Glycoconjugate Journal 16, 801–807, 1999

Lectin analysis of human immunoglobulin G N-glycan sialylation

Martin Dalziel[1], Ian McFarlane[1] and John S. Axford[2]

[1]*ICRF Breast Cancer Biology Group, Thomas Guy House, Guy's Hospital, London, SE1 9RT, UK,* [2]*Academic Unit for Musculoskeletal Disease, St George's Hospital Medical School, London SW17 ORE, UK*

The lectins *Sambucus nigra* agglutinin (SNA) and *Ricinus communis* agglutinin (RCA), specific for α2,6 linked sialylation, and terminal galactose respectively were used to study the occurrence, linkage and distribution of human immunoglobulin G (IgG) sialylation. SNA was shown to bind N-glycan α2,6-linked sialic acid only. Sialidase analysis confirmed that this is the dominant, if not exclusive linkage. Total IgG sialylation was estimated at 1.0 μg SA/mg IgG (or about 0.5 mole per mole) using a biochemical sialic acid assay. SNA displayed strong binding to the IgG Fab fragment in both its native and denatured state. In contrast, SNA failed to bind the IgG Fc fragment in its native form, but displayed strong binding after the Fc was denatured. This allowed the construction of quantitative assays capable of measuring both IgG Fab and Fc α2,6-sialylation without the need for enzymatic peptide digestion.

Keywords: **IgG, Fab, Fc, lectin, sialic acid**

Abbreviations: **AU: *Athrobacter ureafaciens*; BME: β-Mercaptoethanol; BSA: Bovine serum albumin; CP: *Clostridium perfringens*; DTT: Dithiothreitol; ECL: Enhanced chemiluminescence; MAA: *Maackia amurensis* agglutinin; Neu5Ac: N-acetyl neuraminic acid; NDV: Newcastle disease virus; PAGE: Polyacrylamide gel electrophoresis; PBS: Phosphate buffered saline; PTB: 1 x PBS/0.2% Tween 20/1% BSA; PNGaseF: Peptide-N-glycosidase F; RCA: *Ricinus communis* agglutinin; RT: Room temperature; SA: Sialic Acid; SDS: Sodium dodecyl sulphate; SHRP: Streptavidin-horseradish peroxidase; SNA: *Sambucus nigra* agglutinin; TFA: Trifloroacetic acid**

Introduction

Human serum polyclonal IgG consists of, on average, 2.5 N-linked diantennary complex oligosaccharides, two of which are located and highly conserved at asparagine 297 in the Cγ2 domain of the Fc region. Further glycosylation in the Fab region is dependent on variable chain amino acid usage [1].

Sialic acid (SA) occurs exclusively in the N-acetyl neuraminic acid (Neu5Ac) form in humans [2]. The absence of the N-glycolyl neuraminic acid (Neu5Gc) form is due to a partial deletion of the CMP-NeuAc hydroxylase gene [3]. SA is found as the terminal monosaccharide residue in approximately 25–30% of IgG oligosaccharides [1,4]. This low level of terminal sialylation is unusual for a serum glycoprotein where even a partial loss of SA leads to the removal of the glycoprotein via the hepatic asialo-glycoprotein receptor [5]. Analysis of the distribution of sialic acid between the Fab and Fc glycosylation sites shows that in fact the 'exposed' Fab oligosaccharides are normally sialylated and it is only the 'hidden' Fc oligosaccharides which are hyposialylated [1,4,6].

The use of lectins has become increasingly widespread as a convenient, simple and inexpensive method of analysing the oligosaccharide component of various glycoproteins. In particular, lectins such as RCA have been utilised to routinely measure serum IgG hypogalactosylation in patients with rheumatoid arthritis [7]. As SA is a residue that occurs immediately after galactose on N-linked oligosaccharide chains, some studies have attempted to use the lectin SNA [8] in order to gain information about IgG sialylation relative to disease associated IgG hypogalactosylation changes. However, no attempt has been made to understand the nature of SNA binding to serum IgG. This study addresses the issue

*To whom correspondence should be addressed: Martin Dalziel, ICRF Breast Cancer Biology Group, Thomas Guy House, Guy's Hospital, London SE1 9RT, United Kingdom. Tel: + 44 171 955 4877; Fax: + 44 171 955 2026; E-mail: M.Dalziel@icrf.icnet.uk

of lectin sugar residue and linkage specificity of SNA [9] and RCA when used for the study of IgG glycosylation.

Materials and methods

Materials

Biotinylated lectins, *Sambucus nigra* agglutinin, *Maackia amurensis* agglutinin (MAA), and *Ricinus communis* agglutinin were from Vector Laboratories Ltd (Peterborough, UK). Peptide-N-glycosidase F (PNGaseF), *Clostridium perfringens* (CP), *Athrobacter ureafaciens* (AU) and Newcastle Disease Virus (NDV) sialidases were from Oxford Glycosciences (Abingdon, UK). Streptavidin-horseradish peroxidase and enhanced chemiluminescence (ECL) detection system were from Amersham (Amersham, UK). Fab and Fc fragments, isolated from papain digestion of human polyclonal IgG, derived from the pooled serum of a large healthy population ($n > 1000$), were from Chemicon (Harrow, UK). Bovine serum albumin (BSA). Tween 20, standard polyclonal human serum IgG, Neu5Ac, human transferrin, bovine fetuin, phosphate buffered saline (PBS) tablets (pH 7.4), β-mecarptoethanol (βME) and dithiothreitol (DTT) were from Sigma (Poole, UK).

Detection of lectin binding to IgG, Fab and Fc using immunoblotting

10 µg of IgG was loaded on a mini 12.0% SDS-polyacrylamide gel (SDS-PAGE), with 4.0% stacking gel in either a reduced or native state. Reduction was achieved by boiling (5 min) in reducing buffer (100 mM βME/60 mM Tris (pH 6.8)/2.0% sodium dodecyl sulphate (SDS)/10.0% glycerol/0.01% bromophenol blue). Native samples were loaded using native buffer (60 mM Tris - pH 6.8/2.0% SDS/10.0% glycerol/0.01% bromophenol blue) without boiling. SDS-PAGE gels were run for 40-60 minutes (until bromophenol blue marker reached the gel edge) at 200 volts in Tris-Glycine buffer. The gel was then blotted onto nitro-cellulose in Towbin buffer using a Bio-Rad semi-dry electroblotter, 12 volts for 60 min, and subsequently stained with ponceau S in order to identify individual protein bands. The blot was then blocked overnight at 4°C in 1 × PBS (pH 7.4)/0.2% Tween 20/1.0% BSA (PTB solution).

The blot was then incubated with biotinylated SNA or RCA (5 µg/ml in 20 ml PTB solution), for 1 hour at room temperature (RT) with gentle agitation, washed three times (15 min each) with 50 ml of PTB solution, incubated for 1 hour at RT with streptavidin-horseradish peroxidase (SHRP, 1/2000 dilution in 20 ml PTB solution), washed three times as before, and then bound lectin-SHRP detected using ECL (10 ml, 60 seconds) and light sensitive film. Paired Fab and Fc fragments (10 µg) were also run on 12.0% SDS-PAGE (4.0% stack), under native and reducing conditions (as above), blotted and probed with SNA or RCA (all at 5 µg/ml in PTB solution).

Quantitation of IgG sialylation using SNA and RCA

In order to assay IgG SA and galactose residues quantitatively before and after sialidase digestion, linear conditions were established using different amounts of IgG, pre-treated using different conditions (reduction, partial reduction and native) were dot-blotted onto pre-soaked nitro-cellulose and blocked overnight at 4°C in 50 ml PTB. The blots were then incubated with biotinylated SNA or RCA (5 µg/ml in 20 ml PTB solution), for 1 hour at RT, washed three times with 50 ml of PTB solution, incubated for 1 hour at RT with streptavidin-horseradish peroxidase at different concentrations, washed three times and then bound lectin-HRP detected using ECL. Sample intensities were quantitated using a densitometer (Collage software for AppleMac).

Inhibition of lectin binding using acid hydrolysis

Commercial IgG (500 µg) was treated with 0.1 M trifloroacetic acid (TFA) for 1 hour at 80°C. Sample was then dialysed against water overnight at 4°C, after which the sample was lyophilised and protein estimation made using a BioRad BCA assay. Samples, before and after TFA digestion, were analysed by SDS-PAGE immunoblotting and quantitative dot blotting, probed with SNA or RCA as described above.

Inhibition of lectin binding to IgG using sialidase digestion

Human serum polyclonal IgG was incubated at 37°C for 18 hours with NDV, CP or AU sialidase in sodium acetate buffer (50 mM/pH 5.0 and 100 mM/pH 5.5 respectively). IgG in sialidase buffer without sialidase was used as a negative control. Transferrin incubated with CP or AU and Fetuin incubated with CP or NDV sialidase were used as positive controls and then probed with either SNA or MAA. Samples, before and after sialidase digestion, were analysed by SDS-PAGE lectin blots and quantitative dot blotting, probed with SNA or RCA as described above.

Quantitation of released sialic acid by modified Aminoff assay

Sialic acid was quantitated using a modification of the Aminoff assay [10]. Briefly, to each of the sialidase treated and untreated samples 50 µl of 25 mM HIO_4 in 62.5 mM H_2SO_4 was added and incubated for 30 min at 37°C. Next, 50 µl of 2% sodium arsenite in 0.5 M HCl was added followed by 100 µl of 6% thiobarbituric acid, the tubes were then heated at 100°C for 7.5 min and then cooled on ice. Then 400 µl of dimethyl sulphoxide was added and the absorbance at 552 nm was measured. Commercial Neu5Ac was used as an internal standard curve and reference point for quantitation.

Effect of PNGaseF digestion on lectin binding to IgG

500 µg of standard human serum polyclonal IgG was denatured by boiling in 2.5% SDS, 5% βME, in 20 mM sodium phosphate (pH 7.5), 50 mM EDTA, 0.02% sodium

azide for 5 min. After cooling to room temperature, 50 μg of this denatured IgG was then treated with a total of 2.5 units of PNGaseF in 20 mM sodium phosphate (pH 7.5), 50 mM EDTA, 0.02% sodium azide, 0.8% Nonidet p40 at 37°C for 18 hours. Denatured IgG in 20 mM sodium phosphate (pH 7.5), 50 mM EDTA, 0.02% sodium azide, 0.8% Nonidet p40 without PNGaseF was used as a negative control. Samples were then slot blotted and probed with SNA and RCA as described above.

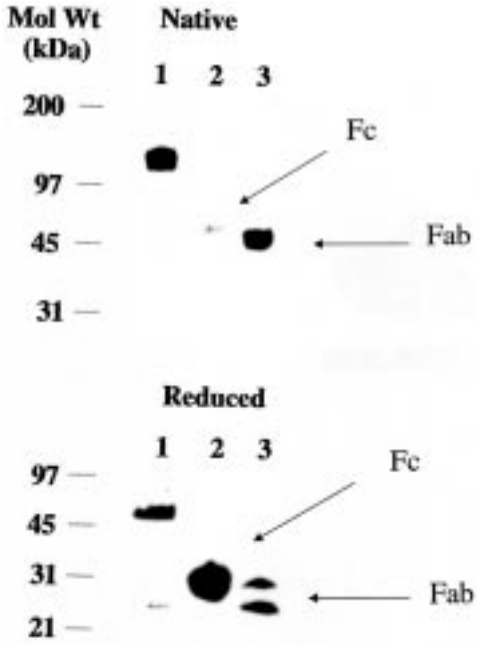

Figure 1. SNA binding to IgG Fab and Fc is dependent on IgG quaternary structure 10 μg of IgG, Fab and Fc were run on a 12% polyacrylamide gel, transferred to nitro-cellulose and probed with SNA as described. A (non reducing conditions). Lane 1, IgG; lane 2, Fc fragment, lane 3, Fab fragment. Molecular weight markers in kilodaltons (kDa) are shown. B (reducing conditions). lane 1, IgG; lane 2, Fc fragment; lane 3, Fab fragment. Molecular weight markers (kDa) are shown.

Results

SNA and RCA binding to IgG, Fab and Fc under native and denaturing conditions

SNA recognises predominantly the heavy chain of polyclonal IgG, although light chain binding was also observed (Figure 1). SNA recognised only the native Fab fragment, whilst negligible native Fc binding was seen (Figure 1). SNA displayed strong staining intensity to both Fc and Fab fragments after they had been reduced. Pre-treatment of Fc with 1.0% DTT or 1.0% βME alone without boiling was found to be sufficient to allow SNA binding to Fc α2,6 sialylation. This staining was to the Fc at its unreduced molecular weight implying the absence of complete disulphide bond disruption (Figure 2). Over 90% of RCA binding was to the Fc, regardless of conditions used, relative to the Fab (Figure 3). i.e. most of the galactosyl residues 'visible' to RCA reside in the Fc of IgG.

Quantitative SNA and RCA assays using native IgG

Native IgG blotted directly to Nitro-cellulose in PBS, representative of Fab sialylation when probed with SNA or

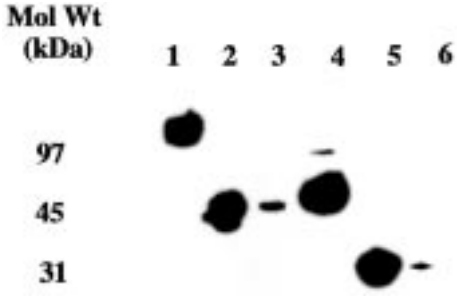

Figure 3. RCA recognises predominantly Fc galactosylation independent of IgG conformation. 10 μg of IgG, Fab and Fc were run on a 12% polyacrylamide gel, transferred to nitro-cellulose and probed with RCA as described, lane 1, native IgG; lane 2, denatured IgG; lane 3, native Fab; lane 4, native Fc; lane 5, denatured Fc; lane 6, denatured Fab. Molecular weight markers (kDa) are shown.

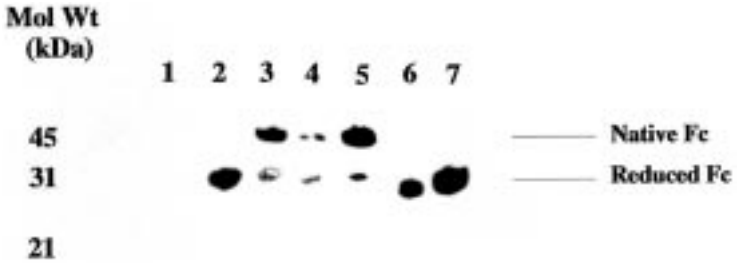

Figure 2. SNA recognises Fc sialylation under partial reducing conditions. 10 μg of Fc pretreated using a variety of conditions, ranging from native to reducing, then run on a 12% polyacrylamide gel, transferred to nitro-cellulose and probed with SNA as described, lane 1, native Fc; lane 2, Fc pretreated with 1.0% βME and boiling; lane 3, Fc pretreated with 0.1% DTT without boiling; lane 4, Fc pretreated with 0.1% βME without boiling; lane 5, Fc pretreated with 1.0% DTT without boiling; lane 6, Fc pretreated with 1.0% βME with boiling; lane 7, Fc pretreated with 1.0% DTT with boiling. Molecular weight markers (kDa) are shown.

RCA, could be quantitated in the 0.0–1.0 μg range (SNA) or 0.0–0.2 μg range (RCA) as described using the lectins at 5 μg/ml and S-HRP at 1/250 dilution. Co-efficient of variance (for SNA), based on quantitating the ability to differentiate between two samples on five separate occasions, was 8.6%.

Creation of denaturing IgG-SNA assay representative of total IgG α2.6 SA

IgG in 1.0% DTT boiled for 5 min, consistently displayed an approximate doubling of staining with SNA, linear range 0.0–0.6 μg, relative to the native assay. This assay used SNA at 5 μg/ml and S-HRP at 1/250 dilution. Co-efficient of variance, based on quantitating the ability to differentiate between two samples on five separate occasions, was 12.1%. Boiling alone reduced SNA staining by over 70% and 1.0% DTT treatment alone, without boiling, had a moderate inhibitory effect on SNA staining to IgG, relative to the native assay (Figure 4). When native and denaturing conditions were applied to SNA binding to the Fab and Fc (0.0 - 1.0 μg standard curves in both cases), the denaturing assay had no effect on the Fab standard curve, but dramatically increased the SNA staining to the Fc from essentially zero to quantitative equivalence with the Fab (Figure 4). Using three separate commercial IgG samples, the average increase in SNA binding to 0.25 and 0.50 μg IgG using 1.0% DTT and boiling, relative to native conditions, was approximately 1.9 and 1.6 fold respectively (data not shown) and 2.5 and 2.0 relative to DTT alone.

These data, taken together, indicate that the increase in SNA staining to IgG after pre-treatment with 1.0% DTT and boiling is due to exposure of the Fc sialylation. This increase is not an artefact of the assay conditions as both boiling and 1.0% DTT alone is actually inhibitory.

Thus IgG can be assayed for both Fab and total (Fab and Fc) sialylation using SNA, although the inhibitory nature of DTT itself as a potential complicating factor must be noted.

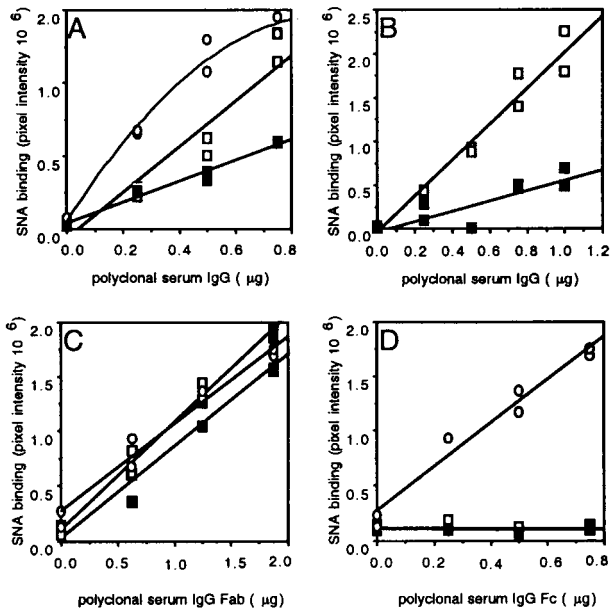

Figure 4. Pre-treatment of IgG with 1.0% DTT and boiling, allows SNA quantitation of both Fab and Fc α2,6 sialylation. A. Effect of boiling IgG in 1.0% DTT before SNA staining. Pretreatment of the IgG sample by boiling in 1.0% DTT gave (open circles), on average, an approximate two fold increase in SNA binding to IgG in the 0.0 - 0.5 μg range relative to native conditions (open boxes). Taking into account the modest inhibitory effect of DTT itself on SNA staining (closed boxes), pretreatment of IgG in 1.0% DTT with boiling increases SNA by over two fold as predicted from the Fab / Fc westerns (see figures 1 and 2). B. Sample boiled in PBS alone reduces SNA staining to IgG by over two fold, native (open boxes), boiled (closed boxes). C. SNA staining to Fab is unaffected by DTT treatment. Fab pretreated by boiling in 1.0% DTT (open circles) or 1.0% DTT alone (closed boxes) showed no difference in quantitative SNA binding relative to untreated native (open boxes). D. SNA staining to Fc is dependent upon 1.0% DTT and boiling pretreatment. SNA binding to native Fc (open boxes) or Fc pretreated with 1.0% DTT alone (closed boxes) was negligible relative to Fc pretreated by boiling in 1.0% DTT (open circles).

Effect of TFA hydrolysis on lectin binding to IgG

SNA but not RCA binding to human IgG was abolished after TFA treatment. RCA binding to IgG was slightly enhanced after TFA treatment (figure 5). TFA inhibition demonstrates that SNA and recognises only the SA component of IgG N-glycans.

Effect of CP, AU and NDV sialidase digestion on lectin binding to IgG

SNA binding to IgG was abolished after pre-treatment of the IgG with AU (α2, 6 preferential cleavage) and CP (cleaves both α2,6 and α2,3-SA) but not NDV (cleaves only α2,3-SA) sialidase (Figure 6). SNA binding to control glycoprotein Transferrin was also abolished by CP sialidase (data not shown). As a control for NDV, the binding of the α2,3-SA specific lectin MAA [11] to fetuin was inhibited by over 70% after NDV digestion (data not shown). These data demonstrate that SNA recognises α2,6 and not α2,3 linked SA on IgG. RCA binding to IgG was used as a sialidase residue specificity control. RCA binding to IgG was slightly increased after CP sialidase pre-digestion but unchanged with AU sialidase pre-treatment (data not shown).

Quantitation of sialidase IgG sialic acid release using Aminoff assay

TFA treatment released, on average, 1.05 μg SA/mg IgG, using the TFA control as a baseline. Using the TFA estimation as the estimate for total IgG sialylation, average sialidase efficiencies were estimated, CP sialidase digestion released 70% of the IgG SA residues (1.05 μg SA/mg IgG) whilst AU digestion was even more effective, releasing on average around 85% of the residues (1.05 μg SA/mg IgG). In contrast, NDV had little effect, releasing around 10% of the residues relative to TFA. It must be noted that the figure of 10% for

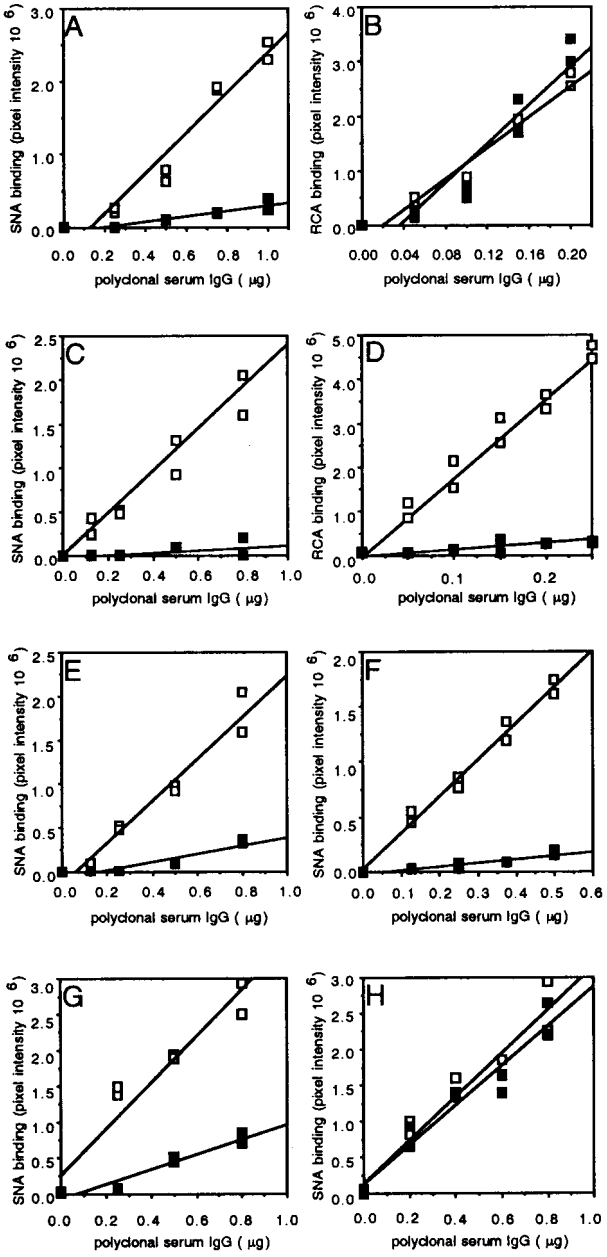

Figure 5. Determination of SNA and RCA IgG binding specificities Standard human IgG was pretreated using TFA, PNGaseF and CP (α2,3 and α2,6 SA), AU (α2,6 SA preferred) or NDV (α2,3 SA only) sialidases as described in materials and methods. A. Quantitative SNA binding to IgG (native assay) treated with (filled box) and without (open box) TFA. B. Quantitative RCA binding to IgG (native assay) treated with (filled box) and without (open box) TFA. C Quantitative SNA binding to IgG treated with (filled box) and without (open box) PNGaseF. D. C. Quantitative RCA binding to IgG treated with (filled box) and without (open box) PNGaseF.E. Quantitative SNA binding to IgG (native assay) treated with (filled box) and without (open box) AU sialidase. F. Quantitative SNA binding to IgG (1.0% DTT denaturation assay), treated with (filled box) and without (open box) AU sialidase. G. Quantitative SNA binding to IgG (native assay) treated with (filled box) and without (open box) CP sialidase. H. Quantitative SNA binding to IgG (native assay) treated with (filled box) and without (open box) NDV sialidase.

NDV digestion was at the lower limit of this Aminoff assay standard curve and thus must be treated with caution. As a control NDV released around 50% fetuin SA residues relative to TFA hydrolysis. These figures correlate well with the observed changes in SNA staining described above. Further, the total SA yield from TFA hydrolysis of IgG is consistent with previous estimation of IgG SA content [12] and the theoretical yield based on 25% sialylation of an average 2.5 N-glycans per IgG molecule [1].

Effect of PNGaseF digestion on lectin binding to IgG

Predigestion of standard human IgG with PNGaseF abolished both SNA and RCA binding to standard human IgG (Figure 5), demonstrating that both the α2,6-SA and galactose residues recognised are both components of IgG N-glycan chains.

DISCUSSION

Using sialidase, PNGaseF and TFA analysis, we have demonstrated that SNA recognises only α2,6-linked sialylation on IgG N-glycans. However, this binding is partially dependent on pre-reduction of the IgG. When IgG is in its native state, only the Fab α2,6-sialylation is recognised by SNA, whilst when IgG is subjected to reducing conditions, both Fab and Fc α2,6-SA residues are made available for SNA binding. Interestingly, even partial reduction that did not separate the component γ chains of Fc still allowed SNA access to these cryptic α2,6-sialylation residues.

SNA recognition of the sequence SA-α2,6-galactose on N-glycans is dependent on the galactose hydroxyl groups at carbon 3 and 4, as well as the SA-α2,6 region [9,13]. Our data has demonstrated that RCA recognition of Fc galactose residues is independent of the IgG conformation. This suggests that, as galactose residues are available for RCA recognition, and assuming both lectins recognise similar exposed regions of galactose, it is the SA portion of the SA-α2,6-galactose sequence that is hidden from SNA recognition.

The observation that SNA can bind Fc α2,6-SA, using mild reducing conditions that do not dissociate the Fc quaternary structure, suggests that these residues are either in solvent but masked from lectin recognition by the Fc quaternary (e.g. reduction of one of the two Fc disulphides may be sufficient to induce a quaternary structure alteration) or, alternatively, they are not in solvent at all, i.e. Fc SA residues associate with the protein. Clearly, the location of these α2,6-SA residues within the Fc is important in understanding why SNA cannot recognise native Fc α2,6-SA.

SNA Fab/Fc staining patterns suggest that IgG contains comparable levels of Fab and Fc α2,6 sialylation. This is to be expected given the greater frequency with which Fab oligosaccharides are sialylated. The Fab has fewer of the sialylation constraints seen in the Fc. Consequently, both mono and di-sialylated N-glycans, both utilising α1,3 and α1,6 arms, are seen in the Fab in contrast to the Fc mono-sialylated

α1,3 arm restriction [14]. SNA staining revealed that Fab light chain contains as much α2,6-sialylation as Fab heavy chain in a normal population. This would indicate that at least two independent Fab N-glycan sites are utilised in this population, one on each chain. In order to estimate the assumed dominance of α2,6-sialylation on IgG, Aminoff assay SA quantitative analysis of IgG sialidase digestion was used. This assay revealed that Neu5Ac reduction post sialidase treatment correlated with loss of SNA binding. This α2,6 domination, or even exclusivity, over α2,3-sialylation is consistent with the known bias that di-antennary N-glycans show towards CMP-NeuAc:Galβ1,4GlcNAc-α2,6 sialyltransferase (ST6Gal I) [15] recognition over the α2,3-sialylating enzymes [16]. Further, the high expression level of ST6Gal I in B-lymphocytes, relative to N-glycan α2,3-sialyltransferases [17], may also contribute.

This phenomenon can also be seen with human IgG monoclonal studies where antibodies have been produced from CHO cells, containing unusually low levels of sialylation (5% and 0% respectively) on their N-glycans [18, 19]. The CHO cell N-glycan repertoire contains only the α2,3-sialylated form due to an inability to express ST6Gal I [20]. Further, studies on human monoclonal IgG from myeloma has found only the α2,6 Neu5Ac form of SA on the N-glycans of both Fab and Fc [21].

Thus, although the potential for human IgG N-glycan α2,3 SA exists, as suggested by some indirect observations [22, 23], it is probably as a minority, if expressed at all, to the α2,6 SA linkage, which, it must be remembered, is itself a minor component of the IgG N-glycosylation.

The dual nature of the SNA-IgG recognition allowed the construction of two quantitative assays for IgG α2,6-sialylation. The first, using native IgG, permits the measurement of Fab α2,6-SA residues, whilst using denatured IgG, both Fab and Fc α2,6-sialylation can be quantitated, from which Fc levels can be deduced. This is important as past attempts at IgG sialylation quantitation, using SNA, were attempted with native IgG (Fab α2,6-SA) and the resultant data mistakenly assumed to represent total IgG sialylation [8, 24].

Acknowledgements

This work was supported by The European Community Concerted Action for Arthritis and Carbohydrate Research.

REFERENCES

1 Rudd PM, Leatherbarrow RJ, Rademacher TW, Dwek RA, Diversification of the IgG molecule by oilgosaccharides, *Molecular Immunology* **28**(12), 1369–78 (1991).
2 Schauer R, Biosynthesis and function of N- and O-substituted sialic acids, *Glycobiology* **1**(5), 449–52 (1991).
3 Irie A, Koyama S, Kozutsumi Y, Kawasaki T, Suzuki A, The molecular basis for the absence of N-glycolyneuraminic acid in humans, *J Biol Chem* **273**, 15866–71 (1998).
4 Rademacher TW, Dwek RA, Structural, functional, and conformational analysis of immunoglobulin G derived asparagine linked oligosaccharides, *Progress in Immunology* V, 95–112 (1983).
5 Iobst S, Drickamer K, Selective sugar binding to the carbohydrate recognition domains of the rat hepatic and macrophage asialoglycoprotein receptors, *J Biol Chem* **271**, 6686–93 (1996).
6 Rademacher TW, Network theory of glycosylation - etiologic and pathogenic implications of changes in IgG glycoform levels in autoimmunity, *Seminars in Cell Biology* **2**, 327–37 (1991).
7 Sumar N, Isenberg DA, Bodman KB, Soltys A, Young A, Leak AM, Round J, Hay FC, Roitt IM, Reduction in IgG galactose in juvenile and adult onset arthritis by a lectin binding method and it's relationship to rheumatoid factor, *Annals of Rheumatic Disease* **50**, 607–10 (1991).
8 Bond A, Jones MG, Hay FC, Human IgG preparations isolated by ion-exchange or protein G affinity chromatography differ in their glycoform profiles, *J Immunol Methods* **166**, 27–33 (1993).
9 Shibuya N, Goldstein IJ, Broekaert WF, Nsimba-Lubaki M, Peeters B, Peumans WJ, The Elderberry (*Sambucus nigra* L.) bark lectin recognises the Neu5Ac(α2–6)Gal/GalNAc Sequence, *J Biol Chem* **262**(4), 1596–1601 (1986).
10 Skoza L, Mohos S, Stable thiobarbituric acid chromophore with dimethyl sulphoxide, *Biochem J* **159**, 457–62 (1976).
11 Wang WC, Cummings RD, The immobilized leukoagglutinin from seeds of *Maackia amurensis* binds with high affinity to complex type Asn linked oligosaccharides containing terminal sialic acid linked α2–3 to penultimate galactose residues, *J Biol Chem* **263**, 4576–85 (1988).
12 Pekelharing JM, Hepp E, Kamerling JP, Gerwing GJ, Leijnse, Alterations in carbohydrate composition of serum IgG from patients with rheumatoid arthritis and from pregnant women, *Annals Rheum Dis* **47**, 91–5 (1988).
13 Fischer E, Brossmer R, Sialic acid-binding lectins: submolecular specificity and interaction with sialoglycoproteins and tumour cells, *Glycoconjugate J* **12**(5), 707–13 (1995).
14 Wormald MR, Rudd PM, Harvey DJ, Chang SC, Scragg IG, Dwek RA, Variations in oligosaccharide-protein interactions in immunoglobulin G determine the site specific glycosylation profiles and modulate the dynamic motion of the Fc oligosaccharides, *Biochemistry* **36**, 1370–80 (1997).
15 Tsuji S, Datta KA, Paulson JC, Systemic nomenclature for sialyltransferases, *Glycobiology* **6**(7), v–vii (1996).
16 Nemansky M, Schiphorst WECM, Van den Eijnden DH, Branching and elongation with lactosaminoglycan chains of N-linked oligosaccharides result in a shift toward termination with α2,3-linked rather than with α2, 6 linked sialic acid residues, *FEBS Letters* **363**, 280–4 (1995).
17 Augener W, Brittenger G, Schiphorst WECM, van den Eijnden DH, Activities and specificities of N-acetylneuraminyltransferases in leukemic cells, *Haematology and Blood Transfusion* **28**, 135–8 (1983).
18 Boyd PN, Lines AC, Patel AK, The effect of the removal of sialic acid, galactose and total carbohydrate on the functional activity of Campath-1H, *Molecular Immunology* **32**, 1311–8 (1995).
19 Routlier FC, Davies MJ, Bergmann K, Hounsell EF, The glycosylation pattern of humanized IgGI antibody (D1.3) expressed in CHO cells, *Glycoconjugate J* **14**(2), 155–309 (1997).

20 Stanley P, Raju TS, Bhaumik M, CHO cells provide access to novel N-glycans and developmentally regulated glycosyltransferases, *Glycobiology* **6**(7), 695–9 (1996).
21 Savvidou G, Klein M, Grey AA, Dorrington KJ, Carver JP, Possible role for peptide-oligosaccharide interactions in differential oligosaccharide processing at asparagine-107 of the light chain and asparagine-297 of the heavy chain in a monoclonal IgG 1 kappa, *Biochemistry* **23**(16), 3736–40 (1984).
22 Masuda Y, Hishikawa Y, Presence of Lewis x and sialyl Lewis x structures in human IgG, *Med Sci Res* **21**, 823 (1993).
23 Goodarzi MT, Axford JS, Varanasi SS, Alavi A, Cunnane G, Fitzgerald O, Turner GA, Sialyl Lewis(x) expression on IgG in rheumatoid arthritis and other arthritic conditions: a preliminary study, *Glycoconjugate J* **15**(12), 1149–54 (1999).
24 Youinou P, Penee YL, Casburn-Budd R, Dueymes M, Letoux G, Lamour A, Galactose terminating oligosaccharides of IgG in patients with primary Sjogrens syndrome, *J Autoimmunity* **5**, 393–400 (1992).

Received 25 January 2000, revised and accepted 4 March 2000