Demonstration of secretory IgA in kidneys of patients with IgA nephropathy

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Abstract

Background. Recently we reported a possible role for secretory IgA (SIgA) in IgA nephropathy (IgAN), as suggested by increased serum levels in patients with active disease and accumulation of SIgA in a glomerular eluate. Therefore, we attempted to find support for these findings by analysis of the presence of SIgA in biopsies of IgAN patients.

Methods. Renal biopsies of 26 patients with biopsy-proven IgAN were analysed for the presence of SIgA and complement proteins.

Results. In 15% mesangial deposition of SIgA was demonstrated, using a specific staining for secretory component (SC) and colocalization with IgA. The presence of SIgA in these biopsies showed a strong correlation with deposition of mannose-binding lectin (MBL) and C4d. Moreover, we observed a strong colocalization between SIgA and MBL or C4d. This local complement activation has previously been linked to more severe renal disease.

Conclusions. Therefore, these data provide additional evidence for a pathogenic role for SIgA in IgA nephropathy.

Keywords: IgA nephropathy; IgA; SIgA; MBL; C4d

Introduction

Primary IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis worldwide. The hallmark of this disease is deposition of IgA in the glomerular mesangium, together with markers of complement activation [1–3]. It is generally thought that deposits of IgA consist of IgA1 which is mostly polymeric [4]. The composition of polymeric IgA in serum is highly diverse and may include CD89/IgA complexes, dimeric IgA, IgA immune complexes and secretory IgA (SIgA) [5–7].

SIgA is the dominant immunoglobulin in external mucosal secretions like in oral, respiratory and intestinal cavities, and is often characterized as a component of the immune systems' first line of defence against pathogens [8]. Next to its presence in mucosal secretions, small amounts of SIgA can also be found in human serum [7,9,10]. Noteworthy, increased serum levels of SIgA have been reported in various diseases like idiopathic membranous nephropathy, idiopathic nephritic syndrome and chronic alcoholic liver disease [11–13], and are associated with more haematuria in IgAN patients [7]. Moreover, polymeric serum IgA of patients with IgAN contains higher SIgA concentrations as compared to healthy controls [14].

Glomerular IgA deposition is associated with activation of the complement system [15], involving the alternative pathway and the lectin pathway of the complement [16]. Recent studies indicate that deposition of mannose-binding lectin (MBL), one of the recognition molecules of the lectin pathway of the complement, in a subpopulation of IgAN patients is associated with a more severe renal injury [16,17], compatible with the observation that MBL bind to polymeric IgA [18].

The aim of the present study was to investigate whether SIgA can be demonstrated in biopsies from patients with IgA nephropathy. The results show that SIgA can be found in a subpopulation of IgAN patients, and that presence of SIgA is associated with the presence of MBL and C4d.

Subjects and methods

Patients and biopsies

Renal biopsies were selected from patients with IgA nephropathy of whom a renal biopsy was taken between January 2001 and December 2003. Patients were selected
when adequate tissue was obtained for diagnostics (at least eight glomeruli in light microscopy sections; complete immunohistology and electron microscopy examination), and when sufficient frozen material was available for additional staining after immunodiagnosis (at least six glomeruli in at least 15 (5 μm thick) tissue sections). Cases with Henoch–Schoenlein purpura, systemic lupus erythematosus, liver cirrhosis or other systemic diseases were excluded. In total, 26 biopsies were selected for evaluation.

Among selected patients, 77% were male and 23% females. Creatinine clearance was calculated according to the Cockcroft formula (range 16–130 ml/min).

Immunofluorescence

For immunofluorescence stainings, unfixed renal tissue was embedded in OCT compound (Sakura Tissue-tek, Bayer), snap-frozen in a mixture of isopentane and dry ice and stored at −80°C. Subsequently, 5 μm sections were placed on slides and stored at −20°C until immunostaining.

We used mouse monoclonal antibodies directed against the following molecules: MBL (mAb 3E7, kindly provided by Prof. Fujita, Fukushima, Japan [19]), and secretory component (SC) (mAb N194-4 from Nordic [7]). Rabbit polyclonal antibodies were applied for detection of IgA (TRITC-labelled anti-human IgA, Dako), C3 (FITC-labelled anti-human C3c, Dako) and C4d (Biomedica [20]). For indirect immunofluorescence, after fixation in cold acetone, tissues were incubated sequentially with the primary antibody and the proper fluorescently labeled secondary antibody (Alexa Fluor 488-conjugated goat anti-mouse Ig for anti-secretory component or Alexa Fluor 546-conjugated goat anti-mouse Ig for anti-MBL, Molecular Probes). Slides were finally mounted with anti-fading aqueous mounting medium (Fluorsave, Calbiochem).

Evaluation of renal tissue

Evaluation of renal tissue was performed blindly by two independent observers. For immunostaining, tissues were scored as negative (0) or positive (1), according to the detection of staining in the majority of the glomeruli, in at least three tubular cross sections per field.

For histology, sections were stained using standard periodic-acid Schiff (PAS), periodic-acid-silver methenamine (PASM), and/or Trichrome techniques. Mesangial proliferation was scored as 1+ when mild (i.e. between four and six cells per mesangial area) and 2+ when intense (more than six cells per mesangial area). Extracapillary proliferation, global sclerosis and segmental sclerosis were calculated as percentage of the total number of glomeruli. Interstitial infiltration and fibrosis were scored 0 when absent, 1+ when mild (involving <30% of the interstitium), 2+ when moderate (30–60% of the interstitium involved), or 3+ when intense (when present in >60% of the renal interstitium). Hyalinosis of the vessel wall was indicated when absent (0) or present (1).

Statistical analysis

Data were compared between IgAN patients showing positive and negative glomerular staining of SIgA, respectively. Frequency analysis was performed using the Fisher exact test. Other comparisons were evaluated using the Mann–Whitney U test. Differences were considered statistically significant when \( P < 0.05 \).

Fig. 1. Glomerular SIgA deposition in IgAN patients. Renal tissue from patients with IgAN was stained for the presence of IgA (red), SC (green) and colocalization of IgA and SC, demonstrating the presence of SIgA (yellow).

Table 1. Clinical and laboratory data from IgA nephropathy patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>SIgA-negative cases ((N = 22))</th>
<th>SIgA-positive cases ((N = 4))</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at renal biopsy</td>
<td>Years (median)</td>
<td>33</td>
<td>26.5</td>
<td>0.0596</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>21–57</td>
<td>24–31</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Female (percentage of cases)</td>
<td>27</td>
<td>0</td>
<td>0.5425</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>Gram/24 hours (median)</td>
<td>1.4</td>
<td>2.1</td>
<td>0.2864</td>
</tr>
<tr>
<td>Macroscopic haematuria</td>
<td>Present (percentage of cases)</td>
<td>37</td>
<td>25</td>
<td>0.6603</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>Micromoles/litre (median)</td>
<td>1.3</td>
<td>1.4</td>
<td>0.4553</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>Millilitres/minute (median)</td>
<td>53</td>
<td>71.5</td>
<td>0.9151</td>
</tr>
<tr>
<td>Serum IgA</td>
<td>Milligram/millilitre</td>
<td>2.40</td>
<td>2.49</td>
<td>0.6698</td>
</tr>
</tbody>
</table>

SIgA-negative cases and SIgA-positive cases are defined on basis of glomerular staining. All data were obtained at the time of renal biopsy.
**Results**

Immunofluorescence staining for SIgA was performed in renal biopsies from 26 IgAN patients. Presence of SIgA was detected with a monoclonal antibody against secretory component (SC), combined with double staining for IgA. As expected, some IgA staining is not colocalized with SIgA, indicating that also other IgA molecules lacking the secretory component are deposited in the glomeruli. Glomerular SIgA positivity was observed in a mesangial pattern in 4 biopsies (15%, Figure 1), whereas glomeruli in 22 biopsies were negative.

Based on the presence (15%) or absence (85%) of glomerular SIgA, two IgAN patient groups were defined and further characterized. SIgA-positive and negative cases had a similar male/female distribution and no difference in renal function (Table 1).

Next we examined the presence of molecules of the complement system (Table 2). In line with our previous study we observed that 19% were positive for MBL and C4d [16]. We observed a strong association between the presence of SIgA and the presence of MBL (\(P = 0.0003\)) and C4d, respectively (\(P = 0.0003\)). Importantly, this association was confirmed by the strong colocalization in renal deposits between SIgA and both MBL and C4d (Figure 2). Furthermore, in line with our previous observation for MBL [16], there was a trend towards more severe mesangial proliferation in SIgA-positive biopsies as compared to SIgA-negative biopsies (Table 2).

**Table 2.** Histological data from IgAN patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>SIgA-negative cases (N = 22)</th>
<th>SIgA-positive cases (N = 4)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesangial proliferation</td>
<td>Intense (percentage of cases)</td>
<td>32</td>
<td>75</td>
<td>0.264</td>
</tr>
<tr>
<td>Extracapillary proliferation</td>
<td>Present (percentage of cases)</td>
<td>27</td>
<td>50</td>
<td>0.543</td>
</tr>
<tr>
<td>Global sclerosis</td>
<td>Percentage of glomeruli (median)</td>
<td>13</td>
<td>23</td>
<td>0.943</td>
</tr>
<tr>
<td>Segmental sclerosis</td>
<td>Percentage of glomeruli (median)</td>
<td>3.5</td>
<td>8</td>
<td>0.972</td>
</tr>
<tr>
<td>Interstitial infiltration</td>
<td>0–3 scale scoring (median)</td>
<td>1</td>
<td>2</td>
<td>0.319</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>0–3 scale scoring (median)</td>
<td>1</td>
<td>1</td>
<td>0.831</td>
</tr>
<tr>
<td>Vascular lesions</td>
<td>Present (percentage of cases)</td>
<td>45</td>
<td>25</td>
<td>0.614</td>
</tr>
<tr>
<td>IgG</td>
<td>Present (percentage of cases)</td>
<td>0</td>
<td>0</td>
<td>1.000</td>
</tr>
<tr>
<td>IgM</td>
<td>Present (percentage of cases)</td>
<td>0</td>
<td>0</td>
<td>1.000</td>
</tr>
<tr>
<td>C3</td>
<td>Present (percentage of cases)</td>
<td>91</td>
<td>50</td>
<td>0.0987</td>
</tr>
<tr>
<td>C4d</td>
<td>Present (percentage of cases)</td>
<td>4.5</td>
<td>100</td>
<td>0.0003</td>
</tr>
<tr>
<td>Glomerular MBL staining</td>
<td>Present (percentage of cases)</td>
<td>4.5</td>
<td>100</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

SIgA-negative cases and SIgA-positive cases are defined on basis of glomerular staining. The two values in bold indicate the significant changes.

**Fig. 2.** Glomerular MBL and C4d deposition with SIgA deposition in IgAN patients. Renal tissues from patients with IgAN were stained for the presence of MBL (red), C4d (red), SIgA (green) and colocalization of SIgA with MBL and C4d in the merge picture (yellow).
Discussion

The present study provides further evidence for a possible pathogenic role for SIgA in IgAN. We demonstrate glomerular deposition of SIgA in biopsies of a subpopulation of IgAN patients. Furthermore, there was a strong association between glomerular SIgA staining and the presence of MBL and C4d, suggesting activation of the lectin pathway of the complement in cases with SIgA deposition.

Deposition of IgA in the mesangial area is the hallmark of patients with IgAN and it is generally thought that this deposition drives a local inflammatory response. Previous research has concentrated on quantitative and qualitative differences of IgA deposited in the kidney. It has been proposed that IgA in renal deposits is mostly high MW of nature and might contain differences in glycosylation which will affect receptor interaction or effector functions. We now show that in a subset of patients SIgA can be demonstrated in the renal biopsies, in line with our previous investigation of a renal eluate [7]. Deposition of SIgA in patients with IgAN has not been widely documented, although it was observed earlier [21,22].

Generation of secretory IgA (SIgA) is a specific process taking place at mucosal surfaces [23]. It has been reported that SIgA adheres selectively to microfold (M) cells irrespective of their antigen-binding specificity [24,25], followed by its transport across the epithelium and targeting of dendritic cells (DC) [26,27]. At present it is unclear how these processes contribute to the appearance of SIgA in circulation [28], as indeed, small amounts of SIgA have also been found in human serum [9,10]. Moreover, increased serum levels of SIgA have been reported in various diseases [11–13] indicating that SIgA may be a marker of clinical interest. We hypothesize that after mucosal challenge the production of SIgA at mucosal sites is increased. This could potentially lead to increased serum SIgA concentrations [7] and thereby, via a presently undefined mechanism, lead to glomerular deposition of SIgA.

Earlier studies have shown that the carbohydrate moieties on SIgA are different when compared to serum IgA [29]. Furthermore, it was shown that MBL can interact with SIgA upon conformational change under acid conditions. This suggests that disruption of the non-covalent interactions between the secretory component and the IgA heavy chain can lead to MBL binding, and subsequently, complement activation via the lectin pathway. Indeed, a difference in interaction with the lectin Vicia villosa was recently observed for IgA1 from breast milk [30]. The data from the present study suggest that in the glomeruli of a subpopulation of IgAN patients, the deposition of SIgA may lead to unmasking the heavy chain of IgA of SIgA leading to MBL binding and complement activation.

Recently, it has been described that MBL deposition in glomeruli is associated with more severe renal disease [16]. In the present study we show a strong co-deposition of SIgA and MBL, suggesting SIgA as a strong cofactor. Furthermore, more haematuria was observed in patients with higher concentrations of SIgA in serum [7]. Moreover, after elution of isolated glomeruli from a patient with IgAN, a 120-fold accumulation of SIgA in the glomeruli was observed. In the present study we provide additional evidence for a pathogenic role of SIgA in a subpopulation of patients with IgA nephropathy.

Acknowledgements. This work was financially supported by the Dutch Kidney Foundation (BDO: C99.1822; AR: PC95).

Conflict of interest statement. None declared.

References


Received for publication: 24.12.06
Accepted in revised form: 4.5.07