The level of serum secretory IgA of patients with IgA nephropathy is elevated and associated with pathological phenotypes

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Abstract

Background. Mucosal infection associated episodic macroscopic haematuria is observed in many patients with IgA nephropathy (IgAN), however, the mechanism has not been elucidated. Recent study suggested that secretory IgA (SIgA) might play an important role in the pathogenesis of IgAN. The aim of this study is to investigate the level of serum SIgA and the deposition of SIgA in glomeruli in IgAN patients with different pathological phenotypes.

Methods. The levels of serum SIgA were detected in 57 patients with IgAN and 48 normal controls. The associations between the levels of SIgA and the pathological phenotypes of IgAN as well as clinical parameters were investigated. Frozen renal sections from 34 of the 57 patients without IgM deposition were immunofluorescence stained and examined by confocal microscopy to detect the co-deposition of IgA and secretory component (SC). The association between deposition of SIgA and the level of serum SIgA was analysed.

Results. The level of serum SIgA in patients with IgAN was significantly higher than that of normal controls. The associations between the levels of SIgA and the pathological phenotypes of IgAN as well as clinical parameters were investigated. Frozen renal sections from 34 of the 57 patients without IgM deposition were immunofluorescence stained and examined by confocal microscopy to detect the co-deposition of IgA and secretory component (SC). The association between deposition of SIgA and the level of serum SIgA was analysed.

Conclusions. It was concluded that mesangial IgA, at least partly, was originated from mucosal immune sites. The levels of serum SIgA were significantly increased in patients with IgAN and were closely associated with pathological phenotypes.

Keywords: IgA nephropathy; pathological phenotype; secretory component; secretory IgA

Introduction

The IgA nephropathy (IgAN) is the most common glomerulonephritis defined by the predominant deposition of IgA in the glomerular mesangium. It is characterized by various pathological phenotypes and variable spectrum of clinical presentation. About 30–40% of these patients will develop progressive renal failure and eventually require either dialysis or kidney transplantation [1].

The pathogenesis of IgAN remains unclear. The clinical association between exacerbation of IgAN and mucosal infections has supported the view that IgAN might be connected with the mucosal immune response. Secretory IgA (SIgA) is mainly in human secretions and represents the major humoral defense mechanism of mucosal areas. Small amounts of SIgA can be found in normal serum and levels of SIgA are elevated in some disorders like liver disease and HIV infection. Moreover, the serum concentration of SIgA was also increased in some patients with IgAN [2,3]. Recently, Oortwijn et al. [4] had established a sandwich enzyme-linked immunosorbent assay (ELISA) to detect the level of SIgA, and found a 120-fold accumulation of SIgA compared to IgA1 in the glomerular eluate of one patient with recurrent IgAN. They further proved that SIgA could bind more to human mesangial cells (HMC) and could induce increased IL-6 production by HMC compared with IgA, which suggested a pathogenic role for SIgA in the progress of IgAN.
However, some other researches favored contrary results in the level of serum SIgA in patients with IgAN [5,6], and the deposition of SIgA could not be found in most of the studies, even in renal cryosections of patients with recurrent IgAN [4]. Therefore, the relationship between mucosal immunity and IgAN is still controversial. In the present study, firstly, the levels of SIgA were detected in sera from healthy controls and IgAN patients with different pathological phenotypes. Then, the associations between the levels of SIgA and pathological phenotypes of IgAN as well as clinical parameters were analysed. Finally, the deposition of SIgA in glomeruli from patients without IgM deposition was investigated and the correlation between deposition of SIgA and the level of serum SIgA was also explored.

Materials and methods

Patients
To detect the serum level of SIgA, 57 patients with renal biopsy-proven IgAN and 48 healthy normal controls with comparable age and gender distribution and negative urinalysis were enrolled. Serum samples from patients were obtained at the time of renal biopsy. Twenty-nine of them had mild mesangial proliferative glomerulonephritis (mIGAN) without overt tubular and interstitial damage in renal histopathology, fulfilling the criteria of Haas-I, a pathologic scheme of IgAN proposed by Haas [7]. The remaining 28 patients had focal proliferative sclerosing IgAN (fpsIgAN) defined as Haas-III or V (see details in Table 1). Informed consent was obtained prior to entry into the study.

To observe SIgA deposition in the kidney, renal cryosections from 34 of the above 57 patients were collected. All the 34 patients were selected based on significant deposition of IgM in glomerular mesangium by routine direct immunofluorescence.

Detection of SIgA in sera by ELISA
In accordance with a previous publication, a sandwich ELISA was used [4]. Mouse monoclonal anti-human secretory component (SC) (Sigma, Saint Louis, USA) diluted to 5.1 μg/ml in 0.05M bicarbonate buffer (PH 9.6) was coated to the wells of one-half of a polystyrene microtitre plate (Costar, Mankato, Minnesota, USA). The wells in the other half were coated with bicarbonate buffer alone to act as antigen-free wells. The volumes of each well for this step and for subsequent steps were 100μl. All incubations were carried out at 37°C for 1 h and the plates were washed three times with 0.01M phosphate-buffered saline (PBS) containing 0.1% Tween 20 (PBST). The plates were then blocked with PBST containing 1% BSA (PBST/BSA). The sera diluted 1:100 were added in duplicate to both antigen-coated and antigen-free wells. Each plate contained a blank control (PBST/BSA) and a positive control. After incubation and washing, rabbit polyclonal anti-human IgA (Dako, Glostrup, Denmark) with a dilution of 1:5000 was added, the wells were then incubated with 1:10 000 diluted horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Zhongshan Biotech, Beijing, China) to detect SIgA in the samples. The reaction was revealed with 0.1 M citrate phosphate buffer (pH 5.0) containing 0.04% o-phenylenediamine and 0.1% H2O2, then the reaction was stopped with 1M H2SO4. The absorbance at 490 nm was recorded in an ELISA reader (Bio-Rad 550, Tokyo, Japan). SIgA purified and isolated from human colostrums (Serotec, Oxford, UK) was used to establish standard curve and as a positive control.

Validation of the specificity and sensitivity of the sandwich ELISA
The specificity of the sandwich ELISA is dependent on the specificity of the anti-SC antibody. To test the specificity of the antibody used in sandwich ELISA, Western blot analysis and direct ELISA were performed. Briefly, in Western blot analysis, 1.0 and 0.5 μg purified SIgA were subjected to 10% SDS-PAGE under reducing conditions followed by transferring to nitrocellulose (NC) membrane (Schleicher & Schuell, NH, USA) by electrophoretic semi-dry blotting system (Amersham Pharmacia, NJ, USA). The NC membrane was blocked at room temperature for 60 min with 4% skimmed milk in 10 mmol/l Tris/HCl buffer with 0.1% Tween-20 (TBST). Then, blots were incubated with mouse monoclonal anti-human SC (Sigma, Saint Louis, USA) diluted at 1.4 μg/ml in TBST/4% skimmed milk and incubated overnight at 4°C. After washing with TBST, blots were then incubated with HRP-conjugated goat anti-mouse IgG (Zhongshan Biotech, Beijing, China) for 1 h at room temperature. After washing, bands were visualized using the enhanced chemiluminescence system (PerkinElmer, USA). In direct ELISA, 2.0 μg/ml SIgA, 2.0 μg/ml BSA, 2.0 μg/ml polymeric IgA (pIgA) (>440 kDa) and 2.0 μg/ml monomeric IgA (mIgA) (150 kDa) isolated from normal human sera with a 16/60 S-300 Sephacryl column

Table 1. The clinical parameters of patients with IgAN

<table>
<thead>
<tr>
<th></th>
<th>Patients with IgAN</th>
<th>Patients with mIgAN</th>
<th>Patients with fpsIgAN</th>
<th>P-value mIgAN vs fpsIgAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>57</td>
<td>29</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.68 ± 9.24</td>
<td>32.04 ± 10.07</td>
<td>30.54 ± 7.85</td>
<td>0.79</td>
</tr>
<tr>
<td>Duration of disease (months)</td>
<td>7(0.5, 240)</td>
<td>5.5(1, 240)</td>
<td>10(0.5, 60)</td>
<td>0.24</td>
</tr>
<tr>
<td>Proteinuria (g/24h)</td>
<td>1.91 ± 1.88</td>
<td>0.66 ± 0.33</td>
<td>3.21 ± 1.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>141.10 ± 99.48</td>
<td>79.67 ± 17.60</td>
<td>205.21 ± 109.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>77.52 ± 33.28</td>
<td>100.95 ± 24.83</td>
<td>53.08 ± 21.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum IgA (mg/ml)</td>
<td>3.11 ± 1.13</td>
<td>3.33 ± 1.31</td>
<td>2.87 ± 0.86</td>
<td>0.19</td>
</tr>
</tbody>
</table>
(Amersham Bioscience, Uppsala, Sweden) were coated to the 96-well polystyrene microtitre plate, after being blocked with PBST/BSA, mouse monoclonal anti-human SC (Sigma, Saint Louis, USA) diluted at 1.4 mg/ml was added. The wells were then incubated with 1:10000 diluted HRP-conjugated goat anti-mouse IgG (Zhongshan Biotech, Beijing, China) to detect SC in the samples.

To test the sensitivity of the sandwich ELISA, purified SIgA and mIgA with different concentrations were measured in the sandwich ELISA as detailed in the earlier method.

**Immunofluorescence staining**

Sections cut at a thickness of 4 μm from frozen renal biopsy tissues were immersed into 0.01 M PBS for 5 min. After blocking the sections with PBS containing 1% BSA, mouse monoclonal anti-human SC (Genetex, San Antonio, USA) diluted at 1:50 in PBS was incubated overnight at 4°C as the primary antibody. Then the sections were washed three times for 10 min in PBS and TRITC-labelled goat anti-mouse IgG (Zhongshan Biotech, Beijing, China) diluted in 1:100 was used as a secondary antibody at 37°C for 30 min. After another three washes, FITC-labelled rabbit anti-human IgA (Dako, Glostrup, Denmark) with a dilution of 1:40 was added at 37°C for 30 min. All the sections were viewed using a confocal microscope (Leica, Germany). A negative control was performed by replacing the primary antibody with an irrelevant antibody of the same isotype and no immunostaining was observed.

**Statistical analyses**

The quantitative data were expressed as mean±SD. For comparison between patients and controls, clinical parameters of patients with mIgAN and fpsIgAN, the Student’s t-test and one-way analysis of variance (ANOVA) test in SPSS (SPSS Inc, Chicago, IL, USA) version 10.0 statistical analysis program were used. The Pearson correlation was used to analyse correlation. A P-value of <0.05 was considered significant.

**Results**

**Specificity and sensitivity of the sandwich ELISA**

Using Western blot analysis, we showed that the monoclonal anti-SC antibody only recognized the 75 kDa SC (Figure 1A). Furthermore, the anti-SC antibody showed specificity for SIgA in the direct ELISA (Figure 1B). For purified SIgA, it could be detected by the sandwich ELISA at a concentration of 73.4 ng/ml. In contrast, SIgA could not be detected in the mIgA preparation even at a very high concentration (Figure 1C).

**Concentrations of SIgA in the sera from patients with IgAN and controls**

The level of serum SIgA in patients with IgAN was significantly higher than that of normal controls (18.51 ± 9.82 μg/ml vs 9.96 ± 5.83 μg/ml, P < 0.001). After the patients were further stratified, the level of serum SIgA was much higher in patients with focal proliferative sclerosing IgAN (fpsIgAN) than that in patients with mild mesangial proliferative IgAN (mIgAN) (23.48 ± 9.44 μg/ml vs 13.72 ± 7.65 μg/ml, P < 0.001) and that in normal controls (23.48 ± 9.44 μg/ml vs 9.96 ± 5.83 μg/ml, P < 0.001). The level of serum SIgA in patients with mIgAN was also significantly higher than that in normal controls (P = 0.034). (Figure 2).

**Association between SIgA and clinical parameters**

The level of serum SIgA correlated with the level of serum creatinine (R = 0.509, P < 0.001), degree of proteinuria (R = 0.643, P < 0.001) and creatinine clearance (R = −0.454, P = 0.002) in patients with IgAN (Figure 3). However, there was no significant
The level of SIgA in serum

Fig. 2. Levels of SIgA in sera of patients with IgAN (mIgAN, mild mesangial proliferative IgAN; fpsIgAN, focal proliferative sclerosing IgAN) and normal controls. (IgAN vs controls, \( P < 0.001 \), fpsIgAN vs controls, \( P < 0.001 \) and fpsIgAN vs mIgAN, \( P < 0.001 \)).

correlation between the serum concentration of SIgA and level of serum IgA, age of patients and duration of disease.

Glomerular deposition of SIgA in patients with IgAN

Mesangial IgA deposition was detected in all the 34 patients. Significant mesangial SC deposits were detected only in 11 of them. The renal deposition of IgA and SC could be largely merged as observed with confocal microscope. (Figure 4)

Associations between deposition of SIgA and renal pathological phenotypes as well as the level of serum SIgA

Among the 11 patients with significant SC deposits, six were with mIgAN and five with fpsIgAN. Although the level of serum SIgA in patients with SC deposits was higher than those without SC deposits, the difference was not significant (21.53 ± 13.00 µg/ml vs 18.89 ± 8.81 µg/ml, \( P = 0.690 \)) (Figure 5)

Discussion

Although chronic renal failure develops in a considerable number of patients with IgAN, the exact aetiology including the origin of IgA deposited in the glomeruli and the mechanism of IgA deposition has not yet been clearly elucidated.

Most studies suggested that IgA deposited in the kidney of patients with IgAN originated from systemic immune sites. However, still much evidence indicated that IgAN was closely related to the human mucosal immunity: (i) Patients with IgAN often showed episodes of macroscopic haematuria or deteriorated urinary sediments after upper respiratory tract infections. (ii) Elevated serum and salivary levels of SIgA were found in IgAN, especially in those with episodic macroscopic haematuria [5], and IgA production in both systemic and mucosal IgA systems was increased after systemic immunization in IgAN [8]. (iii) Mesangial IgA is predominantly pIgA, and pIgA is normally of mucosal origin. (iv) Clearance of SIgA was found defective in patients with IgAN and bile duct ligation in mice could promote SIgA deposited in the glomerular mesangium [9,10]. (v) Studies confirmed that human tonsil tissues, as one of the main mucosal associated lymphoid tissues, had a close relationship with IgAN. For example IgA, especially IgA1, secreting cells in tonsils were increased in patients with IgAN [11]; the antibodies eluted from renal tissues of patients with IgAN could bind to tonsillar cells [12]; erythrocyte count in the urinary sediments increased after tonsillar stimulation and even tonsillectomy could improve IgA deposition and mesangial proliferation [13]. Moreover, recently, Oortwijn et al. [4] found a 120-fold accumulation of SIgA compared to IgA1 in the glomerular eluate of one patient with recurrent IgAN. They also proved that more SIgA could bind to HMC followed
by increased production of IL-6 compared with IgA through a series of studies.

The vast majority of human IgA is produced at mucosal surfaces and is almost exclusively pIgA with both subclasses, and the relative proportion of subclasses varies at different mucosal sites. Mucosal pIgA is rapidly transported across the adjacent epithelial barrier into external secretions with very little entering the blood. This active transport is mediated by the polymeric immunoglobulin receptor (pIgR), which is expressed on the basolateral surfaces of epithelial cells and recognizes J-chain-containing immunoglobulins (pIgA and IgM). Once bound, the pIgR–pIgA complex is internalized and secreted at the luminal side of the epithelial cells. Such transported pIgA retains a portion of the pIgR (secretory component, SC), forming SIgA. SIgA is composed of two monomers, linked by J-chain and wrapped by SC around the dimer. Small quantities of IgA are also made in systemic immune sites, most notably the bone marrow. IgA originated from bone marrow is mainly monomeric IgA1 and secreted into the circulation.

To explore the possible link between mucosal immunity and the development of IgA nephropathy, the levels of SIgA in sera were measured and their association with renal pathological phenotypes was investigated. It was found that the level of serum SIgA was significantly higher in patients with IgAN compared with that in healthy individuals. After stratification of the patients by renal histopathology, it was found that the levels of serum SIgA in patients with IgAN were associated with the pathological phenotypes; the level of serum SIgA in patients with fIgAN was much higher than that in patients with mIgAN, though the level of serum SIgA was also significantly increased in mIgAN. This discovery again supported the association between mucosal immunity and the development of IgAN, more importantly, it shed new light on the possibility that the serum level of SIgA might be linked to the renal inflammation and injury, which had been also demonstrated in the current study by the observation that the level of SIgA deposits and patients without (A) and patients without (B). (A vs B, P = 0.690).

Fig. 4. Mesangial deposition of SIgA in two patients with IgAN. Mesangial deposition of (A) IgA, (B) secretory component and (C) merging of IgA and secretory component in one patient with IgAN. Mesangial deposition of (D) IgA, (E) secretory component and (F) merging of IgA and SC in the second patient. (magnification 400×).

Fig. 5. Comparison of serum SIgA levels between patients with SIgA deposits (A) and patients without (B). (A vs B, P = 0.690).
serum SIgA was closely associated with the level of serum creatinine, degree of proteinuria and creatinine clearance in IgAN patients. The mechanisms of increasing the level of serum SIgA in IgAN might include the following: (i) excessive production of SIgA in submucosal tissue with partial backward flowing to blood, (ii) the free SC secreted into blood that might bind polymeric IgA originating from bone marrow, spleen or lymphoid tissues, (iii) abnormal clearance of serum SIgA by hepatic asialoglycoprotein receptors. However, the exact reason for elevated serum SIgA in IgAN is still unknown and further study is needed.

To further determine the possible role of mucosal immunity in the pathogenesis of IgAN, we employed frozen renal tissues from 34 patients without IgM deposits to investigate the deposition of SIgA in glomeruli. Although significant deposition of SIgA was found only in 11 patients, it was suggested that at least in some patients, a proportion of the mesangial IgA might originate from mucosal immune sites. It was also demonstrated by Suzuki et al. [14] that SC was detected in 13 out of the 191 renal biopsy specimens from patients with IgAN by immunofluorescent microscopy in 1990.

To investigate whether the deposition of SIgA correlated with the level of serum SIgA and renal histopathology, we compared the levels of serum SIgA and pathological phenotypes in the 11 patients with SIgA deposition with the other 23 patients without SIgA deposition. However, no significant differences could be found. This might be due to the limited cases; therefore, more renal tissues from patients with different histopathological phenotypes of IgAN were needed for further investigation.

In summary, we found the deposition of SIgA in glomerular mesangium in some patients with IgAN. The level of serum SIgA was associated with the pathological phenotypes of IgAN. These findings suggested that SIgA might play an important role in the pathogenesis of IgAN. However, the mechanisms still need to be investigated in future studies.

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References


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