Differential Glycosylation of Polymeric and Monomeric IgA: A Possible Role in Glomerular Inflammation in IgA Nephropathy

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IgA nephropathy (IgAN) is characterized by mesangial deposition of polymeric IgA1 (pIgA1) and complement. Complement activation via mannose-binding lectin and the lectin pathway is associated with disease progression. Furthermore, recent studies have indicated a possible role for secretory IgA. IgAN is associated with abnormalities in circulating IgA, including aberrant O-linked glycosylation. This study characterized and compared functional properties and N-linked glycosylation of highly purified monomeric IgA (mIgA) and pIgA from patients with IgAN and control subjects. Total serum IgA was affinity-purified from patients (n = 11) and control subjects (n = 11) followed by size separation. pIgA but not mIgA contained secretory IgA, and its concentration was significantly higher in patients with IgAN than in control subjects. Both in patients with IgAN and in control subjects, IgA binding to the GalNAc-specific lectin Helix Aspersa and to mannose-binding lectin was much stronger for pIgA than for mIgA. Furthermore, binding of IgA to mesangial cells largely was restricted to polymeric IgA. Binding of pIgA to mesangial cells resulted in increased production of IL-8, predominantly with IgA from patients with IgAN. Quantitative analysis of N-linked glycosylation of IgA heavy chains showed significant differences in glycan composition between mIgA and pIgA, including the presence of oligomannose exclusively on pIgA. In conclusion, binding and activation of mesangial cells, as well as lectin pathway activation, is a predominant characteristic of pIgA as opposed to mIgA. Furthermore, pIgA has different N-glycans, which may recruit lectins of the inflammatory pathway. These results underscore the role of pIgA in glomerular inflammation in IgAN.


Primary IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis. The disease leads to progressive renal failure in a substantial proportion of patients. The hallmark of this disease is deposition of IgA in the glomerular mesangium, together with markers of complement activation (1,2). It generally is thought that this mesangial IgA mainly consists of IgA1 and is mostly polymeric (3). The composition of polymeric forms of IgA (pIgA) in serum is diverse and may include dimeric IgA, secretory IgA (sIgA), CD89 (FcαRI)/IgA complexes, IgA immune complexes, and IgA–fibronectin complexes (4–6). Serum dimeric IgA consists of two IgA molecules linked with J chain, whereas sIgA in addition contains secretory component, derived from the mucosal epithelium.

Deposition of circulating IgA in the mesangium leads to renal inflammation, potentially involving direct interactions of IgA with resident and infiltrating cells in the glomerulus, as well as complement activation. The inflammatory process results in renal injury. Although the mechanism of IgA deposition in the renal mesangium of patients with IgAN has been a subject of intensive research during the past decades, the pathogenesis of IgAN is still incompletely characterized. A number of studies provided evidence for a mesangial IgA receptor, which is involved in mesangial cell activation by IgA in vitro (7–9).

Globular IgA deposition is associated with activation of the complement system (5), involving the alternative pathway and the lectin pathway of complement (10). Recent studies indicate deposition of mannose-binding lectin (MBL), a major recognition molecule of the lectin pathway of complement, in a subpopulation of patients in association with a more severe renal injury (10,11), whereas in vitro studies demonstrated binding of MBL to polymeric serum IgA (12). Globular complement activation can enhance renal injury via the proinflammatory effects of the complement activation cascade.

Studies in patients who had IgAN and received a renal transplant showed recurrence of mesangial IgA deposition in a high number of cases (13). Conversely, the accidental transplantation of a kidney with mesangial IgA deposition into a recipient without IgAN resulted in spontaneous disappearance of
IgA deposits after transplantation (14). These studies strongly suggest that IgAN is a systemic disease rather than a disease of the kidney.

On basis of these data, abnormalities in IgA are hypothesized to be involved in the pathogenesis of IgAN. Therefore, circulating IgA from patients with IgAN has been studied extensively. Serum from patients with IgAN contains higher concentrations of IgA (15,16). Recently, our group showed low concentrations of circulating SlgA in patients with IgAN and control subjects, whereas patients with IgAN and a high serum concentration of SlgA showed more hematuria (17). Furthermore, SlgA accumulated in glomerular IgA deposits, suggesting a pathogenic role for SlgA in IgAN (17). Several studies also focused on IgA glycosylation, showing aberrant O-glycosylation on circulating IgA from patients with IgAN, resulting in increased Tn antigen (GalNAcα1-Ser/Thr) residues (18,19).

This undergalactosylated IgA1 may lead to generation of circulating IgG–IgA1 complexes (20). O-linked glycans are present on IgA1 but not IgA2, whereas IgA1 and IgA2 heavy chains both contain several N-glycosylation sites (21). The galactosylation of the N-glycans is not different between patients with IgAN and control subjects (22). However, the complete structure of N-linked glycans on IgA has not been studied in IgAN.

Functional studies with purified IgA from patients with IgAN suggested an increased interaction of IgA with mesangial cells from patients with IgAN as compared with IgA from healthy individuals (23), although this still is controversial (24). Furthermore, after stimulation of mesangial cells with IgA from patients with IgAN, the production of proinflammatory cytokines and chemokines was shown to be increased (25), possibly involving (26,27) undergalactosylation of IgAN IgA (28).

The aim of our study was to characterize and compare the molecular composition and functional properties of monomeric and polymeric serum IgA from patients with IgAN and control subjects. Therefore, we analyzed highly purified total serum IgA from patients and control subjects in a number of aspects that potentially are important in the pathogenesis of IgAN, including interaction with lectins and mesangial cells. The results show clear functional differences between naturally occurring polymeric and monomeric serum IgA both for patients and control subjects. The most obvious difference that was noted between IgA that was isolated from patients with IgAN and from control subjects was an increased fraction of SlgA in plgA from patients with IgAN. Furthermore, we demonstrate that plgA differs from monomeric IgA (mIgA) in its composition of N-linked glycans.


down cellulose column of 15 ml was loaded with human IgA; provided by Dr. J. van den Born, Free University Medical Center, Amsterdam, The Netherlands) coupled to cyanogen bromide–activated Sepharose 4FF (Amersham, Roosendaal, The Netherlands). A column of 15 ml was loaded with γ-globulin precipitate corresponding to 10 ml of serum, using 0.5× PBS as a running buffer, followed by washing with 90 ml 0.5× PBS. Fractions of 3 ml were collected. For removal of nonspecifically bound proteins, the column was washed with 70 ml of 1 M NaCl. Finally, bound IgA was eluted with 100 ml of 0.1 M glycine/0.3 M NaCl (pH 2.8). Fractions were neutralized with 1 M Tris (pH 8.0). Fractions that contained IgA, as assessed by ELISA (29), were pooled, dialyzed against PBS that contained 2 mM EDTA, and applied to a mixture of protein G/anti-human IgM (HB57)-Biogel A5 to remove residual contaminating IgG and IgM, followed by concentration and size separation with a HiLoad 16/60 HR200 Superdex prep grade gel filtration column (120 ml; Amersham Pharmacia), run in 50 mM NH₄HCO₃. Fractions were assessed for the presence of IgA and total protein. On basis of the protein profile, IgA-containing fractions were pooled into plgA (eluted at 44 to 50 ml) and mlgA (eluted at 50 to 60 ml). These pools were analyzed for total IgA, IgA1, and IgA2 content using ELISA (29). The percentage of plgA was quantified using calculation of the area under the curve on the basis of the gel filtration profile.


down cellulose column of 15 ml was loaded with human IgA; provided by Dr. J. van den Born, Free University Medical Center, Amsterdam, The Netherlands) coupled to cyanogen bromide–activated Sepharose 4FF (Amersham, Roosendaal, The Netherlands). A column of 15 ml was loaded with γ-globulin precipitate corresponding to 10 ml of serum, using 0.5× PBS as a running buffer, followed by washing with 90 ml 0.5× PBS. Fractions of 3 ml were collected. For removal of nonspecifically bound proteins, the column was washed with 70 ml of 1 M NaCl. Finally, bound IgA was eluted with 100 ml of 0.1 M glycine/0.3 M NaCl (pH 2.8). Fractions were neutralized with 1 M Tris (pH 8.0). Fractions that contained IgA, as assessed by ELISA (29), were pooled, dialyzed against PBS that contained 2 mM EDTA, and applied to a mixture of protein G/anti-human IgM (HB57)-Biogel A5 to remove residual contaminating IgG and IgM, followed by concentration and size separation with a HiLoad 16/60 HR200 Superdex prep grade gel filtration column (120 ml; Amersham Pharmacia), run in 50 mM NH₄HCO₃. Fractions were assessed for the presence of IgA and total protein. On basis of the protein profile, IgA-containing fractions were pooled into plgA (eluted at 44 to 50 ml) and mlgA (eluted at 50 to 60 ml). These pools were analyzed for total IgA, IgA1, and IgA2 content using ELISA (29). The percentage of plgA was quantified using calculation of the area under the curve on the basis of the gel filtration profile.


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Table 1. Clinical characteristics of the patients with IgAN and control subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with IgAN</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Male/female</td>
<td>8/3</td>
<td>9/2</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>48</td>
<td>33</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L; mean [range]</td>
<td>190 (82 to 366)</td>
<td>ND</td>
</tr>
<tr>
<td>Proteinuria (0 to 3+; median)</td>
<td>1+</td>
<td>ND</td>
</tr>
<tr>
<td>Erythrocyturia (0 to 5+; median)</td>
<td>1+</td>
<td>ND</td>
</tr>
</tbody>
</table>

*IgAN, IgA nephropathy; ND, not determined.
preparation of MBL in complex with its associated serine proteases (MASP). MBL binding was studied by ELISA, in which 5 μg/ml IgA or human serum albumin (HSA) as a control was coated, followed by blocking with PBS/BSA, incubation with MBL (2 μg/ml), and detection of MBL binding as described (12). For inhibition experiments, MBL was preincubated with MgEGTA (10 mM), d-mannose, or l-mannose (100 mM; Sigma, St. Louis, MO).

**Activation of C4 via the Lectin Pathway**

Activation of C4 by MBL–MASP complexes was measured as described previously (12). In brief, incubation of MBL–MASP complexes on coated IgA was followed by incubation with purified C4 and detection of C4 binding.

**Helix Aspersa Binding**

IgA was assayed for binding to biotinylated *Helix Aspersa* (HAA; Sigma) lectin, known to recognize terminal GalNAc. NUNC Maxisorp plates were coated with 5 μg/ml IgA or HSA as a control, in carbonate buffer (pH 9.6), overnight at room temperature. After washing with PBS/Tween and blocking for 1 h with PBS/1% BSA, wells were incubated with 5 μg/ml biotinylated HAA in PBS/1% BSA/0.05% Tween. Binding of HAA was detected with horseradish peroxidase–conjugated streptavidin (Zymed, Invitrogen, Brech, The Netherlands). Enzyme activity of horseradish peroxidase–conjugated streptavidin was visualized using PE-conjugated goat anti-mouse IgG1 (Southern Bio-technology, Birmingham, AL), and fluorescence intensity was assessed by flow cytometry (FACS Calibur, Cell Quest Software; BD Biosciences, Alphen aan den Rijn, The Netherlands). Dead cells, identified by propidium iodide uptake, were excluded from analysis.

**Flow Cytometry**

Cells were washed with FACS buffer (0.5× PBS that contained 1% BSA/2.8% glucose/0.01% NaN₃) and incubated with mlgA and plgA. After incubation for 1 h at 4°C, cells were washed and incubated for 1 h at 4°C with monoclonal anti-IgA mAb 4E8 (IgG1) (29). IgA binding was visualized using PE-conjugated goat anti-mouse IgG1 (Southern Bio-technology, Birmingham, AL), and fluorescence intensity was assessed by flow cytometry (FACS Calibur, Cell Quest Software; BD Biosciences, Alphen aan den Rijn, The Netherlands). Dead cells, identified by propidium iodide uptake, were excluded from analysis.

**Cytokine Analysis**

Production of IL-8 and monocyte chemoattractant protein-1 (MCP-1) was measured in supernatants of cultured mesangial cells. Before stimulation, cells were transferred to 96-wells plates (Costar, Corning, NY) at a density of 15 × 10³ cells per well and cultured overnight in culture medium with 0.5% serum. Cells were cultured in the presence or absence of mlgA and plgA for 72 h, in concentrations as indicated. The concentration of IL-8 and MCP-1 in culture supernatants was measured by ELISA as described previously (30,31).

**N-Glycan Analysis**

The IgA heavy chains were isolated on SDS-PAGE under reducing conditions and visualized by Coomassie staining. The N-glycans were released from these excised gel bands by PNGase F, labeled with the fluorophore 2-aminobenzamide, and analyzed by normal-phase HPLC with exoglycosidase sequencing as described previously (32).

**Statistical Analyses**

Statistical analysis was performed using the Mann-Whitney test and the Wilcoxon signed rank test. The Spearman rank correlation coefficient was used to analyze correlations. Differences in N-glycan composition were evaluated using the t test. Differences were considered statistically significant at P < 0.05.
of pIgA to mesangial cells and IL-8 production after co-culture (R = 0.6450, P = 0.0038; Figure 2C). Furthermore, stimulation with IgA clearly enhanced production of MCP-1, and MCP-1 production correlated with IL-8 production after stimulation with different IgA samples (R = 0.59, P = 0.01). These functional data indicate intrinsic differences between mIgA and pIgA.

Interaction of pIgA with HAA Lectin

It has been reported that O-glycans of patients with IgAN contain more Tn antigen (GalNAc-Ser/Thr) compared with control subjects (18,33). Terminal GalNAc can be detected by specific lectins, including HAA. We investigated the binding of HAA to IgA by ELISA (Figure 3). The binding of HAA to IgA from healthy individuals and patients with IgAN was four-fold higher for pIgA than for mIgA (P = 0.0003). However, we could not observe a difference in HAA binding between patients and control subjects.

Figure 1. Purification of monomeric IgA (mIgA) and polymeric IgA (pIgA). (A) Affinity purification of IgA with an anti-IgA column. First peak is flow through (protein, no IgA), the second peak is after washing with 0.5 M NaCl (protein, no IgA), and the third peak is after acid elution. This peak contains IgA as detected with ELISA. (B) Size fractionation of IgA on a HiLoad 16/60 HR 200 Superdex prep grade gel filtration column. All fractions were measured for total protein and the presence of total IgA by ELISA. IgA was pooled in pIgA (44 to 50 ml) and mIgA (50 to 60 ml) as indicated.

Figure 2. Increased binding and stimulation of mesangial cells with pIgA. (A) Normal human mesangial cells were incubated with different molecular forms of IgA (200 μg/ml) from patients with IgAN and control subjects and assessed for IgA binding by flow cytometry. Depicted is the mean fluorescence intensity after subtracting the isotype control. (B) Human mesangial cells (15 × 10⁶ cells/well) were stimulated with different molecular forms of IgA (200 μg/ml). After 72 h, supernatants were harvested and tested for IL-8. Horizontal lines indicate the median; dotted line represents the detection limit. IL-8 was undetectable in cultures without IgA. P < 0.01, pIgA versus mIgA (A and B). (C) Correlation between production of IL-8 after stimulation of mesangial cells with IgA and the binding of IgA to mesangial cells.
MBL Binds Exclusively to pIgA, Resulting in C4 Activation

We and others showed that IgAN is associated with complement activation via the lectin pathway (10,34). Therefore, the binding of MBL to purified IgA from patients with IgAN and control subjects was studied, showing that MBL binds to pIgA but not to mIgA with a high interindividual variation, both for patients with IgAN and for control subjects (Figure 4A). Using parallel detection of immobilized IgA on plates, we confirmed that equal amounts of IgA were present in coated wells, indicating that differences in coating could not explain the observed differences (data not shown). Binding of MBL to IgA was completely inhibitable by d-mannose and MgEGTA but not by l-mannose (Figure 4B), confirming that the C-type lectin domain of MBL was involved in binding to IgA.

Binding of MBL to pIgA from patients with IgAN and from control subjects resulted in activation of purified C4 (Figure 4C), presumably involving C4 cleavage by MBL-associated MASP-2. This activation of C4 showed a strong correlation with MBL binding (R = 0.98, P < 0.0001 for pIgA).

Treatment of IgA with Neuraminidase Enhances Its Interaction with HAA

To examine whether sialic acids, commonly present on N-linked and O-linked glycans of IgA, might hamper the interaction of IgA with HAA and/or MBL, we treated immobilized IgA with neuraminidase. After treatment of IgA, the interaction with HAA increased significantly (3.8-fold for control mIgA, 3.1-fold for control pIgA, 3.2-fold for control pIgA, 3.0-fold for control pIgA; P < 0.004; Figure 5A), suggesting the presence of sialylated Tn antigen, because the removal of the sialic acid exposes the GalNAc (Tn) epitope. In contrast, the binding of MBL to IgA was hardly affected by neuraminidase, only showing a minor increase after treatment of pIgA (1.1-fold; Figure 5B), consistent with the known specificity of MBL for glycans that present 3,4 cis hydroxyls such as mannose, to which sialic acids do not attach.
Molecular Composition of mIgA and pIgA from Patients with IgAN and Control Subjects

The results presented indicate major functional differences between mIgA and pIgA. We therefore investigated the molecular composition of mIgA and pIgA from patients with IgAN and control subjects. The size distribution of IgA from patients with IgAN and control subjects was similar (mean % pIgA: controls 18.9%, patients 18.8%; \( P < 0.0001 \)). The IgA preparations were assessed for IgA1 and IgA2 content by ELISA. As described before (35), IgA2 is a minor constituent of human serum IgA. However, the relative amount of IgA2 was significantly higher in pIgA (20 ± 4.1%) as compared with mIgA (9.2 ± 4.7%; \( P < 0.0001 \); Figure 6A), suggesting that circulating IgA2 is more likely to be produced as polymeric complexes than circulating IgA1. The relative amount of IgA2 was similar in IgA from patients and control subjects.

Subsequently, we measured SlgA in mIgA and pIgA from patients with IgAN and control subjects. In agreement with the molecular size of SlgA, SlgA is present exclusively in pIgA (Figure 6B). SlgA comprised <1% of total polymeric serum IgA. However, the proportion of SlgA that is present in pIgA from patients with IgAN is 2.5 times higher than in pIgA from control subjects (\( P = 0.0152 \)).

pIgA Shows a Different Composition of N-Linked Glycans Compared with mIgA

Recent studies showed that MBL is able to bind to N-linked glycans of IgG (36) and IgM (37). On the basis of the known structures of N-linked and O-linked glycans on IgA, it is more likely that MBL would bind to N-linked glycans than to the O-linked glycans. Furthermore, information on N-linked glycosylation of IgA in IgAN is not available. Therefore, we characterized the N-glycans of 6 mIgA and 6 pIgA preparations in detail.

Heavy chains and light chains of mIgA and pIgA were separated by SDS-PAGE (Figure 7). N-linked glycans were released via an in-gel digestion of the heavy-chain and light-chain bands of IgA using PNGase F. Isolated glycan samples were...
labeled with 2-aminobenzamide and run on normal-phase HPLC. Consistent with earlier data (32), light chains of IgA were found not to be glycosylated (data not shown). The elution pattern of heavy chains is shown in Figure 8A. The most prominent peaks, present between glucose units 8 and 10, represent complex glycans that are sialylated, as was demonstrated by a neuraminidase digestion (Figure 8B, abs). No obvious differences could be observed between N-glycans from patients and from control subjects. However, upon comparison of glycans from mlgA and plgA, a single peak at GU 6.2 was present in all plgA samples but absent in mlgA. Digestion with mannosidase (Figure 8B, jbm) demonstrated that this is an oligomannose structure (Man5), as schematically drawn in Figure 8A.

Using sequential enzyme digestions (Figure 8B), the glycan structures on these samples were identified and quantified (Table 2). Comparison of mlgA and plgA revealed that 3.3% of the total glycan pool contained Man5 in plgA, whereas this structure is absent in mlgA. Furthermore, the double-sialylated glycans are underrepresented in plgA as compared with mlgA (29 and 37%, respectively; \( P = 0.001 \)), resulting in a shift to smaller glycan structures on plgA. There were no significant differences between patients and control subjects. Together, the results indicate that N-linked glycosylation of the IgA heavy chain is significantly different between mlgA and plgA (Table 2).

Discussion
Deposition of IgA in the renal mesangium is the primary characteristic of IgAN and is responsible for glomerular inflammation and finally the development of renal failure. On the basis of earlier observations of mesangial IgA, this IgA is believed to be largely plgA1. In this study, we show that plgA, as opposed to mlgA, from patients with IgAN and from healthy control subjects shows increased binding to and activation of mesangial cells and has a superior capacity to bind the complement-activating lectin MBL. These aspects of plgA are most likely to be involved in induction of glomerular deposition and inflammation. Furthermore, we provide evidence that plgA is differently glycosylated from mlgA, as suggested by lectin binding studies and demonstrated by a direct identification of N-linked glycans. It is noteworthy that the only obvious difference observed between IgA from patients with IgAN and con-
Comparisons between IgA that was isolated from patients and from healthy control subjects were reported previously (19, 22, 28, 38–40). Most experiments were performed with either fractionated total serum without an IgA purification step (28, 39, 40) or with pooled serum IgA purified with Jacalin, a lectin that binds Gal\(^{1–3}\)GalNAc (39–41). In our study, we purified IgA from individual patients and control subjects with an anti-IgA mAb. With this method, we prepared mIgA and pIgA that contains total and highly pure serum IgA without a preceding selection for certain IgA glycoforms. In contrast to methods using Jacalin, this method also enabled us to isolate the IgA2 that is present in serum.

Previous studies described that the binding of pIgA to mesangial cells was higher than that of mIgA (23, 28), although this could not be reproduced by others (24). Moreover, the binding of patient IgA and that of \textit{in vitro} degalactosylated IgA was higher than that of control IgA (24). In this study, we confirm a prominent increase in mesangial cell binding of pIgA over mIgA, but we did not detect a difference between patient and control IgA. Our studies further establish the proinflammatory properties of pIgA and demonstrate that IgA-induced chemokine production correlates with the interaction of IgA with the mesangial cell surface. It is most likely that next to the increased binding of pIgA, also a more efficient receptor cross-linking will contribute to its proinflammatory action. In addition, the observed biochemical properties of pIgA might contribute to this process. However, unlike previous investigations (27), this property could not be attributed specifically to IgA that was derived from patients with IgAN.

Next to the direct effects of IgA on mesangial cells, activation products of the complement system, involving both the alternative pathway and the lectin pathway, are likely to drive the local inflammatory process. Activation of the lectin pathway of complement via an interaction between MBL and IgA has been described on IgA heavy chains of monomeric and polymeric IgA.

### Table 2. Analysis of N-glycans of IgA heavy chains of monomeric and polymeric IgA

<table>
<thead>
<tr>
<th>Major Structure(^b)</th>
<th>GU</th>
<th>Monomeric</th>
<th>Polymeric</th>
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<tr>
<td></td>
<td></td>
<td>Control</td>
<td>IgAN</td>
<td>Mean</td>
</tr>
<tr>
<td>A2B</td>
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<td>Man 5</td>
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</table>

\(\text{a}\) N-glycans were identified on IgA heavy chains of monomeric and polymeric IgA from six individuals (three patients with IgAN and three control subjects, as indicated), using sequential exoglycosidase digestion as indicated in Figure 8. For each structure, the percentage of total N-glycans is indicated. Differences between monomeric and polymeric IgA are evaluated by paired \(t\) test. GU, glucose units.

\(\text{b}\) All N-glycans have two core GlcNAcs; F, core fucose linked \(\alpha1–6\) to inner GlcNAc; Man(x), number (x) of mannose on core GlcNAcs; A, number (x) of antenna (GlcNAc) on trimannosyl core; B, bisecting GlcNac linked \(\beta1–4\) to inner mannose; Gx, number (x) of galactose on antennae; Sx, number (x) of sialic acids on antennae.
shown before (12), and in this study, we confirm and extend these data by showing that binding of MBL is a common feature of pIgA but not mIgA that was isolated from different donors. Because ligand recognition by MBL requires multiply presented carbohydrates, MBL binding could be favored by the structure of pIgA. Binding of MBL leads to activation of C4 presumably via activation of the C4-cleaving enzyme MASP-2. In a healthy situation, the binding of MBL to pIgA could be involved in host defense. However, in IgAN, lectin pathway activation via pIgA is unfavorable (10).

Many studies on IgA from patients with IgAN focused on glycosylation. IgA is glycosylated extensively, via both N-linkages (IgA1 and IgA2) and O-linkages (IgA1) (21). It was observed consistently that serum IgA from patients with IgAN contains smaller O-linked glycans, with less sialylation and galactosylation, than IgA from healthy control subjects (18). Previous investigations suggested that this predominantly was the case for mIgA (40). Our experiments using HAA, a lectin that is used commonly to detect terminal GalNac on non-galactosylated O-linked glycans, suggested the presence of terminal GalNac (Tn antigen) predominantly on pIgA, from both patients and control subjects. This is in agreement with a previous study that showed reactivity of HAA with high molecular weight serum proteins (28) and with data that were provided by Leung et al. (40). Binding of HAA was strongly increased by neuraminidase treatment, suggesting a high frequency of non-galactosylated O-linked glycans on IgA that expose terminal GalNac after enzymatic removal of sialic acid.

A detailed quantitative analysis of N-linked glycans on IgA heavy chains of mIgA and pIgA revealed several significant differences between these molecular forms of IgA. In this respect, pIgA consistently contained an oligomannose structure that was undetectable on all mIgA preparations and showed significantly less glycans with two terminal sialic acid residues.

Recent studies showed that MBL can bind to certain glycoforms of human IgM, involving GlcNAc-terminated glycans and oligomannose structures on the IgM heavy chains (37). Therefore, the presence of specific glycans on pIgA but not mIgA also might be involved in its recognition by MBL. At present, it is unknown whether a specific glycosylation pattern of the heavy chain of pIgA is involved in the polymerization of IgA and/or whether this merely is related to the conditions that are present during production of the different forms of IgA. Earlier studies from our group indicated that polymeric serum IgA contains dimeric IgA linked with J chain, as well as complexes of mIgA linked via other mechanisms (42). In the first case, polymerization takes place in the B cell, and the presence of oligomannose, which is a premature glycan structure, might suggest the endoplasmic reticulum as a possible location for polymerization, which prevents further synthesis of the glycan structure by steric hindrance. In the latter case, polymerization might take place outside the B cell.

Part of the observed differences in glycosylation between mIgA and pIgA also might be explained by the presence of SlgA in the polymeric fraction of serum IgA, because the SlgA heavy-chain N-glycosylation is very different from that of monomeric serum IgA (32). SlgA has approximately 8% oligomannose structures and 60% glycans with exposed GlcNAc with <15% for all glycans sialylated (32), compared with monomeric serum IgA, in which most of the glycans are sialylated. However, SlgA comprises only <1% of total polymeric serum IgA.

We observed that polymeric serum IgA contains more IgA2 than mIgA. This could be because IgA2 polymerizes more easily than IgA1 or that IgA2-producing B cells preferentially secrete pIgA. Bone marrow–derived IgA as present in serum largely is monomeric and of the IgA1 subclass, whereas mucosal IgA largely is polymeric, containing J chain and secretory component (43), and contains a substantial fraction of IgA2 (35). Therefore, an increased fraction of IgA2 and the presence of SlgA in circulating pIgA may suggest its production by the mucosal immune system. Quantitative measurement of the presence of SlgA in polymeric serum IgA suggests that only a minor part of pIgA contains a secretory component. We hypothesize that this SlgA is derived from the mucosal surface. Circulating dimeric IgA without a secretory component could have been produced partially in mucosal lymphoid tissue and directly transported toward the circulation.

Although SlgA requires transepithelial transport for the attachment of a secretory component to dimeric IgA, the presence of low concentrations of circulating SlgA has been described before (17,44,45). Moreover, increased serum levels of SlgA have been reported in various diseases (17,46–48). In contrast to previous studies, we now determined the SlgA concentration in highly purified polymeric serum IgA. Our data demonstrate a clear relative increase in SlgA in pIgA from patients with IgAN compared with control subjects. We recently reported a preferential interaction of SlgA with mesangial cells and showed glomerular accumulation of SlgA in IgAN (17). Therefore, these results further support a role for SlgA in the pathogenesis of IgAN.

Taken together, the presented data suggest that a part of circulating pIgA has a mucosal origin. There is accumulating evidence that the pathogenesis of IgAN is related to aberrant production of IgA. In this respect, in vivo studies indicated that patients with IgAN have a disturbed mucosal immune response, which was restricted to production of antibodies of the IgA1 subclass (49). Our observation that SlgA is increased in the pIgA fraction of patients with IgAN further supports a role for abnormal mucosal immunity. Because IgAN is a slowly progressive disease, it is well conceivable that only a minor subfraction of circulating IgA in patients with IgAN is abnormal and that this IgA gradually accumulates in the mesangial area. We hypothesize that this abnormal IgA is derived at least partially from the mucosal immune system. Because our data strongly indicate that large-sized IgA is especially able to interact with mesangial cells and to induce complement activation, the gradual deposition of such proinflammatory IgA eventually may lead to renal disease.

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