Glycoconjugate Journal (1996) **13:** 505–511

Aberrant glycosylation of IgA from patients with IgA nephropathy

DIMITRA BAHARAKI[1], MARYVONNE DUEYMES[1], RÉGINE PERRICHOT[2], CHRISTELLE BASSET[1], ROZENN LE CORRE[1], JACQUES CLÈDES[2], and PIERRE YOUINOU[1]*

[1]*Laboratory of Immunology and* [2]*Department of Nephrology, Brest University Medical School Hospital, BP824, F29609 Brest Cedex, France*

Received 30 August 1995, revised 1 November 1995

Despite the prominent role of IgA, particularly IgA_1, in the pathogenesis of IgA nephropathy (IgAN), the precise role of this molecule in the process remains unclear. Four biotin-conjugated lectins in sandwich-type enzyme-linked immunosorbent assays were devised to determine the glycosylation profiles of total IgA and its subclasses. We took advantage of differential binding properties of these lectins to sugar residues to dissect the oligosaccharide chains *O*-linked to the hinge and those *N*-linked to the Fc region of total IgA and IgA subclasses in 47 patients with IgAN and an equal number of controls. The proportion of sialylated IgA_1 was higher in patients compared with controls ($p < 0.02$), whereas IgA_2 in patients appeared less well sialylated. A reduction of galactose in pathological IgA as detected by RCA-I became significant after treatment of the molecule with neuraminidase ($p < 0.01$). Defective galactosylation was also observed for patient IgA_1 when it was probed with ECL, a lectin that has a specificity for Gal 1,4 *N*-acetylglucosamine groupings on *N*-linked oligosaccharides. The RCA and ECL results, therefore, suggest that increased sialylation on the IgA_1 is on *O*-linked oligosaccharides in the hinge region. This was partly confirmed by a small increase in the binding of PNA to IgA_1 from the patient group. This lectin binds preferentially to Gal 1,3 *N*-acetylgalactosamine groups that are found on *O*-linked oligosaccharides.

Keywords: IgA nephropathy, IgA, glycosylation

Introduction

In humans, IgA occurs in two isotypes, IgA_1 and IgA_2, with serum IgA represented predominantly by monomeric molecules of the IgA_1 subclass [1]. First described by Berger and Hinglais [2], IgA nephropathy (IgAN) is now recognized as the commonest type of glomerulonephritis worldwide [3]. Clinical, immunological and histological features of IgAN of the form secondary to Henoch-Schönlein purpura (HSP), are similar to those of the primary type of the disorder [4]. This is characterized by mesangial deposits of IgA, which appears to be mostly IgA_1 [5].

Despite the prominent role of IgA in the pathogenesis of the disease, the causative peculiarities of this molecule remain enigmatic. A number of immunological disturbances resulting in IgA overproduction have been identified [6], but could not be a prerequisite, inasmuch as elevated levels of IgA, IgA-rheumatoid factor and IgA-containing immune complexes do not coincide with mesangial deposition in various settings, such as acquired immunodeficiency syndrome [7] or primary Sjögren's syndrome [8]. Instead, glomerular IgA precipitation could be due to a qualitative abnormality of the molecule. It is noteworthy that the eluted mesangial IgA [9], as well as its plasma counterpart [10], have a restricted anionic charge which could denote changes in their carbohydrate moieties. The structural features of the oligosaccharides are subclass specific [11]. The IgA_1 subclass (Fig. 1) is unusual among glycoproteins in having two types of oligosaccharide linkages to the peptide chain, the *N*-glycosidic linkage of *N*-acetylglucosamine (GlcNAc) to asparagine in the Fc region [12], and the *O*-glycosidic linkage of *N*-acetylgalactosamine (GalNAc) to serine in the hinge region [13]. André *et al.* [14] were the first to show that IgA in IgAN has unusual glycosylation

*To whom correspondence should be addressed.

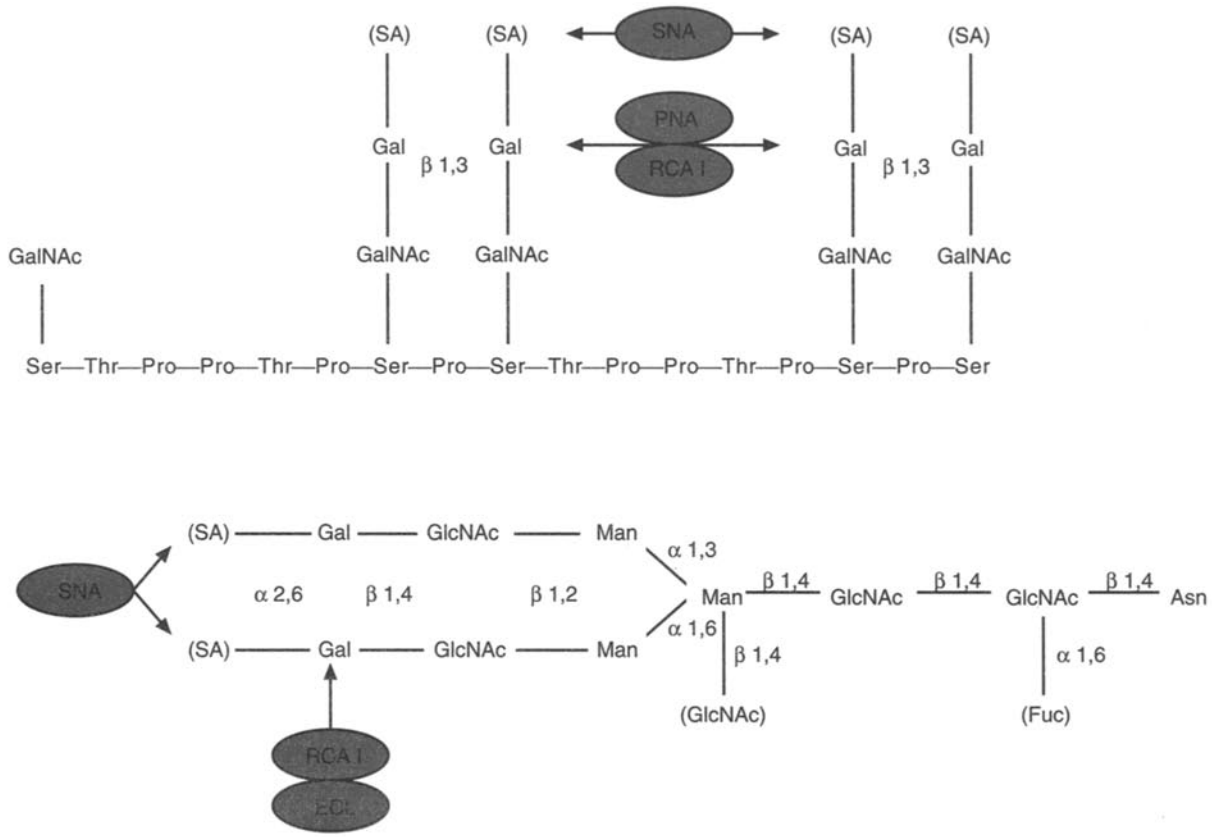

Figure 1. Structure of the *O*-linked and *N*-linked oligosaccharides of IgA_1 and IgA_2. The binding sites of *Sambucus nigra* agglutinin (SNA) to sialic acid (SA), *Ricinus communis* agglutinin I (RCA-I) to galactose (Gal), *Erythrina cristagalli* lectin (ECL) to Gal β1,4 *N*-acetylglucosamine (GlcNAc) and peanut agglutinin (PNA) to Galβ1,3*N*-acetyl-galactosamine (GalNAc) are indicated. The sugar residues in parentheses are not present on all molecules.

demonstrated by reduced binding to jacalin, a lectin specific for galactose (Gal) linked to GalNAc in the β1,3 configuration. Abnormal *O*-linked glycosylation has also been described [15, 16] and associated with raised peripheral blood mononuclear cell β1,3 Gal transferase [16, 17].

In this study enzyme-linked immunosorbent assays (ELISA) were used to further determine the glycosylation profile of total IgA and subclasses by taking advantage of differential binding properties of four lectins to sugar residues [18]. *Sambucus nigra* agglutinin (SNA) which is specific for sialic acid (SA) attached to terminal Gal in the α2,6 and to a lesser degree, α2,3 linkage. *Ricinus communis* agglutinin I (RCA-I) which has an affinity for terminal β1,3 and β1,4 Gal. *Erythrina cristagalli* lectin (ECL) which has a specificity for Gal and the highest binding activity towards Gal β1,4 GlcNAc. Peanut agglutinin (PNA) which binds preferentially to Gal β1,3 GalNAc. In some patients, we found an excessive sialylation of oligosaccharides attached to the Fc region, combined with a defective galactosylation. These abnormalities might affect not only the IgA_1 clearance, but also its glomerular deposition.

Materials and methods

Patients and controls

Serum was obtained from 47 patients with biopsy-proven IgAN. This diagnosis was considered if the immunofluorescence study indicated IgA deposition either in a predominantly mesangial pattern, or equally distributed between the mesangium and the capillary wall. Forty-four patients had the idiopathic form of the disease and the remaining three IgAN secondary to HSP. There were 14 women and 33 men, whose age ranged from 28 to 67 years (mean 41). All patients, but three, had elevated levels of serum IgA. Twelve of them presented end-stage renal failure. Normal controls consisted of 47 healthy subjects who were members of the staff or residents of a home for the elderly. As glycosylation varies with age, they were matched by age and sex with the patients.

Sialylation IgA assay

As depicted in Fig. 2, microtitre plates were coated ($5\ \mu g\,ml^{-1}$) with a polyclonal $F(ab')_2$ anti-human IgA (Jackson Immuno-Research, West Grove, PA), a monoclonal anti-human IgA1 (Boehringer Mannheim,

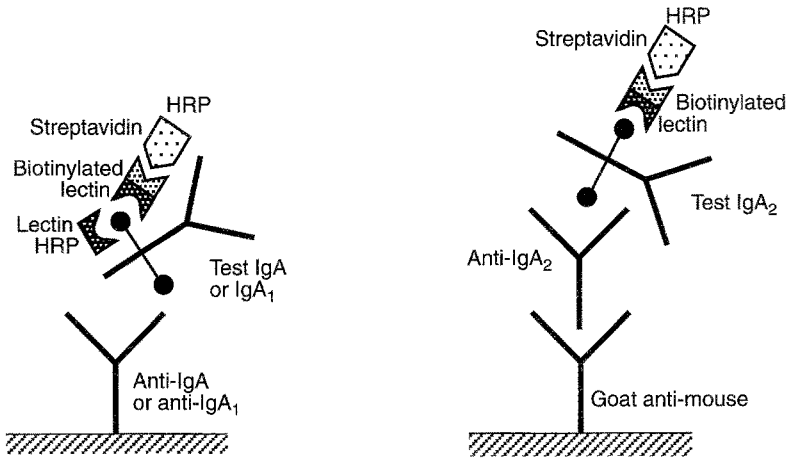

Figure 2. Enzyme-linked immunosorbent assays based on using anti-IgA, anti-IgA_1 or anti-IgA_2 antibodies as capturing agents and horseradish peroxidase (HRP)-labelled lectin or biotinylated lectin with a second layer of HRP-conjugated streptavidin as detecting agents.

Germany), or a polyclonal rabbit $F(ab')_2$ anti-mouse IgG (Dako, Versailles, France) which captured a monoclonal for anti-human IgA_2 (Boehringer). This was carried out in carbonate-bicarbonate buffer, pH 9.7, for 3 h at 30 °C. After coating, each washing step consisted of three washes of phosphate-buffered saline (PBS) supplemented with 0.05% (*v/v*) Tween-20, followed by blotting dry. Plates were blocked with 200 μl of PBS containing 3% (*w/v*) bovine serum albumin by incubating for 90 min at 30 °C.

To reduce the background reaction between the carbohydrate on the capturing antibody and the detecting lectins, the former was exposed to an oxidation step [18]. This treatment opens up the ring structure of the terminal SA and Gal using periodate, as demonstrated by the rapid loss of binding to SNA and RCA-I, respectively (unpublished observations). The degree of oxidation also altered to varying degrees, the binding of the antibodies for IgA. Therefore, a compromise between the time of oxidation and the background lectin reaction was reached. Plates were oxidized with 200 μl of 50 mM sodium periodate in 0.05 M citrate buffer, pH 4, for 10 min, and washed a further five times.

Then, 100 μl of test serum, diluted 1:100 in PBS supplemented with 0.1% (*v/v*) Tween-20 (PBS-T), was added to saturate the plates, and incubated for 60 min at 30 °C. Plates were washed, 100 μl of biotinylated SNA (Vector Laboratories, Burlingame, CA), diluted 1:4000 in PBS-T, was added and incubated for 60 min at 37 °C. After washing, 100 μl of horseradish peroxidase (HRP)-conjugated streptavidin (Amersham, Little Chalfont, UK), diluted 1:4000 in PBS-T supplemented with 3% polyethylene glycol 6000 (Merck, Chelles, France) was added and incubated for 60 min at 37 °C. Plates were washed and incubated with 100 μl of 1,2 *O*-phenylenediamine substrate (Dako) in 0.1 M citric acid-phosphate buffer, pH 5, plus 5 μl of 30% (*v/v*) hydrogen peroxide per 12 ml of substrate. The reaction was stopped with 50 μl of 1.5 M sulfuric acid and the optical density (OD) read at 492 nM using an ELISA spectrophotometer.

A pool of normal sera was passed over a goat $F(ab^1)_2$ anti-human IgA column and the total IgA eluted with glycine-HCl, pH 2.4, and applied to a jacalin column (Vector) which is specific for IgA_1 [19]. The column was washed through several times with Tris-HCl (pH 7.5) and the final effluent contained IgA_2, but not IgA_1. This was established by IgA_1- and IgA_2-specific ELISAs IgA_1 was eluted with 0.8 M galactose in Tris-HCl buffer (pH 7.5). The eluate and the effluent served as positive controls in the IgA_1 and the IgA_2 tests, respectively, and the serum from an IgA-deficient patient (generous gift from Jean-Louis Preud'homme, Poitiers, France) was used as a negative control.

Galactosylation IgA assay

As previously demonstrated by inhibition experiments [20], several lectins specifically identify Gal, provided this sugar residue terminates the oligosaccharide chain. Using the method described for SA, binding of IgA to RCA-I was 80% inhibited by 0.8 mg ml^{-1} Gal, but it was not inhibited by SA or GlcNAc [21]. Biotinylated-RCA-I (Vector) was used, diluted 1:8000 in PBS-T, and after washing away unbound lectin, the bound lectin was detected using a second layer of HRP-conjugated streptavidin in the total IgA and the IgA_2 assays. HRP-labelled ECL (Vector Labs), diluted 1:1000, and HRP-labelled PNA (Makor Chemicals Ltd, Tel Aviv, Israel), diluted 1:500, were used to identify Gal 1,4 GlcNAc in the Fc region and Gal β1,3 GalNAc in the hinge region of IgA_1, respectively. At saturating levels of Ig the lectin binding was proportional to the amount of Ig present (unpublished observations). Whilst these assays detected sugars present on nanograms of Ig (approximately 0.30–0.75 ng

of oligosaccharide), the correlation between lectin reactivity and the amount of protein present was better when higher amounts of glycopeptide were present.

Defective binding could be due to the masking effect of SA or to absence of Gal. In order to make a distinction between these two possibilities, Gal was exposed by treating the IgA molecules with neuraminidase. Five ml of serum were incubated overnight at room temperature with 150 μl of 0.067 U ml^{-1} of Clostridium perfringens type V neuraminidase (Sigma Chemical Co., St Louis, MO) in 50 ml of 0.2 mmol l^{-1} of citrate buffer, pH 5. One unit liberated 1 μM of SA per min at pH 5.0 at 37 °C. The removal of SA was demonstrated by the above sialylation assay. Serum treated with heat-denatured neuraminidase in identical buffer conditions served as a negative control. Despite different levels of sialoglycoproteins in different sera, pilot experiments showed that the amount of enzyme was adequate to remove SA in both the control and patient sera.

Statistics

The backgrounds for SNA and RCA-1 were 0.04 and 0.18 respectively and these were subtracted from each measurement as appropriate. All measurements were performed in triplicate and these results were averaged. Patients and control sera were dispensed at random on to every plate. Intra-plate and inter-plate variation coefficients were under 10%. The assays were thus reproducible. All comparisons were made using the Mann-Whitney U test for unpaired data and the Wilcoxon's signed rank test for paired data. The correlations were assessed using the Spearman test.

Results

Sialylation of total IgA, IgA_1 and IgA_2

Figure 3 shows the ODs for the different groups using SNA. These were 0.84 (median 0.86, range 0.65–0.92) and 0.68 (median 0.65, range 0.46–0.83) for patient and control total IgA respectively ($p < 0.04$); 0.65 (median 0.63, range 0.27–0.98) and 0.49 (median 0.47, range 0.17–0.77) for IgA_1 respectively ($p < 0.02$); and 0.70 (median 0.80, range 0.32–1.28) and 0.88 (median 0.92, range 0.33–1.50) for IgA_2 respectively ($p < 0.05$). The larger variations in the control group were probably due to different glycoforms, whereas in the disease group, it was probably caused by different degrees of disease activity. Assuming that the upper cut-off point (mean ± 2SD) in the control group is 1.16 for total IgA, 0.81 for IgA_1 and 1.54 for IgA_2, then there are six patients where the sialylation of IgA_1 was outside the normal range. Although this finding did not correlate with the total level of serum IgA in each specimen ($r = 0.07$), five of them presented with end-stage renal failure. Oversialylation of IgA_1 appeared to be counter-balanced by defective sialylation of IgA_2.

Galactosylation of total IgA

The binding of RCA-1 to IgA_1 is shown in Fig. 4. The patient mean ODs were 0.88 (median 0.92, range 0.5–1.69) and 1.18 (median 1.20, range 0.89–1.50) before and after treatment with neuraminidase respectively (delta P; $p < 0.0001$), and the control mean ODs, 0.86 (median 0.86, range 0.52–1.20) and 1.47 (median 1.39, range 1.02–1.58) (delta C; $p < 0.0001$). Although the binding of RCA-I to IgA was not significantly different in the patient

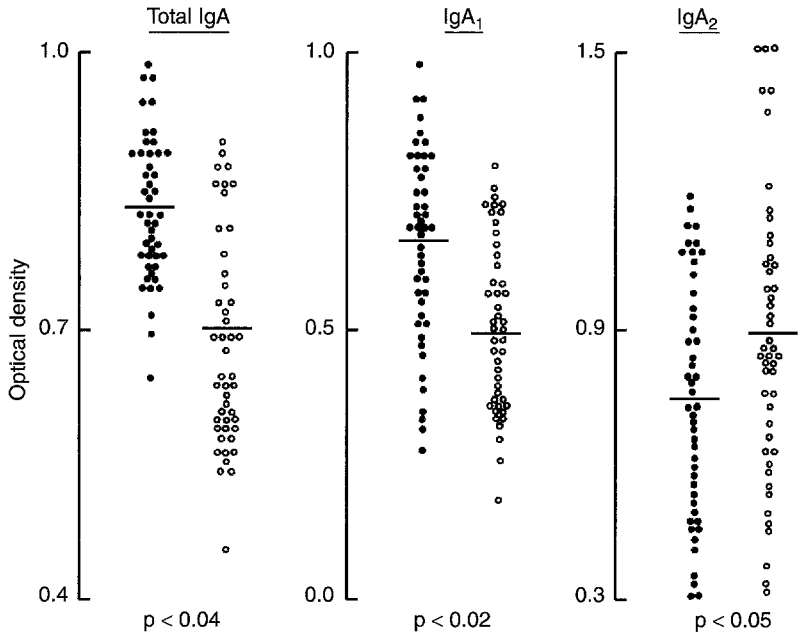

Figure 3. Sialylation of total IgA, IgA_1 and IgA_2 as detected by SNA in 47 patients with IgA nephropathy (closed symbols) and 47 controls (open symbols). Horizontal lines in figures 3 to 5 are mean values.

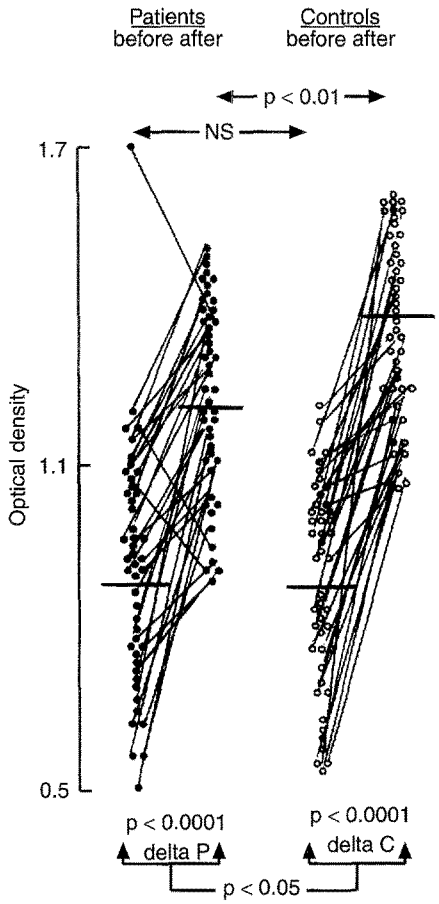

Figure 4. Galactosylation of IgA_1 before and after neuraminidase treatment of the immunoglobulins, evaluated using RCA-I.

and control groups before neuraminidase treatment, after treatment, it was significant ($p < 0.01$). Also, the increase in RCA-I binding caused by neuraminidase treatment was significantly higher in the control group (delta P vs delta C; $p < 0.05$).

Fc dependent galactose deficiency in IgA_1

Reduced galactosylation in the Fc region of IgA_1 was demonstrated in the patient group using ECL (Fig. 5). All specimens were pretreated with neuraminidase. The mean OD for the patients was 1.06 (median 1.07, range 0.55–1.58) compared with 1.32 (median 1.32, range 0.83–1.68) for the controls, $p < 0.02$. If the bottom cut-off point was taken as 0.75 (mean −2SD), the level of the IgA_1 hinge region galactosylation was abnormally low in seven patients. Interestingly, four of them also had an excess of SA in IgA_1. The measurements made with PNA showed that galactosylation in the hinge region of IgA_1 was slightly increased. The mean OD was 0.30 (median 0.25, range 0.13–0.36) in the patients, compared with 0.26 (median 0.22, range 0.10–0.34) in the controls, $p < 0.05$.

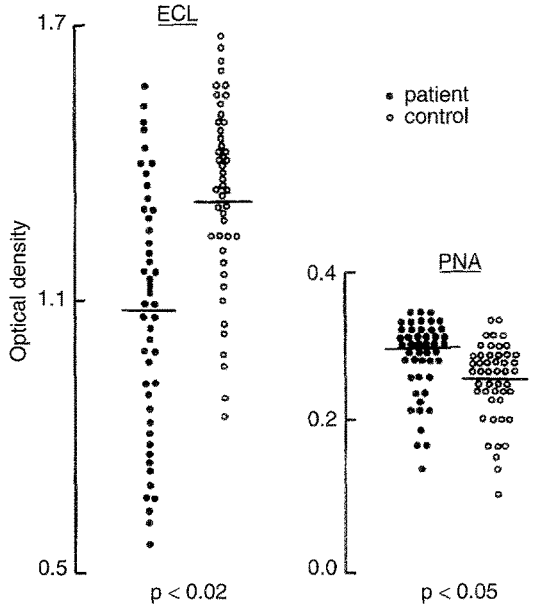

Figure 5. Galactosylation of IgA_1 from 47 patients with IgA nephropathy and 47 normal controls measured using ECL and PNA lectins. All preparations were previously treated with neuraminidase. ECL was used to detect changes in Fc galactosylation and PNA was used to detect changes in hinge galactosylation.

Galactosylation of IgA_2

In contrast to IgA_1, galactosylation of neuraminidase-treated IgA_2 (as detected by RCA-I) was comparable in the patients (0.86 SD ± 0.18) and the controls (0.87 SD ± 0.17).

Discussion

There is a dearth of information about the factors leading to mesangial IgA deposition. The IgA-dependent carbohydrates could play a pivotal role in this respect. The major emphasis of this study therefore, was to investigate the oligosaccharide chains attached to the hinge and the Fc regions of the molecule. Our approach was to develop assays in which antibody was used to catch IgA or IgA subclasses, and a sugar-specific lectin was used to identify the terminal sugar residues on the captured molecule. A mild oxidation step was included in the assay to reduce direct interaction between antibody and lectin. This was based on the procedure described by Kinoshita *et al.* [18]. A number of assays of this type have been previously reported [22]. These assays appear to be sensitive (approximately, 0.30–0.75 ng of oligosaccharide), specific (shown by inhibition experiments) and reproducible (with intra-plate and inter-plate variation coefficients of under 10%).

The proportion of sialylated IgA_1 was abnormally high in patients. This could be due to hinge *O*-glycosylation as

well as Fc *N*-glycosylation. The excess of SA is likely on both chains. However, after neuraminidase treatment, decreased Gal was detected by RCA-I (which detects Gal in the Fc and hinge regions) and by ECL (which detects Gal in Fc region) which suggests that the increase SA was attached to GalNAc in the hinge region.

From the SNA results IgA_2 seemed to be less well sialylated in the patients although IgA_2 does contain SA-GalNAc. Our finding of a normal amount of RCA-I-binding to IgA_2 suggests that SA is more deficient than Gal in IgA_2. A positive relationship has been previously shown between the concentrations of Gal and SA in normal IgA_2 proteins [11]. This interpretation is in accordance with the virtual absence of IgA_2 within the mesangial deposits.

Lectin-binding alterations of the patients' IgA_1 molecules have previously been reported [14–16]. These were also consistent with the results of direct chemical analyses of IgA from IgAN patients [15]. However, it was worth knowing whether the previously Gal deficiency was associated with both the *O*- and *N*-linked oligosaccharide chains. Given that most of the oligosaccharides reported for IgA have been determined using myeloma proteins, normal immunoglobulins may differ in glycosylation to myeloma proteins.

Caution should, thus, be exercised when identifying sugar residues on particular parts of IgA with the different lectins. The explanation why after neuraminidase treatment, the amount of Gal is significantly lower in the patient group is unclear. The glycosylation of serum IgA is more heterogeneous [23] than that reported for the myeloma protein [12, 13]. The structures vary in antennary oligosaccharides complexity and charge due to non-, mono-, di- and tri-sialylated composition. Nor can the large number of glycoforms result from a polyclonal population, since a similar heterogeneity was found for hybridoma IgG [24]. If neutral forms of IgA predominated in a subgroup of patients, Gal residues could be cleaved from IgA by small amounts of contaminating β-galactosidase and neuraminidase [25].

Nephropathy could arise from derangements of the genes encoding the IgA heavy chains. Restriction fragment length polymorphism analysis of the Cα1 and Cα2 flanking regions indicated that there may be two separate associations of IgAN with the Ig switch region [26]. Hence, it is feasible that the number of locations for GalNAc is augmented, and, consequently, that of Gal residues predisposed to accept SA. Of great interest was the relation found between levels of oversialylated IgA and the progression of the disease to end-stage renal failure, so that, conceivably, renal failure *per se* and not IgAN may explain the observed differences. In order to conclude that the findings are specific for IgAN, a group of patients with other glomerular diseases would provide an ideal negative control.

Nonetheless, evidence that carbohydrates are important in the IgA deposition come from other sources. Early studies [27] established that IgA present in normal serum reacts with a liver receptor specific for asialoglycoprotein (ASGP-R). This structure recognizes the *O*-linked carbohydrate unit of IgA_1 that are desialylated [15]. Oversialylated IgA_1 would not therefore, be properly cleared, so that its serum half-life would be extended. Undoubtedly, a better understanding of IgA catabolism is of importance in determining the pathogenesis of IgAN. Alternatively, the expression of monocyte IgA Fc receptors might be defective, as recently reported in patients with alcoholic cirrhosis [28].

The glomerular mesangium is composed of matrix and mesangial cells. The binding of IgA to the matrix relies on a carbohydrate/lectin interaction which can be inhibited by a variety of sugar residues. For example, SA is extremely efficient in preventing IgA from fixing to laminin [29]. Increased sialylation of IgA1 results in increased anionic charge on the molecule, thereby making the molecule more likely to precipitate. An additional IgA receptor in rat and human mesangial cells has been recently described [30]. Again, the IgA oligosaccharides are essential to its function. Indeed, preincubation of cells with Gal or GalNAc caused a significant reduction of the IgA binding.

Herein, a number of IgA glycosylation abnormalities have been established in patients with IgAN. Yet, the mechanisms remain elusive, since the bone marrow cells are the primary source of pathological IgA [31]. Further investigations need to be carried out on sialyl and galactosyl transferase activities in these cells.

Acknowledgements

The secretarial expertize of A. Paul is greatly appreciated. We are grateful to J. Keusch for fruitful discussion. Thanks are also due to J.L. Preud'homme for providing reagents.

References

1. Mestecky J, Russell MW (1986) *Monogr Allergy* **19**: 277–301.
2. Berger J, Hinglais N (1968) *J Urol Nephrol* **74**: 694–95.
3. Julian BA, Waldo FB, Rifai A, Mestecky J (1988) *Am J Med* **84**: 129–32.
4. Nakamoto Y, Assano Y, Kasuhiro D, Fujioka M, Iida H, Kida H, Kibe Y, Hatori N, Takeuchi J (1978) *Q J Med* **188**: 496–516.
5. Conley ME, Cooper MD, Micheal AF (1980) *J Clin Invest* **66:** 1432–36.
6. Sakai H, Miyasaki M, Endoh M, Nomoto Y (1989) *Clin Exp Immunol* **78**: 378–82.

7. Jackson S, Dawson LM, Kotler DP (1988) *J Clin Immunol* **8**: 64–68.
8. Bendaoud B, Pennec YL, Lelong A, Le Noac'h JF, Magadur G, Jouquan J, Youinou P (1991) *J Auntoimmunity* **4**: 177–94.
9. Monteiro RC, Halbwacks-Mecarelli L, Roque-Barreira MC, Noël LH, Berger J, Lesavre P (1985) *Kidney Int* **28**: 666–71.
10. Monteiro RC, Chevailler A, Noël LH, Lesavre P (1988) *Clin Exp Immunol* **73**: 300–6.
11. Tomana M, Niedermeier W, Mestecky J, Skvaril F (1976) *Immunochemistry* **13**: 325–28.
12. Baenziger J, Kornfield S (1974) *J Biol Chem* **249**: 7260–69.
13. Baenziger J, Kornfield S (1974) *J Biol Chem* **249**: 7270–81.
14. André PM, Le Pogamp P, Chevet D (1990) *J Clin Lab Analysis* **4**: 114–19.
15. Mestecky J, Tomana M, Crowley-Nowick PA, Moldoveanu Z, Julian BA, Jackson S (1993) *IgA nephropathy : the 25th year* (Béné MC, Faure GC, Kessler M eds) pp. 172–82 Basel: Karger.
16. Allen AC, Harper SJ, Feehally J (1994) *Glycosyl Dis* **1**, 215 (abstract).
17. Allen AC, Harpe SJ, Feehally J (1996) *Clin Exp Immunol* (in press).
18. Kinoshita N, Suzuki S, Matsuda Y, Taniguchi N (1989) *Clin Chim Acta* **179**: 143–52.
19. Kennedy DM, Skillen AW, Self CH (1993) *Clin Exp Immunol* **94:** 447–51.
20. Hashim OH, Kobayashi K, Taniguchi N (1992) *Biochem Int* **27**: 423–29.
21. Casburn-Budd R, Youinou P, Hager H, Elkington D, Baxter I, Berthelot JM, Le Goff P, Isenburg DA (1992) *J Rheumatol* **19**: 1070–74.
22. Turner GA (1992) *Clin Chim Acta* **208:** 149–71.
23. Field MC, Amatayakul-Chantler S, Rademacher TW, Rudd PM, Dwek RA (1994) *Biochem J* **299**: 261–75.
24. Rademacher TW, Homans SW, Parekh RB, Dwek RA (1986) *Biochem Soc Symp* **51:** 131–48.
25. Adler Y, Lamour A, Jamin C, Menez J-F, Le Corre R, Shoenfeld Y, Youinou P (1995) *Clin Exp Rheumatol* **13**: 315–19.
26. Demaine AG, Rambausek M, Knight JF, Williams DG, Welsh KI, Ritz E (1988) *J Clin Invest* **81**: 611–14.
27. Stockert RJ, Kressner MS, Collins JC, Sternlieb I, Morell AG (1982) *Proc Natl Acad Sci USA* **79**: 6229–31.
28. Silvain C, Patry C, Launay P, Lehuen A, Monteiro RC (1995) *J Immunol* **155**: 1606–18.
29. Coppo B, Amore A, Gianoglio B, Reyna A, Peruzzi L, Roccatello D, Alessi D, Sena LM (1993) '*IgA nephropathy: the 25th year*' (Béné MC, Faure GC, Kessler M, eds) pp. 162–71 Basel: Karger.
30. Gómez-Guerrero C, González E, Egido J (1993) *J Immunol* **151**: 7172–81.
31. van den Wall Bake AWL, Daha MR, Valentin RM, van Es LA (1987) *Sem Nephrol* **7**: 329–31.