O-Glycosylation of Serum IgA1 Antibodies against Mucosal and Systemic Antigens in IgA Nephropathy

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In IgA nephropathy (IgAN), serum IgA1 with abnormal O-glycosylation deposits in the glomerular mesangium. The underlying mechanism of this IgA1 O-glycosylation abnormality is poorly understood, but recent evidence argues against a generic defect in B cell glycosyltransferases, suggesting that only a subpopulation of IgA1-committed B cells are affected. For investigation of whether the site of antigen encounter influences IgA1 O-glycosylation, the O-glycosylation of serum IgA1 antibodies against a systemic antigen, tetanus toxoid (TT), and a mucosal antigen, Helicobacter pylori (HP), was studied in patients with IgAN and control subjects. Serum IgA1 was purified from cohorts of patients with IgAN and control subjects with HP infection and after systemic TT immunization. The IgA1 samples were applied to HP- and TT-coated immunoplates to immobilize specific antibodies, and IgA1 O-glycosylation profiles were assessed by binding of the O-glycan–specific lectin Vicia villosa using a modified ELISA technique. Although total serum IgA1 had raised lectin binding in IgAN, the O-glycosylation of the specific IgA1 antibodies to TT and HP did not differ between patients and control subjects. In both groups, IgA1 anti-HP had higher lectin binding than IgA1 anti-TT. This study demonstrates that IgA1 O-glycosylation normally varies in different immune responses and that patients produce the full spectrum of IgA1 O-glycoforms. IgA1 with high lectin binding was produced in response to mucosal HP infection in all subjects. The raised circulating level of this type of IgA1 in IgAN is likely to be a consequence of abnormal systemic responses to mucosally encountered antigens rather than a fundamental defect in B cell O-glycosylation pathways.


IgA nephropathy (IgAN) is one of the most common patterns of glomerulonephritis and is characterized by the mesangial deposition of polymeric IgA1 (1). IgA1 deposition is associated with variable degrees of glomerular injury and renal dysfunction and leads to progressive renal failure in almost one third of cases. There now is convincing evidence that mesangial IgA1 is derived from a small fraction of the total circulating pool of IgA and that this pathogenic IgA has particular propensity for mesangial deposition and mesangial cell activation (2).

One of the most consistent abnormalities of serum IgA1 in IgAN is aberrant O-glycosylation (3). The hinge region of each IgA1 heavy chain possesses up to six O-glycans (4) based on inner core N-acetylgalactosamine (GalNAc) units, each of which may or may not carry galactose and sialic acid, giving rise to many different IgA1 O-glycoforms with varying degrees of O-galactosylation and sialylation (Figure 1). In IgAN, IgA1 O-galactosylation is reduced, leading to increased frequency of truncated O-glycans and enhanced exposure of terminal GalNAc. The functional effects of altered IgA1 hinge region O-glycosylation are imprecisely understood. Experimental data suggest that aberrantly glycosylated IgA1 molecules have an increased tendency to self-aggregate (5), form antigen–antibody complexes with IgG antibodies that are directed against IgA1 hinge epitopes (6), and aggregate with extracellular matrix components (5), all producing complexes that are prone to mesangial trapping. IgA1 eluted from IgAN glomeruli is aberrantly O-glycosylated and is more abnormal in this regard than serum IgA1, strongly implicating altered glycosylation in the process of IgA deposition (7,8).

There also is increasing in vitro evidence for both enhanced binding of IgA immune complexes (IgA-IC) to human mesangial cells and exaggerated receptor-mediated mesangial cell activation by IgA-IC (9–11), especially those that contain undergalactosylated IgA1 (12). Consistent with these findings, clinical studies have shown that undergalactosylated IgA1 is a major component of circulating IgA-IC in IgAN and that the extent of the glycosylation defect may correlate with the degree of glomerular injury at the time of renal biopsy (13,14). In light of this evidence, we believe that aberrant IgA1 O-glycosylation drives the processes of IgA deposition and glomerular injury in IgAN. At present, little is known about the control of IgA1 O-glycosylation in B lymphocytes and plasma cells. We recently showed no undergalactosylation of serum IgD in IgAN, arguing that the O-glycosylation defect is not generic in B lineage cells in IgAN but arises only after class switching to IgA (15). Taken with other published data, this finding suggests a defect in IgA-committed B cells after antigen encounter. Clearly, this defect is not expressed uniformly in all
IgA-committed B lineage cells, because abnormalities of B cell function and serum IgA in IgAN are mild and the pathogenic fraction of serum IgA is small. We therefore hypothesize that the abnormality is restricted to specific B cell populations and that the principal factor that determines the galactosylation profile of IgA antibodies might be the context in which the B cell encounters antigen. The site of antigen encounter is known to influence the phenotype of both T and B lymphocytes through their subsequent expression of cell surface homing receptors and ultimately their functional capabilities (16,17). We therefore studied the pattern of IgA1 O-glycosylation in patients with IgAN and normal subjects, examining specifically IgA1 that was generated after antigen encounter within the systemic compartment (tetanus toxoid [TT]) and at the mucosal surface (Helicobacter pylori [HP]).

Materials and Methods

Unless otherwise stated, all reagents and consumables that were used in this study were purchased from Sigma Chemical Co. (Poole, UK). Horseradish peroxidase–conjugated antibodies and orthophenylenediamine (OPD) ELISA substrate tablets were purchased from DakoCytomation (Ely, UK), and jacalin agarose, biotinylated Vicia villosa (VV) lectin, and horseradish peroxidase–conjugated avidin were from Vector Laboratories (Peterborough, UK).

Participants and Samples

A total of 25 patients with IgAN and 23 healthy control subjects were included in the experiments reported here. All patients had biopsy-proven IgAN and had microscopic hematuria and/or proteinuria, but proteinuria was <2.5 g/24 h in all cases. At the time of the studies, no participant had an intercurrent illness, and none of the patients with IgAN had macroscopic hematuria. The median time from renal biopsy to entry into the studies for patients with IgAN was 4 yr (range 0.2 to 10). None of the patients or control subjects was receiving immunosuppressive treatments at the time of the study or had received treatment in the 24 mo before study entry. Twelve patients with IgAN (six men; mean age 34 yr; range 21 to 59) and 12 control subjects (six men; mean age 36 yr; range 25 to 59) received subcutaneous immunization with TT. All of these individuals had received TT not less than 5 yr before during routine immunizations. Each of these individuals gave 20-ml venous blood samples at the time of immunization and 2 wk later. Serum was separated from the blood samples and stored at −80°C until required. This study was approved by the LNR Research Ethics Committee, and the participants all gave their full informed consent to participation.

A different group of 13 patients with IgAN (nine men; mean age 54 yr; range 32 to 68) and 11 control subjects (10 men; mean age 40 yr; range 30 to 55) with HP infection confirmed by 13C-urea breath test also were studied. A single serum sample was obtained with informed consent from these participants and stored at −20°C until required. We described these individuals and other investigations that were carried out on the same serum samples in a previously published report (18).

The study was conducted with the approval of Leeds General Infirmary Ethical Committee. Finally, we studied paired serum and breast milk samples that were donated by four lactating women between 25 and 35 yr of age, without known renal or other systemic disease.

Serum Antibody Levels

The serum antibody levels of the HP-infected patients and control subjects used in this study were reported previously (18). IgG, IgA, and IgA1 antibodies against TT were measured in serum samples from the immunized individuals by antigen-specific ELISA as described previ-
ously (19,20), using simple TT (National Institute of Biologic Standards and Control, Potters Bar, UK) to coat the plates. Standard curves were constructed from serial dilutions of a standard (made by pooling aliquots of serum that was taken from three high-responding individuals 2 wk after immunization) and used to calculate the antibody concentrations of the test sera in arbitrary units per milliliter. The intra-assay and interassay coefficients of variation were <5%.

IgA1 Purification

IgA1 was purified from serum by affinity chromatography on agarose-bound jacalin essentially as described previously (21,22). Serum was mixed with an equal volume of 45% ammonium sulfate in PBS, and after discarding the supernatant, the precipitate was dissolved in the original serum volume of 0.175 M Tris HCl buffer (pH 7.5) and added to the same packed volume of jacalin-agarose. After mixing slowly for 1 h at room temperature, the agarose was washed repeatedly to remove unbound proteins and the IgA1 was eluted in half the original volume of 1 M galactose by mixing gently for an additional 1 h at room temperature. Finally, the IgA1 preparations were passed through a PD10 gel filtration column to exchange the buffer to PBS.

Lectin-Binding Assays

To compare the O-glycosylation profiles of serum IgA1 antibodies to different antigens, we measured the binding of the O-glycan–specific lectin from VV to antigen-immobilized IgA1 using our previously published ELISA method (21,22). This lectin recognizes terminal O-linked GalNAc, and IgA1 samples with lower terminal galactosylation show higher lectin binding. We measured the lectin binding of IgA1 antibodies against TT in the immunized individuals and against HP in the infected individuals.

We also wished to compare the lectin binding of IgA1 antibodies against TT and HP in the same serum samples. Because none of the HP-infected individuals had received recent tetanus immunization, their anti-TT levels were too low for lectin-binding assays. However, none of the immunized individuals (four patients with IgAN and five control subjects) had serum IgA and IgG anti-HP titers similar to those of the HP-infected group; therefore, we measured the lectin binding of IgA1 antibodies to both antigens in these individuals.

The lectin binding of total serum IgA1 in the samples first was measured to ensure that the TT and HP groups did not differ in this regard. Purified IgA1 samples in PBS were applied to replicate wells of immunoplates at approximately 10 μg/ml, at which concentration the protein binding capacity of the wells were saturated and the amount of IgA1 immobilized on each well was constant for all of the samples. After overnight incubation and washing four times in PBS that contained an additional 0.4 M NaCl and 0.1% Tween 20, biotinylated VV lectin and monoclonal anti-IgA1 (both 1:1000 in PBS) were applied to parallel wells in duplicate for 90 min at room temperature. After further washing, peroxidase-conjugated avidin or peroxidase-conjugated anti-mouse as appropriate, and monoclonal anti-IgA1 to duplicate plates, followed by peroxidase-conjugated avidin or peroxidase-conjugated anti-mouse as appropriate, and monoclonal anti-IgA1 to duplicate plates, followed by peroxidase-conjugated avidin or peroxidase-conjugated anti-mouse as appropriate. The assay then was carried out as above, applying biotinylated VV lectin and monoclonal anti-IgA1 to duplicate plates, followed by peroxidase-conjugated avidin or peroxidase-conjugated anti-mouse as appropriate, and developing with OPD/H2O2 substrate. Results were read as absorbance at 492 nm, and then, the lectin binding was expressed as A492 lectin binding/A492 anti-IgA1 binding.

Lectin binding of antigen-specific IgA1 in the samples was measured in a similar assay system. In this case, the immunoplates first were coated with TT as for ELISA above or with a sonicated and ultracentrifuged HP preparation, as described previously (18). After overnight incubation at 4°C and washing, purified IgA1 samples were applied to duplicate wells and incubated overnight at 4°C. The samples all were diluted in PBS to achieve equivalent absorbances on TT and HP when developed with anti-IgA1 in preliminary screening experiments. The target anti-IgA1 absorbance chosen lay within the range that we previously established as giving constant values of lectin binding/anti-IgA1 binding for a given sample (7), meaning that minor variations in the amount of IgA1 that is bound to the plate do not affect the results obtained. The assay then was carried out as above, applying biotinylated VV lectin and monoclonal anti-IgA1 to duplicate plates, followed by peroxidase-conjugated avidin or peroxidase-conjugated anti-mouse as appropriate, and developing with OPD/H2O2 substrate. Results were read as absorbance at 492 nm, and again, the lectin binding was expressed as A492 lectin binding/A492 anti-IgA1 binding for each antibody type.

Lectin Binding of IgA1 from Paired Serum and Breast Milk Samples

To investigate further the differences in VV lectin binding to IgA1 from systemic and mucosal sources, we studied four paired samples of serum and expressed breast milk. The IgA1 concentrations in the samples were measured by ELISA, and the samples were diluted in PBS to

![Figure 2. Serum antibody responses after booster immunization with tetanus toxoid (TT; all participants). Twenty-four participants (12 patients with IgAN and 12 matched control subjects) were given subcutaneous booster immunizations with TT. All participants had previously received this vaccine not less than 5 yr before the study. Serum was obtained at the time of immunization (for baseline antibody levels) and 14 d later, and IgG and IgA antibody levels were measured by antigen-specific ELISA. Both IgG (top) and IgA (bottom) increased significantly after immunization, demonstrating the characteristics of a secondary immune response.](image-url)
equivalent IgA1 levels before carrying out total IgA1-lectin binding assays as described previously.

**Statistical Analyses**

Parametrically distributed data were analyzed by *t* test, and nonparametric data were analyzed by Mann-Whitney *U* test. Comparison of lectin binding of IgA1 antibodies to two different antigens in the same serum samples was by paired *t* test, and correlation was by regression analysis. In all cases, *P* < 0.05 was considered to be statistically significant.

**Results**

**Serum Responses to TT Immunization**

Measurement of serum antibody levels against TT before and 2 wk after simultaneous immunization with these antigens showed patterns typical of secondary immune responses against recall antigens. In all of the participants, titers of both IgG- and IgA-specific antibodies against TT rose dramatically after immunization (Figure 2). There was no difference between the IgAN and control groups in the serum levels of IgG, IgA, or IgA1 antibodies at either time point (Table 1). We also attempted to measure IgA2 antibodies against TT, but only trace amounts were detectable in a very few individuals (data not shown).

**O-Glycosylation of Serum IgA1 Antibodies against TT and HP**

We compared the O-glycosylation of serum IgA1 antibodies in two separate groups of participants: Anti-TT in the 24 immunized individuals (12 control subjects and 12 patients) and anti-HP in a different group of 24 individuals (11 control subjects and 13 patients). We first measured the VV lectin binding of total serum IgA1 in these two groups. Including both patients and control subjects together, there was no difference in the lectin binding of serum IgA1 of the TT and HP groups, indicating that the groups were comparable to one another and neither group had an unusual pattern of IgA1 O-glycosylation overall (Figure 3). However, when we compared antigen-specific serum IgA1 antibodies in these two groups, we found that IgA1 anti-HP had significantly and strikingly higher lectin binding than IgA1 anti-TT (Figure 3).

We then compared the lectin binding of total and specific IgA1 in patients with IgAN and control subjects. As expected, the total serum IgA1 of patients with IgAN had higher lectin binding than that of control subjects, demonstrating that these patients display the usual abnormality of IgA1 O-glycosylation. However, there was no difference between patients and control subjects in the lectin binding of IgA1 antibodies against either TT or HP (Figure 4), with anti-TT having low lectin binding and anti-HP high lectin binding in both control subjects and patients with IgAN (A$_{492}$VV/A$_{492}$ anti-IgA1, controls: anti-TT 0.31 ± 0.03; anti-HP 0.63 ± 0.05 [P < 0.001]; IgAN: anti-TT 0.35 ± 0.04; anti-HP 0.61 ± 0.09 [P < 0.02]).

Finally, we identified nine participants (five control subjects, four patients with IgAN) in the TT immunized group who coincidently also had high titers of serum antibodies against HP. Therefore, in these individuals, we were able to compare the lectin binding of specific IgA1 antibodies with both antigens in the same individual. In all cases, serum IgA1 anti-HP had higher lectin binding than the serum IgA1 anti-TT of the same individual (Figure 5). However, we found no correlation between the O-glycosylation patterns of anti-TT and anti-HP in this small group of individuals (*r* = 0.477, *P* = 0.194).

**O-Glycosylation of Serum and Milk IgA1**

We compared the binding of VV lectin with IgA1 from serum and milk samples from four normal breastfeeding women. In all four cases, the lectin binding of milk (mucosally secreted)

### Table 1. Serum antibody responses after booster immunization with TT

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Serum Antibody Responses after Booster Immunization with TT (Mean AU/ml ± SEM)</th>
<th>Control Subjects (<em>n</em> = 12)</th>
<th>Patients with IgAN (<em>n</em> = 12)</th>
<th><em>P</em></th>
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<tbody>
<tr>
<td><strong>IgG</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>at immunization</td>
<td>149 ± 38</td>
<td>110 ± 23</td>
<td>NS</td>
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<tr>
<td>14 d later</td>
<td>342 ± 60</td>
<td>1250 ± 439</td>
<td>NS</td>
<td></td>
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<tr>
<td><strong>IgA</strong></td>
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<tr>
<td>at immunization</td>
<td>722 ± 151</td>
<td>593 ± 155</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>14 d later</td>
<td>1700 ± 387</td>
<td>2704 ± 739</td>
<td>NS</td>
<td></td>
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<tr>
<td><strong>IgA1</strong></td>
<td></td>
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<tr>
<td>at immunization</td>
<td>44 ± 9</td>
<td>62 ± 29</td>
<td>NS</td>
<td></td>
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<tr>
<td>14 d later</td>
<td>214 ± 91</td>
<td>179 ± 45</td>
<td>NS</td>
<td></td>
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</table>

*Twelve patients with IgA nephropathy (IgAN) and 12 matched control subjects were given subcutaneous booster immunizations with tetanus toxoid (TT). All participants had previously received this vaccine not less than 5 yr before the study. Serum was obtained at the time of immunization (for baseline antibody levels) and 14 d later. IgG, IgA, and IgA1 antibody levels were measured by antigen-specific ELISA. Antibodies of all three classes increased significantly after immunization in all participants, but there was no difference between patients with IgAN and control subjects in the levels of any antibody at any time point.*
and, more recently, underexpression of the chaperone protein Cosmc, the availability of O-galactosyltransferase (C1Gal-T) (23). This reaction requires the coexpression of a chaperone protein Cosmc, the availability of which may be a limiting factor in the O-galactosylation process (24). In IgAN, IgA1 seems to be undergalactosylated, leading to increased exposure of terminal GalNAc. Defective expression or function of C1Gal-T has been suggested as a basis for this, and, more recently, underexpression of the chaperone protein Cosmc by peripheral blood cells has been demonstrated (25). However, altered IgA1 O-galactosylation cannot be attributed fully to a straightforward lack of galactosylating activity in affected individuals. Any defect must be restricted to IgA1-producing B cells or subpopulations thereof, because other O-glycosylated proteins such as C1 inhibitor (22) and IgD (15) do not share the under-O-galactosylation of IgA1.

In IgAN, increased systemic (26) and decreased mucosal (27, 28) production of polymeric IgA suggests an imbalance between these two distinct immune compartments, but hitherto it has been unclear how this relates to O-glycosylation abnormalities. In this study, we have addressed this issue by investigation in patients with IgAN and control subjects of the O-glycosylation of serum IgA1 antibodies against a systematically administered antigen TT and the mucosal antigen HP.

HP colonizes the stomach, and serum IgA antibodies against it predominantly are polymeric in both patients with IgAN and control subjects, a property that typifies mucosally rather than systemically derived IgA. The site of synthesis of the circulating antibodies to this mucosal antigen is not clear, but the polymeric nature of the IgA antibody indicates that the B cells that are responsible are mucosal or under mucosal influences. By contrast, TT is administered by the subcutaneous route and therefore encountered systemically.

For this study, we developed assays to measure the binding of the O-glycan-specific VV lectin to IgA1 antibodies that were immobilized on TT- or HP-coated immunoplates. Lectins cannot give precise structural information about IgA1 O-glycans because they are not totally specific for their target sugars, and their binding may be influenced by other physicochemical factors in the system. However, assays similar to those described here have been used widely for IgA1 O-glycosylation analysis because they are well suited to the simultaneous analysis of multiple small samples and consistently identify variations in overall IgA1 O-glycosylation profile in different test samples (29). The more precise methods, such as chromatography (21) and mass spectroscopy (30–33), are technically challenging and require larger sample sizes; analysis of antibodies of defined antigen specificity is not possible by such methods at the present time.

The TT and HP antibodies were from different groups of patients and control subjects. As expected, both patient groups had higher lectin binding to total serum IgA1 than did control subjects, indicating that these patient groups display the typical O-glycosylation abnormality often reported in IgAN (3). However, the lectin binding of the antigen-specific IgA1 antibodies to TT or to HP did not differ in patients and control subjects. This perhaps unexpected finding indicates that the O-glycosylation of antigen-specific IgA1 antibodies is normal in IgAN.

However, we found that in both patients with IgAN and control subjects, the lectin binding of IgA1 anti-HP was strikingly and significantly high, whereas that of IgA1 anti-TT was low, indicating that antibodies of different specificities have markedly different O-glycosylation profiles. This observation indicates that the production of the alternative IgA1 O-glycoforms is controlled differentially in different immune responses. Thus, serum normally contains a mixture of IgA1

![Figure 3. O-glycosylation profiles of IgA1 antibodies in TT-immunized and Helicobacter pylori (HP)-infected groups. IgA1 was purified from serum, and the O-glycosylation profile of each IgA1 sample was assessed by the binding of the O-glycan-specific lectin Vicia villosa (VV) in modified ELISA. The cohorts that were immunized with TT (□) and infected with HP (■) are compared with one another, with both patients and control subjects included together. (Left) Lectin binding of total serum IgA1 applied directly to uncoated immunoplates. There is no difference in the overall O-glycosylation profile of total serum IgA1 between the TT and HP cohorts, showing that they are comparable to one another for the purposes of this study. (Right) Lectin binding to antigen-specific IgA1 antibodies that were immobilized on antigen-coated immunoplates. IgA1 antibodies to HP have significantly higher lectin binding than IgA1 antibodies to TT, demonstrating that antibodies that are produced in these immune responses have distinct and different O-glycosylation profiles.]
molecules with varying O-glycosylation profiles, which at least partly represent different ongoing immune responses.

It is widely recognized that total serum IgA1 in IgAN has a characteristic profile of higher VV lectin binding than in control subjects (22,34), which was assumed to demonstrate a fundamental “abnormality” of IgA1 O-glycosylation in these patients, perhaps as a result of deficient glycosyltransferase function. However, in this study, we found that IgA1 anti-TT had low VV lectin binding in both patients with IgAN and control subjects, showing that the patients are able to produce the full range of IgA1 O-glycoforms and that the overall high lectin binding of their total serum IgA1 is not due to an intrinsic abnormality of the O-glycosylation process.

Furthermore, we have shown that healthy control subjects produce IgA1 anti-HP molecules with high VV binding. Indeed, patients with IgAN and control subjects do not differ in this regard, demonstrating that this type of O-glycosylation pattern is a constituent of normal serum rather than being peculiar to IgAN. The raised lectin binding of total serum IgA1 in IgAN therefore is much more likely to be due to an increased proportion of a particular type of IgA1 than the presence of a truly abnormal glycoform.

The factors that control IgA1 O-glycosylation are poorly understood, but the results of this study clearly show that this process is differentially regulated in the two immune responses investigated. This may be due to the kinetics of the respective responses at the time point at which the blood samples were taken. TT was given as single-booster immunization, and the antibodies were studied 2 wk later during the active phase of a secondary immune response that was initiated after a long period of quiescence since the previous exposure to the antigen. The HP group had active infection and would have been subject to continuous antigen exposure for some time before the point of study. It is plausible that the IgA1 O-glycoforms produced may change during the development of immune responses. Some evidence for this is provided by our recent observation that the O-glycosylation patterns of serum IgD (restricted to primary immune responses) and IgA1 (mostly produced during secondary responses) are distinct from one another, despite that both of these Ig isotypes carry the same type of O-linked sugar chains (15). It is therefore possible that IgA1 with high VV binding is an indicator of a certain stage of development of an IgA response, which is overrepresented in IgAN. There is some evidence for aberrant development of IgA1 immune responses in IgAN, provided by the observation that IgA affinity maturation fails to occur during the systemic response to TT (35). It is possible that altered IgA1 O-glycosylation may be a further reflection of the same defect of functional immunity.

Another explanation for the marked contrast in the O-glycosylation of IgA1 antibodies to TT and HP is that the site of antigen exposure profoundly influences the O-glycoforms that are produced. HP is a mucosal antigen, and the high VV lectin binding pattern of IgA1 antibodies against it seems likely to be an additional facet of their mucosal nature. This is supported further by the observation that IgA1 from human breast milk samples, another mucosal source, also has high VV binding compared with serum IgA1 from the same individuals. This may be due to the different routes of antigen presentation that operate in mucosal and systemic immune induction sites,
which lead to particular microenvironments that affect the development of locally activated B cells (36). In the mucosa, the mode of antigen presentation influences the key "decision" as to whether tolerance or an inflammatory immune response should ensue. Nonprofessional antigen-presenting cells probably play a larger role in induction of mucosal immune responses than they do in systemic sites and may exert downstream effects on the characteristics of the antibodies produced. Mucosal immune induction preferentially promotes B cell class switching to IgA and also J chain coexpression to produce polymeric rather than monomeric IgA antibodies. The same influences also may affect B cell glycosyltransferase expression with the resultant production of IgA1 with a characteristic "mucosal" O-glycosylation pattern.

There are speculative but plausible reasons for why the preferred IgA1 O-glycoform may vary according to origin. In the mucosa, neutralization and exclusion of potentially pathogenic antigens are preferred to acute inflammatory reactions. Polymeric antibodies dominate here, forming large immune complexes that are readily excluded. IgA is particularly suited to this role because it is noninflammatory compared with IgM (37). Particular IgA1 O-glycoforms may be favorable in this context by contributing to complex formation via lectin-like interactions. Indeed, undergalactosylated IgA1 from patients with IgAN has been shown to be "sticky," or prone to complex formation (5,6).

Whereas IgA1 antibodies to systemic TT display low VV binding and those to mucosal HP display high VV binding in both patients with IgAN and control subjects, total serum IgA1 is intermediate in this respect, containing as it does a mixture of systemic and mucosal antibodies. The raised VV binding of total serum IgA1 in IgAN is probably a consequence of an increased proportion of circulating mucosal-type IgA1 antibodies in these patients. Our previous published study of this group of patients with IgAN showed that their serum levels of IgA1 anti-HP are higher than those of control subjects (18), and other groups have demonstrated the same for other mucosal antigens (38,39).

This excess of serum IgA1 antibodies to mucosally encountered antigens in IgAN is intriguing, because the IgA production at mucosal sites (28) and the mucosal response to mucosal antigen exposure (27) are reduced in these patients. Overall, there is a clear disturbance of mucosal antigen handling in IgAN, resulting in inappropriately high and persistent circulating levels of polymeric IgA1 against mucosal antigens, which also carries an O-glycosylation pattern that is more suited to antibodies that are destined for mucosal secretion. Together, the polymeric nature and "sticky" O-glycoform may create an IgA1 phenotype that is particularly prone to glomerular deposition.

In summary, the results of this study demonstrate that both patients with IgAN and control subjects produce IgA1 with a wide range of O-glycosylation patterns, and this is at least partly dictated by the nature of the immune response involved. The "undergalactosylated" form of IgA1 is not restricted to IgAN but also is produced by control subjects in certain conditions. Equally, patients with IgAN are able to produce well-galactosylated IgA1 O-glycoforms, as seen in response to systemic TT. Therefore, the overrepresentation of the "undergalactosylated" form of IgA1 in the serum of patients with IgAN is not due to a basic abnormality of IgA1 O-glycosylation by B cells but is a further reflection of disturbed IgA immune responses in these patients.
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