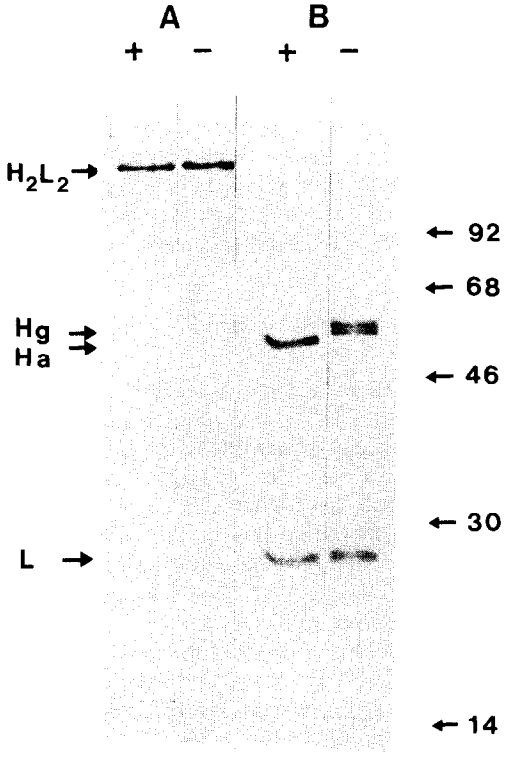

Fig. 2. Fluorograph of a 10% acrylamide SDS–PAGE gel of non-reduced (A) and reduced (B) [^{3}H]leucine-labelled IgG2a produced in the presence (+) and absence (−) of tunicamycin. The positions of mol. wt standards are arrowed ($\times 10^{-2}$). H, IgG2a heavy chain; L, IgG2a light chain; Ha, aglycosylated H-chain; Hg, glycosylated H-chain.

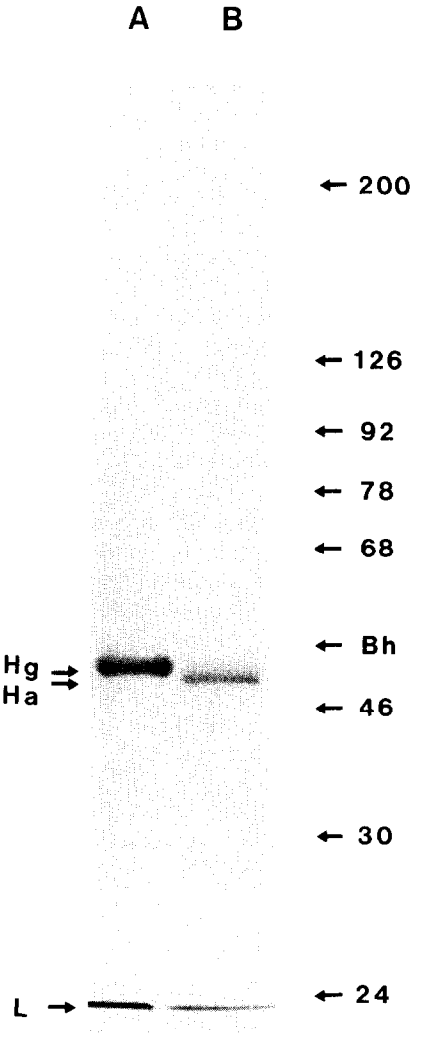

Fig. 3. SDS–PAGE (9% acrylamide) of the final purified glycosylated (A) and aglycosylated (B) IgG2a. The samples were reduced with 2-mercaptoethanol. The gel was stained for protein using Coomassie blue. Hg, position of glycosylated heavy chain; Ha, position of aglycosylated heavy chain; L, position of light chain; Bh, position of bovine IgG heavy chain. The positions of various mol. wt standards are arrowed ($\times 10^{-2}$).

K3 protein contains only a single *N*-linked oligosaccharide per H-chain (data not shown).

Since the altered migration on SDS–PAGE is due to the presence of oligosaccharide the gel profiles were used to assess the extent of aglycosylation of the IgG2a. In conjunction with the differential incorporation of radiolabelled glucosamine (Fig. 1) the aglycosylated IgG2a preparation contained no detectable contaminating glycosylated components. In addition neither the glycosylated nor aglycosylated preparation showed the presence of any other protein bands. The total yield of aglycosylated IgG2a was ~1 mg. This represents a yield of 30% (~150,000-fold purification).

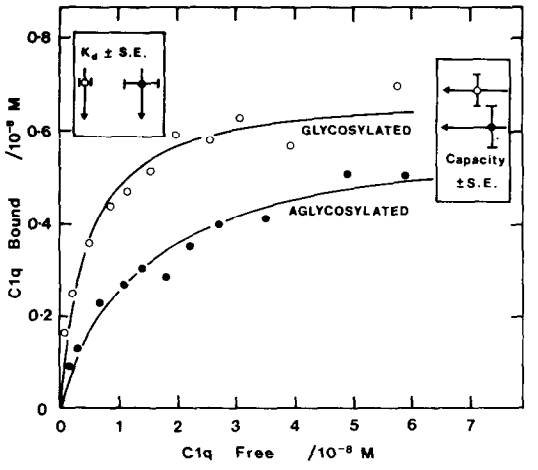

Fig. 4. Binding of [^{125}I]-C1q to glycosylated (○) and aglycosylated (●) IgG2a–DNP–Affigel 701. The curves represent the computed best fit to the data which gave the following values for the parameters:

	Capacity ± SE (nM)	$K_d \pm$ SE (nM)
Glycosylated IgG2a	6.8 ± 0.3	4.3 ± 0.9
Aglycosylated IgG2a	6.0 ± 0.5	14.0 ± 3.0

Binding of C1q to native and aglycosylated IgG2a

The binding of C1q to glycosylated and aglycosylated IgG2a bound to DNP–Affigel 701 (Leatherbarrow and Dwek, 1984) is shown in Fig. 4 together with the best-fit binding curves to the data and values for K_d and capacity. Also tabulated are SEs of these parameters, which were obtained from the fitting procedure. It is found that both systems have the same capacity to bind C1q. There is a slight difference, however, in the dissociation constants, the aglycosylated IgG binding C1q approximately three-fold weaker than the glycosylated IgG.

Activation of C1 by glycosylated aglycosylated IgG2a

Binding of C1q alone is not sufficient to activate C1. Both tryptophan-modified (Allan and Isliker, 1974*a*, *b*) and glutaraldehyde cross-linked (Folkerd *et al.*, 1980) IgG retain the ability to bind C1q and yet do not activate the bound C1. Therefore it is conceivable that aglycosylated IgG may bind and yet not activate C1 and so its ability to perform this activation was examined.

The time-course of C1 activation (cleavage of C1r and C1s) at 37°C by glycosylated and aglycosylated IgG2a–DNP–Affigel 701, and by a control sample of DNP–Affigel 701 alone, is shown in Fig. 5. Both IgGs were found to cause activation of the bound C1 with respect to the control sample although the rate of activation by the aglycosylated IgG2a is slower by a factor of three [a further control of C1 alone showed an identical activation profile to C1 plus DNP–Affigel 701 (data not shown)]. This reduction is predicted as the aglycosylated IgG2a was found to bind less C1 (30% specifically bound compared with 42% to the aglycosylated IgG2a). The difference in binding of C1 is a consequence of the reduction in C1q binding. The work of Hughes-Jones and Gorick (1982) has shown that the amount of C1/C1q bound affects the rate of activation of C1, presumably due to a proximity effect.

Binding to human monocyte Fc receptors

The assay system utilised inhibition of binding of pooled human IgG to human monocytes. Pooled human IgG is essentially equivalent to polyclonal IgG1 owing to the weak binding of IgG2a and the low relative serum concn of the other subclasses (Kurlander and Batker, 1982; Fries *et al.*, 1982). Prior to using mouse IgG2a in this assay, however, it was necessary to establish that the IgG2a bound to the monocyte Fc receptor with the same affinity as human IgG. Figure 6 shows the inhibition of binding of human [^{125}I]-IgG by unlabelled human IgG of mouse IgG2a (K3). It is evident that the immunoglobulins behave similarly and therefore allows the use of this system to compare the inhibition by native and aglycosylated mouse IgG.

The ability of glycosylated and aglycosylated IgG to inhibit the binding of ^{125}I-labelled human IgG to human monocytes is shown in Fig. 7. It was found that there is at least a 50-fold difference in the inhibitory capacity (K_{50}) of the two proteins. Since densimetric gel scanning or double-labelling experiments do not detect any glycosylated contaminant, we conclude that this cannot be present at more than about 3%. Nevertheless such an amount of glycosylated IgG, if present, could easily account for the residual activity.

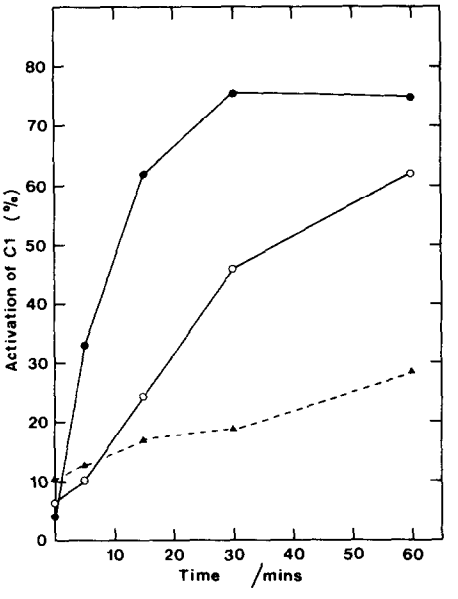

Fig. 5. Time-course of activation of C1 by: (●) glycosylated IgG2a–DNP–Affigel 701, (○) aglycosylated IgG2a–DNP–Affigel 701, and (▲) DNP–Affigel 701 alone.

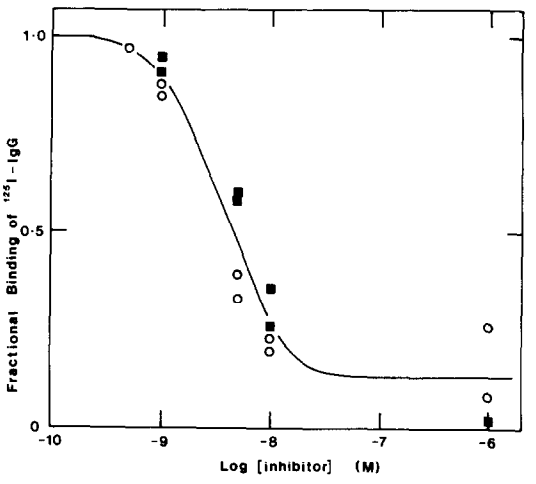

Fig. 6. Comparison of the inhibitory effects of monomeric human IgG and mouse IgG2a on binding of human [^{125}I]-IgG (1 nM). (■) Human IgG and (○) mouse IgG2a. The results shown are from two different experiments normalised so that the fractional binding [^{125}I]-IgG = 1.0 in the absence of inhibitors. This corresponds to 10–30% binding of the total [^{125}I]-IgG [see Woof *et al.* (1984) for a typical Scatchard plot of human IgG binding to monocytes]. The numbers of cpm bound at 0 and 100% inhibition were 81 ± 4 and 9 ± 2 respectively.

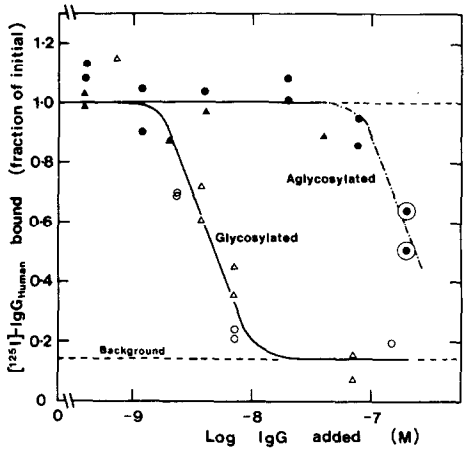

Fig. 7. Inhibition of human [^{125}I]-IgG by glycosylated and aglycosylated mouse IgG2a. The figure is a compilation of two separate experiments (denoted by circles and triangles). The points marked ◉ were performed using an uncentrifuged preparation and therefore may contain aggregates which would cause greater inhibition than an aggregate-free preparation of the same concn. The values for the apparent inhibition constants (K_{50}) are: glycosylated, 5×10^{-9} M; aglycosylated, $>2.5 \times 10^{-7}$ M. The numbers of cpm bound at 0 and 100% inhibition were 43 ± 3 and 9 ± 2 respectively.

DISCUSSION

Several workers have used glycosidase treatment of IgG to examine the role of the carbohydrate in antibody function. While all agree that antigen binding is unaffected, the following effects on effector functions have been reported. Williams *et al.* (1973) found that upon removal of 60% of the carbohydrate from rabbit IgG by glycosidase digestion three out of the four preparations tested exhibited no change in complement-fixing, opsonic or agglutinating activities: however, one preparation completely lost both complement-fixing and opsonic activity. Koide *et al.* (1977), again using glycosidase digestion, claimed 84% removal of carbohydrate from rabbit anti-sheep red blood cell IgG, and obtained the following results: no effect was found on haemagglutination, significant effects were noted on rosette formation, antibody-dependent cell-mediated cytotoxicity, and complement fixation. Winkelhake *et al.* (1980) have described the use of an endoglycosidase which was claimed to remove all the complex-type oligosaccharides from IgG in a single step, resulting in a loss of C1q-binding activity. The enzyme used was β-aspartyl *N*-acetylglucosaminyl amidohydrolase from hen oviduct, isolated as described by Tarentino and Maley (1969). However, in this latter paper the authors clearly show that the specificity of their enzyme is such that for the Asn–GlcNAc linkage to be cleaved the Asn must possess both free amino and carboxy termini–addition of amino acid(s) at either end totally prevents activity. In view of this it is unlikely that it could act on an Asn–GlcNAc linkage in a whole protein, and therefore claims of deglycosylation of intact IgG by this method must be viewed with caution.

Claims of a role for the oligosaccharides in facilitating hepatic uptake of immune complexes (Thornberg *et al.*, 1980) have recently been withdrawn (Baynes, 1983). Nose and Wigzell (1983) have recently reported that aglycosylated mouse IgG2b possessed significantly impaired activity in cell binding and complement fixation. In addition, immune complexes formed from aglycosylated IgG were removed comparatively slowly from the circulation, although no indication was given as to whether the aggregate size remained the same in each case.

In this paper we describe the interaction of an aglycosylated monoclonal anti-DNP mouse IgG2a with C1q, C1 and human monocytes. The results show that while no difference is observed in the binding capacity for C1q, the dissociation constant for the interaction is increased approximately threefold. This difference in C1q binding accounts for the reduction in aglycosylated IgG2a binding to C1 (30% bound compared with 42% for native IgG2a) and the consequent decrease in the rate of C1 activation under the assay conditions used (1 nM C1). That C1 is activated however, by aglycosylated IgG2a suggests that the oligosaccharide has no major role in either the binding or activation of this component. It should be noted that the slight difference in C1q binding constant which occurs when the oligosaccharide is absent leads to differences in binding being most noticeable at low C1q concns. Since it is usually at low effective C1q concns that whole com-

plement fixation assays are performed, this may lead to the differences noted by other workers when assaying their carbohydrate-depleted IgG (Koide *et al.*, 1977; Nose and Wigzell, 1983). In addition this effect could be magnified by the nature of the complement cascade process. This indicates the necessity of performing a complete binding curve and using individual components if a meaningful comparison of binding ability is to be made.

The domain of IgG responsible for monocyte binding is controversial (Okafor *et al.*, 1974; Barnett-Foster *et al.*, 1980; Ciccimarra *et al.*, 1975; Abramson *et al.*, 1970; Holm *et al.*, 1974; Yasmeen *et al.*, 1976; Ovary *et al.*, 1976; Birshtein *et al.*, 1982). Recently we have shown that an extensively immunoaffinity purified pFc′ does not inhibit the IgG monocyte interaction and that earlier reports of pFc′ inhibition probably arise from minor (<0.1%) IgG contamination (Woof *et al.*, 1984). We also find that human myeloma IgGs with either C_H2 or C_H3 domain deletions are unable to bind to monocyte Fc receptor (Woof *et al.*, 1984). These results indicate that in contrast to C1q binding where the site seems to be accountable for by structures located entirely on a single domain (Colomb and Porter, 1975; Painter *et al.*, 1982; Burton *et al.*, 1980) monocyte Fc receptor binding requires a greater level of structural integrity if the binding site is to be maintained.

In our study the interaction of mouse IgG2a with human monocyte Fc receptor is markedly affected by the loss of the C_H2 domain oligosaccharides. The loss of monocyte-binding activity may occur by several mechanisms. First, the aglycosylated IgG may have partially or wholly lost the necessary binding site (i.e. the oligosaccharide itself or a structure maintained or caused by its presence). Second, it is possible that the aglycosylated IgG is degraded during the incubation period. In common with many aglycosylated proteins (Olden *et al.*, 1982) the susceptibility of IgG to proteolysis is markedly increased (>60-fold) upon removal of the carbohydrate (Leatherbarrow, 1983). It is well known that cells of the mononuclear phagocyte series secrete proteases (Schneider and Baggiolini, 1978; Adams, 1981; Werb, 1981)—indeed proteolysis of IgG by macrophages has been observed, and implicated in B-cell activation (Morgan and Weigle, 1980; Morgan *et al.*, 1982). The integrity of the IgG under the conditions used here was determined by incubating [^{3}H-leu]–IgG with monocytes under assay conditions. At the end of the incubation period the IgG was examined by SDS–PAGE and the bands located by autoradiography. It was found that no detectable proteolysis of aglycosylated IgG had occurred during the assay (results not shown). Third, it is possible that aglycosylated mouse IgG2a binds to the human monocytes at a different site from human IgG and therefore is no longer able to displace it. Incubation of aglycosylated [^{3}H-leu]–IgG2a alone with the monocytes was performed and the amount bound determined directly. These binding data are given in Table 1. These results show that there is less direct binding of aglycosylated IgG than glycosylated IgG to the monocytes. We have therefore concluded that the effects observed are due primarily to a reduction in the strength of the interaction of aglycosylated IgG with monocyte Fc receptor.

That cross-species effects are probably not important in this assay is reflected in the similar affinities of human monocytes for human IgG1, human IgG3, rabbit (Woof *et al.*, 1984) and mouse IgG2a. The differences in oligosaccharides between the species and the oligosaccharide heterogeneity within the monoclonal IgG2a itself would, at the outset, suggest that the oligosaccharide is not the main structural determinant in the binding of IgG to monocyte Fc receptors. Indeed preliminary data using glycopeptides at concns up to 45 μM confirm that monomeric oligosaccharides will not act as inhibitors. However it should be noted that the Fc contains two interacting oligosaccharides. It is possible that the essentially *dimeric* interaction between the oligosaccharide from each C_H2 domain results in or contributes to a monocyte-binding site.

The reduction in human monocyte binding observed here is also consistent with that reported for the binding of deglycosylated rabbit IgG to human monocyte Fc receptors (Koide *et al.*, 1977). Nose and Wigzell (1983) also report a loss in activity for interaction of aglycosylated IgG2b with mouse macrophage Fc receptors—although the Fc receptors for mouse and human are not necessarily the same. For the present, we conclude that the decreased monocyte Fc receptor affinity caused by aglycosylated IgG2a is compatible with an effect on the overall Fc structure in such a way as to perturb the Fc receptor binding

Table 1. Direct binding of [^{3}H-leu]–IgG (glycosylated and aglycosylated) to human monocytes[a]

	% binding	"Specific" binding
[^{3}H]glycosylated	22.4 ± 2.0	10.7 ± 2.4
[^{3}H]glycosylated + excess normal IgG2a	11.7 ± 0.4	
[^{3}H]aglycosylated	15.5 ± 1.3	3.5 ± 1.4
[^{3}H]aglycosylated + excess normal IgG2a	12.0 ± 0.1	

[a]The results are from duplicate experiments. The "specific" binding is given as the amount bound minus the amount bound in the presence of excess normal IgG (i.e. minus the non-specifically bound or entrapped IgG). Approximately the same concns of glycosylated and aglycosylated IgG were used.

site. It cannot be ruled out however that the oligosaccharide is not playing some more positive role in the interaction. It is not possible from our studies to determine the extent of any structural perturbation arising from aglycosylation, but the continued binding of C1q and protein A (Leatherbarrow and Dwek, 1983; Nose and Wigzell, 1983) by aglycosylated IgG indicates that any alterations may be quite subtle. These observations are in accord with previous data (Klein *et al.*, 1981; Woof *et al.*, 1984) from which it appears that Fc receptor–IgG interactions are more sensitive to structural perturbations in IgG than are C1q–IgG interactions. The differential effect of aglycosylation on C1q and monocyte Fc receptor binding is therefore further evidence for different sites on IgG for these two receptor systems. One speculative explanation for the rather different effects would be for the monocyte Fc receptor binding site to be located across domains, i.e. involving C_H2–C_H2. Such an arrangement may be expected to display more sensitivity to slight structural perturbations than a site located on a single domain. Further interpretations of these results must however await a more detailed mapping of the Fc receptor site.

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