Abnormal IgA glycosylation in Henoch-Schönlein purpura restricted to patients with clinical nephritis

Alice C. Allen, Frank R. Willis, T. James Beattie and John Feehally

Department of Nephrology, Leicester General Hospital, Gwendolen Road, Leicester LE5 4PW, and Renal Unit, Royal Hospital for Sick Children, Yorkhill, Glasgow G3 8SJ, UK

Abstract

Background. Glomerular deposition of IgA1 is a common feature of Henoch-Schönlein purpura, and is indistinguishable from that seen in IgA nephropathy. Serum IgA1 is abnormally O-glycosylated in IgA nephropathy, and this may contribute to mesangial IgA1 deposition and the development of glomerular injury. This altered O-glycosylation of IgA1 can be detected by its increased binding to the lectin Vicia villosa.

Methods. To investigate whether IgA1 is abnormally glycosylated in Henoch-Schönlein purpura, the binding of Vicia villosa lectin to serum IgA1 was studied in the following subject groups: IgA nephropathy; adults and children with Henoch-Schönlein purpura and nephritis; children with clinically diagnosed Henoch-Schönlein purpura but no renal involvement; adults and children with non-IgA associated glomerulonephritis; and matched controls.

Results. The abnormality of lectin binding seen in IgA nephropathy was also found in both adults and children with Henoch-Schönlein purpura and nephritis. However, the lectin binding of serum IgA1 from children with Henoch-Schönlein purpura lacking renal involvement did not differ from controls, and similarly, no abnormality of lectin binding was seen in patients with non-IgA associated glomerulonephritis.

Conclusions. These data indicate that the abnormality of IgA1 O-glycosylation seen in IgA nephropathy is also found in Henoch-Schönlein purpura, but only in those subjects with renal involvement, while IgA1 O-glycosylation is normal in patients with other forms of renal disease. These findings lend strong support to a role for altered IgA1 O-glycosylation in the pathogenesis of IgA-associated glomerular disease.

Key words: glomerulonephritis, IgA1 hinge region, IgA nephropathy, O-glycosylation, Vicia villosa lectin

Introduction

Henoch-Schönlein purpura (HSP) is a form of systemic vasculitis characterized by IgA deposition in affected blood vessels. HSP can occur at any age, but is most common in children. Renal involvement is common and in the glomerular mesangium there is IgA1 deposition which is indistinguishable from that found in IgA nephropathy (IgAN). The mechanisms of mesangial IgA1 deposition and subsequent glomerular injury in IgAN and HSP are unknown. Altered IgA1 glycosylation has recently received attention as a candidate physicochemical abnormality in the pathogenesis of IgAN [1], but has not been studied in HSP.

IgA1 is an unusual serum protein in possessing a series of O-linked glycans in the hinge region of the molecule (Figure 1A), a feature which distinguishes it from IgA2 and other serum immunoglobulins [2]. Each of the O-linked glycans is based on N-acetylgalactosamine (GalNAc) O-linked to serine residues; O-glycosylation of threonine residues, though theoretically possible, has not been described in IgA1. The glycan chains may be elongated with the further addition of galactose (Gal) in β1,3 linkage with GalNAc, and a variable degree of sialylation (Figure 1B) [3,4].

Altered O-glycosylation of serum IgA1 in IgAN has been described in a number of studies [5–10], but the abnormality has yet to be defined precisely. Most of the evidence for this abnormality has come from reports of altered binding of IgA1 to various lectins. Though lectins cannot provide exact structural information about the abnormality, they are useful and convenient tools with which to detect altered glycosylation. We have used the lectin from Vicia villosa (VV) to demonstrate abnormal O-glycosylation of serum IgA1 in IgAN [8] and the Wiskott-Aldrich syndrome [11]. VV lectin binds to terminal O-linked GalNAc, and shows higher binding to IgA1 from patients with IgAN than controls. This finding suggests that the abnormality may take the form of a reduction in the degree of galactosylation of GalNAc moieties in IgAN, resulting in increased exposure of GalNAc.
Materials

All chemicals were purchased from Sigma Chemical Co., Poole, UK, except where otherwise specified. Antibodies and biotinylated *Vicia villosa* lectin and peroxidase-conjugated avidin D from Vector Laboratories, Peterborough, UK.

Subjects and samples

Details of the patient and control groups are given in Table 1.

Paediatric subjects

**HSP**: Of 24 children with HSP nephritis (HSPN), half had undergone renal biopsy, and the rest had unequivocal clinical evidence of renal disease: persistent proteinuria and/or haematuria with or without impairment of renal function. The 22 children with HSP but no nephritis (HSPN0) all had a clinical diagnosis of HSP, but no proteinuria, haematuria, hypertension or renal impairment.

**PSGN**: Seven children with acute nephritic syndrome due to post-streptococcal glomerulonephritis were also studied.

**Control**: 22 age and sex-matched children were recruited from healthy volunteers. Venous blood was obtained from the subjects with informed consent. The serum was separated from the blood samples and stored in aliquots at −20°C.

Preparation of ammonium sulphate precipitates

Aliquots of 200 μl serum were added to 200 μl 56% ammonium sulphate in PBS, rotated for 1 h at ambient temperature, and centrifuged for 20 min at 10,000 g. The supernatants were discarded and the precipitates redissolved in 1 ml PBS.

IgA ELISA

The IgA concentration of the ammonium sulphate precipitate samples was measured by sandwich ELISA. Briefly, 96 well immunoplates were coated with 100 μl per well rabbit anti-human IgA in 0.1 M carbonate/bicarbonate buffer, pH 9.6, overnight at 4°C. The plates were washed four times in

To lectin binding. This interpretation of our data is consistent with the findings of other investigators [5–7,9], though more detailed structural information is still required to confirm precisely the glycan abnormality of IgA1.

Altered O-glycosylation of IgA1 has the potential to affect the interactions of the molecule in various ways. It may compromise the efficiency of hepatic clearance of circulating IgA1 by the asialoglycoprotein receptor which displays high affinity for O-linked glycans [12]. Furthermore, altered IgA1 O-glycosylation may favour mesangial deposition or the initiation of glomerular inflammatory processes by promoting self-aggregation of the molecule in various ways. O-glycosylation of threonine residues is also possible, but has not been described in IgA1. GalNAc is O-linked to serine or threonine, and is frequently further substituted by galactose in the β1,3 configuration. Mono- or di-sialylation (N-acetyl neuraminic acid; NeuNAc) may also occur. Sugars not consistently present are shown in italics: Gal and NeuNAc.

Fig. 1. (A) IgA1 molecule, showing the position of the hinge region O-glycans. The hinge region lies between the CH1 and CH2 domains. On the right is the amino acid sequence of the hinge region, with the serine-linked O-glycosylation sites marked by arrows. O-glycosylation of threonine residues is also possible, but has not been described in IgA1. (B) Structure of the O-linked glycan moiety of IgA1. GalNAc is O-linked to serine or threonine, and is frequently further substituted by galactose in the β1,3 configuration. Mono- or di-sialylation (N-acetyl neuraminic acid; NeuNAc) may also occur. Sugars not consistently present are shown in italics: Gal and NeuNAc.

role for altered IgA1 glycosylation in the pathogenesis of IgA-associated glomerulonephritis.

Subjects and methods

**Materials**

**Subjects and samples**

Details of the patient and control groups are given in Table 1.

**Paediatric subjects**

**HSP**: Of 24 children with HSP nephritis (HSPN), half had undergone renal biopsy, and the rest had unequivocal clinical evidence of renal disease: persistent proteinuria and/or haematuria with or without impairment of renal function. The 22 children with HSP but no nephritis (HSPN0) all had a clinical diagnosis of HSP, but no proteinuria, haematuria, hypertension or renal impairment.

**PSGN**: Seven children with acute nephritic syndrome due to post-streptococcal glomerulonephritis were also studied.

**Control**: 22 age and sex-matched children were recruited from hospital staff. Venous blood was obtained from the subjects with informed consent. The serum was separated from the blood samples and stored in aliquots at −20°C.

**Preparation of ammonium sulphate precipitates**

Aliquots of 200 μl serum were added to 200 μl 56% ammonium sulphate in PBS, rotated for 1 h at ambient temperature, and centrifuged for 20 min at 10,000 g. The supernatants were discarded and the precipitates redissolved in 1 ml PBS.

**IgA ELISA**

The IgA concentration of the ammonium sulphate precipitate samples was measured by sandwich ELISA. Briefly, 96 well immunoplates were coated with 100 μl per well rabbit anti-human IgA in 0.1 M carbonate/bicarbonate buffer, pH 9.6, overnight at 4°C. The plates were washed four times in

...
Table 1. Details of study groups

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Number of subjects</th>
<th>Male/female (m/f)</th>
<th>Median age (years)</th>
<th>Age range (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paediatric Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSPN</td>
<td>24</td>
<td>14 M 10 F</td>
<td>7.0</td>
<td>3–12</td>
</tr>
<tr>
<td>HSPN&lt;sub&gt;0&lt;/sub&gt;</td>
<td>22</td>
<td>10 M 12 F</td>
<td>5.5</td>
<td>3–13</td>
</tr>
<tr>
<td>PSGN</td>
<td>7</td>
<td>5 M 2 F</td>
<td>7.5</td>
<td>5–12</td>
</tr>
<tr>
<td>Controls</td>
<td>22</td>
<td>10 M 12 F</td>
<td>7.0</td>
<td>4–16</td>
</tr>
<tr>
<td>Adult Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSPN</td>
<td>31</td>
<td>17 M 14 F</td>
<td>37.5</td>
<td>16–71</td>
</tr>
<tr>
<td>IgAN</td>
<td>9</td>
<td>2 M 7 F</td>
<td>32.0</td>
<td>23–51</td>
</tr>
<tr>
<td>Non-IgA GN</td>
<td>11</td>
<td>6 M 5 F</td>
<td>49.0</td>
<td>22–78</td>
</tr>
<tr>
<td>Controls</td>
<td>28</td>
<td>13 M 15 F</td>
<td>36.0</td>
<td>21–78</td>
</tr>
</tbody>
</table>

HSPN, Henoch-Schönlein purpura with nephritis; HSPN<sub>0</sub>, Henoch-Schönlein purpura without renal involvement; PSGN, Post-streptococcal glomerulonephritis; IgAN, IgA nephropathy; Non-IgA GN, Mesangial proliferative glomerulonephritis without IgA deposition.

Washing buffer (PBS/0.3 M NaCl/0.1% Tween 20) using an automated plate washer (Denley, Billingshurst, UK), and excess protein binding sites blocked with 100 μl/well 2% bovine serum albumin (BSA) in PBS for 1 h at ambient temperature. After further plate washing, the samples, appropriately diluted in PBS, were applied to the plates at 50 μl/well in duplicate, and standard curves constructed on each plate with serial dilutions of a human immunoglobulin calibrator (The Binding Site, UK). The plates were incubated overnight at 4°C, washed again, and 50 μl/well horseradish peroxidase-conjugated rabbit anti-human IgA diluted in PBS applied for 2 h at ambient temperature. After final washing, the colour was developed with 50 μl/well OPD substrate solution with 0.4% v/v 30% H<sub>2</sub>O<sub>2</sub>, stopping the reaction after 10 min with 75 μl/well 1M H<sub>2</sub>SO<sub>4</sub>. The absorbance of the wells at 492 nm was read with an automated plate reader. The mean absorbance of each sample was calculated from the duplicates. Since the absorbance values obtained varied between assays according to the time the substrate was allowed to develop, a series of normal samples run in each assay were used to provide a standardization factor by which the samples were adjusted to compare results obtained on different occasions. The lectin binding results were expressed as arbitrary units (AU). Intra-assay coefficients of variation (CVs) were calculated from replicates of one sample run on one plate, and on different plates within the same assay run.

Statistics

Mean lectin binding results for the various patient and control groups shown in Table 1 were compared by Student’s t-test.

Results

Calibration of the assay system

The intra-plate CV for this assay was 3.0%, and the inter-plate CV 5.2%.

Dose response sugar inhibition curves were constructed to demonstrate the specificity of VV lectin binding to IgA in this assay system (Figure 2), and showed that VV binding was highly sensitive to GalNAc inhibition. At the 50% inhibition level,
GalNAc was 125-fold more potent that Gal, while GlcNAc and Glic did not inhibit to this degree at all.

**Samples**

The samples were applied to the anti-IgA coated plates at saturating concentrations, so that the wells captured a constant amount of IgA from each sample. VV lectin binding did not correlate with IgA concentration in any of the subject groups (data not shown).

**VV lectin binding in paediatric and adult controls and HSPN patients**

IgA1 lectin binding was significantly higher in adult controls than paediatric controls (4.7 ± 0.14 vs 4.1 ± 0.10 AU, P = 0.002), but the lectin binding of IgA1 from adults with HSPN did not differ from that of children with HSPN (5.3 ± 0.18 vs 5.5 ± 0.43, P = NS). Both adult and paediatric HSPN patients showed significantly higher IgA1-lectin binding than their respective control groups (adults 5.3 ± 0.18 vs 4.7 ± 0.14, P = 0.007; children 5.5 ± 0.43 vs 4.1 ± 0.10, P = 0.005).

**VV lectin binding of IgA1 in paediatric subjects**

IgA1 from children with HSPN showed significantly raised VV lectin binding than IgA1 from children with HSPN<sub>0</sub>, PSGN, and control children. The lectin binding of IgA1 from children with HSPN<sub>0</sub> or PSGN did not differ from the controls (Figure 3).

**VV lectin binding in adult subjects**

IgA1 from both adults with HSPN and with IgAN showed significantly higher lectin binding than IgA1 from subjects with non-IgA GN or control subjects; lectin binding of IgA1 from non-IgA GN did not differ from controls (Figure 4).

**Discussion**

This study confirms our previous findings of increased binding of *Vicia villosa* lectin to the O-linked glycans of serum IgA1 in IgA nephropathy [8]. Serum IgA1 from both adults and children with HSPN also displayed raised lectin binding in comparison to matched control groups. This finding strengthens the evidence for a common pathogenic basis to mesangial IgA1 deposition and the subsequent development of glomerulonephritis in IgAN and HSPN.
In contrast to HSPN, we found no abnormality of VV lectin binding in the serum IgA1 of children with clinically-diagnosed HSP who lacked renal involvement; there was also normal IgA1-VV binding in both adults and children with glomerulonephritis not associated with IgA deposition (non-IgA mesangial proliferative GN and post-streptococcal GN respectively). Therefore altered O-glycosylation of IgA1 is not merely a marker of glomerular disease, but is specific to subjects with IgA-related glomerulonephritis. The observation that in HSP altered IgA1 O-glycosylation only occurs in those with renal involvement lends considerable support to a role for this abnormality in the development of renal IgA deposits.

Lectin binding cannot precisely define the glycosylation abnormality, but raised binding of the GalNAC-specific lectin VV suggests increased exposure of GalNAC. The most likely explanation for this is a reduction in terminal galactosylation and sialylation of the O-linked glycan moieties. Both of these have indeed been indicated by some preliminary studies using chromatography techniques to analyse sugars released from the IgA1 of patients with IgAN [6,14]. Reduced galactosylation is also indirectly suggested by our finding of reduced activity of the O-galactosylating enzyme β1,3 galactosyltransferase in the B cells of patients with IgAN [15].

The high density of O-glycans in the hinge region of IgA1 is a distinctive structural feature of the molecule. O-glycans tend to confer an extended conformation upon the peptide backbone to which they are linked [16], and in the case of IgA1, may maintain the spatial distance between the CH1 and CH2 domains. The degree of sialylation of the O-glycans also has the potential to exert important effects upon this pivotal area of the molecule, since sialic acid carries a high negative charge, but the hinge region is devoid of charged amino acids. Thus alterations in the hinge region O-glycans could result in subtle changes in the 3D structure and charge of the molecule, affecting its behaviour, handling, and interactions with other molecules.

Definitive structural analysis of these O-glycans is now required, as well as study of the functional effects of altered IgA1 hinge sugars. Such studies will help to establish whether altered IgA1 hinge region glycosylation is indeed pathogenic in the development of mesangial IgA1 deposits in IgA nephropathy and HSP nephritis.

Acknowledgements. This work was supported in part by the National Kidney Research Foundation, UK (Grant R44/2/94). The authors thank Dr D O’Donoghue, Hope Hospital, Salford, UK, and Dr M de Caestecker, Manchester Royal Infirmary, Manchester, UK, for providing serum samples from their patients.

References

1. Allen AC. Abnormal glycosylation of IgA—is it related to the pathogenesis of IgA nephropathy? Nephrol Dial Transplant 1995; 10: 1121–1124
5. Andre PM, le Pogamp P, Chevet D. Impairment of jacalin GalNAc. The most likely explanation for this is a binding to serum IgA in IgA nephropathy. J Clin Lab Anal 1990; 4: 115–119
12. Stockert RJ, Kressner MS, Collins JC, Sternlieb I, Morell AG. IgA interaction with the asialoglycoprotein receptor. Proc Natl Acad Sci USA 1982; 79: 6229–6231

Accepted in revised form: 19.12.97

Received for publication: 14.7.97