Collectin11 and Complement Activation in IgA Nephropathy

Min Wei,1,2,3,4,5 Wei-yi Guo,1,2,3,4,5 Bo-yang Xu,1,2,3,4,5 Su-fang Shi,1,2,3,4,5 Li-jun Liu,1,2,3,4,5 Xu-jie Zhou,1,2,3,4,5 Ji-cheng Lv,1,2,3,4,5 Li Zhu,1,2,3,4,5 and Hong Zhang1,2,3,4,5

Abstract

Introduction

IgA nephropathy is the most common primary GN worldwide (1). It is characterized by the glomerular mesangial deposition of IgA, which is mainly IgA1 immune complexes, and finally leads to mesangial cell activation and tissue injury (2). During this process, complement activation was suggested to play an essential role in the local inflammatory response and kidney injury, especially through the alternative and lectin pathways (3-7).

Activation of the complement lectin pathway is initiated by the binding of pattern recognition molecules to specific carbohydrate moieties (8,9). The initiators of lectin pathway include mannose-binding lectin, ficolins, and collectin11 (10). Ficolins and mannose-binding lectin have been found to codeposit with IgA1 in the kidneys of approximately 25% of patients (4).

Collectin11 is a recently described initiator of lectin pathway activation (11,12). It belongs to the C-type lectin superfamily, which is the same as mannose-binding lectin. Structurally, both collectin11 and mannose-binding lectin contain a carbohydrate recognition domain at the C terminus to recognize the carbohydrate moiety and a collagen-like domain at the N terminus that binds to the serine protease mannose-binding lectin-associated serine protease 1/2 (MASP1/2). Collectin11 showed high affinity for D-mannose and L-fucose, which are calcium dependent (11,13). Other than carbohydrates, collectin11 was able to interact with nucleic acids and some proteins in a calcium-independent manner (14,15). Unlike mannose-binding lectin, which is mainly produced by hepatocytes, collectin11 is ubiquitously expressed in the human body (11). In the kidney, collectin11 was mainly detected in proximal and distal tubules as well as cells within the glomeruli (13).

Collectin11 and Complement Activation in IgA Nephropathy

In vitro, cultured normal human mesangial cells were shown to express collectin11 (11). A series of recent studies indicated a pathogenic role of collectin11 in AKI (16-18). Collectin11 was upregulated in kidney tubular epithelial cells following kidney stress and aggravated kidney damage by activation of lectin pathway in situ.

Design, setting, participants, & measurements

The deposition of collectin11 and other complement proteins was detected in glomeruli of 60 participants with IgA nephropathy by immunoﬂuorescence. In vitro, human mesangial cells were treated with IgA1-containing immune complexes derived from participants with IgA nephropathy. Then, the expression of collectin11 in mesangial cells was examined by quantitative RT-PCR and immunofluorescence. The codeposition of collectin11 with IgA1 or C3 on mesangial cells was detected by immunofluorescence and proximity ligation assays.

Results

In total, 37% of participants with IgA nephropathy (22 of 60) showed codeposition of collectin11 with IgA in the glomerular mesangium. Using an injury model of mesangial cells, we demonstrated that IgA1-immune complexes derived from participants with IgA nephropathy increased the secretion of collectin11 in mesangial cells with the subsequent deposition of collectin11 on the cell surface via the interaction with deposited IgA1-immune complexes. In vitro, we found that collectin11 bound to IgA1-immune complexes in a dose-dependent but calcium-independent manner. Furthermore, deposited collectin11 initiated the activation of complement and accelerated the deposition of C3 on mesangial cells.

Conclusions

In situ–produced collectin11 by mesangial cells might play an essential role in kidney injury in a subset of patients with IgA nephropathy through the induction of complement activation.
Given the importance of the lectin pathway in IgA nephropathy and the relatively high expression level of collectin11 in the kidney, we hypothesized that collectin11 might play a role in the kidney injury in IgA nephropathy through the activation of complement via lectin pathway.

Materials and Methods

Participants
In total, 60 patients with IgA nephropathy were enrolled between April 2017 and June 2017 from the Peking University First Hospital for immunofluorescence staining. The diagnosis of IgA nephropathy depends on the demonstration of glomerular mesangial deposition of granular IgA by immunofluorescence as well as electron-dense materials by electron microscopy. Clinical and laboratory data were collected from medical records at the time of kidney biopsy. Meanwhile, biopsy samples of four patients with FSCS as well as paracarcinoma kidney tissue specimens from a patient with kidney cell carcinoma were enrolled as controls. The study complied with the Declaration of Helsinki principles and was approved by the Peking University First Hospital ethics committees. Written informed consent was obtained from all participants.

Purification of Immunoglobulin A1–Immune Complexes and Preparation of Galactose-Deficient Immunoglobulin A1
Plasma from 20 patients with IgA nephropathy and 20 age- and sex-matched healthy controls was pooled for IgA1-immune complexes purification as previously described (19). In addition, another 11 patients and four healthy controls were recruited for IgA1-immune complexes purification from each individual. The purity of IgA1-immune complexes was detected by Coomassie blue staining and western blot (Supplemental Material). To obtain galactose-deficient IgA1 (Gd-IgA1), monomeric IgA1 was treated with neuraminidase (Sigma) and galactosidase (Sigma) to remove terminal sialic acid and galactose from O-glycans.

Enzyme-Linked Immunosorbent Assay Protocol
For characterization of the interaction between IgA1 and collectin11 in vitro, microplates were coated with 2 µg/ml recombinant collectin11 (R&D). IgA1 diluted in BVB2+ buffer (20) was then added and incubated for 1 hour. Plates were incubated with biotin-labeled mouse anti-human IgA1 antibody (BIORAD) and, subsequently, HRP-streptavidin (Sigma) for 1 hour. Under some conditions, coated plates were preincubated with mann (Sigma) or directly incubated with IgA1 in calcium-free BVB2+/EGTA buffer (BVB2+ buffer without CaCl2 and supplemented with 10 mM EGTA).

For detection of binding between IgA1 and mannos-binding lectin, microplates were coated with IgA1. After blocking, mannos-binding lectin diluted in BVB2+ buffer was added. Plates were then incubated with rabbit anti-human mannos-binding lectin antibody (BIORAD) and, subsequently, HRP-goat anti-rabbit IgG (Sigma) for 1 hour. Under some conditions, mannos-binding lectin was preincubated with mann and then added to plates or directly incubated in calcium-free BVB2+/EGTA buffer.

The concentration of collectin11 in cell supernatant was detected using the ELISA kit (Novus Biologicals) according to the manufacturer’s instructions.

Cell Culture and Treatment with Immunoglobulin A1–Immune Complexes
Primary human mesangial cells (ScienCell) were cultured in medium containing 2% FBS. After serum starvation, mesangial cells were treated with 100 µg/ml IgA1-immune complexes or PBS. For the complement activation assay, 10% factor-B-depleted human serum (Comp Tech), used as the source of complement proteins, was added for another 1 hour after IgA1-immune complexes treatment for 24 hours.

Immunofluorescence
Slides from the frozen kidney biopsy tissue were incubated sequentially with primary antibody at 4°C overnight and fluorophore-conjugated secondary antibodies for 1 hour at room temperature. For detection of endogenous collectin11 in cultured mesangial cells, cells were fixed in 4% paraformaldehyde and permeabilized with 0.2% Triton X-100. To detect IgA1-immune complexes on mesangial cells, cells were fixed but not permeabilized and then subjected to immunostaining.

The primary antibodies used included goat polyclonal anti-human collectin11 antibody (Santa Cruz), mouse monoclonal anti-human MASP2 antibody (Santa Cruz), rabbit polyclonal anti-human C1q antibody (Dako), rabbit polyclonal anti-human C3c antibody (Dako), mouse monoclonal anti-human Bb antibody (Abcam), mouse monoclonal anti-human mannose-binding lectin antibody (Hy Hutch Biotech), mouse monoclonal anti-human MASP1/3 antibody (Hy Hutch Biotech), mouse monoclonal anti-human L-ficolin antibody (Hy Hutch Biotech), rabbit polyclonal anti-human C4d antibody (Biomedica), mouse monoclonal anti-human C5b-9 antibody (Abcam), and rabbit monoclonal anti-human IgA1 antibody (ab193187; Abcam). The secondary antibodies were Alexa Fluor 488–conjugated donkey anti-goat IgG (Invitrogen), Alexa Fluor 488–conjugated goat anti-mouse IgG or goat anti-rabbit IgG (Jackson ImmunoResearch), and Cy3-conjugated goat anti-mouse IgG or goat anti-rabbit IgG (Jackson ImmunoResearch).

Small Interfering RNA Transfection
For transfection, 1×10⁶ cells per microliter were resuspended in Amaxa Basic SMC nucleofector solution (Lonza), and 1 µg siRNA targeting collectin11 or nontargeting control siRNA (RiboBio, Guangzhou, China) was added. The Nucleofector 2b Device and the program D-033 were used for nucleofection. After transfection, cells were seeded in full medium, and transfection efficiency was determined by quantitative PCR after 24 hours.

Results
Collectin11 Was Deposited in the Mesangium in Participants with Immunoglobulin A1 Nephropathy
The clinical features of 60 participants are listed in Table 1. The 60 biopsied participants with IgA nephropathy had a mean (±SD) age of 37±13 years, mean eGFR of 82±30 ml/
Table 1. Clinical and histologic features of participants with IgA nephropathy at the time of kidney biopsy

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All</th>
<th>Lectin Pathway (+)</th>
<th>Lectin Pathway (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>60</td>
<td>38</td>
<td>22</td>
</tr>
<tr>
<td>Age, yr</td>
<td>37±13</td>
<td>37±14</td>
<td>38±12</td>
</tr>
<tr>
<td>Men, %</td>
<td>38 (63)</td>
<td>22 (58)</td>
<td>16 (73)</td>
</tr>
<tr>
<td>Urine protein, g/24 h</td>
<td>0.9 (0.4–2.1)</td>
<td>1.0 (0.4–1.8)</td>
<td>0.9 (0.4–3.0)</td>
</tr>
<tr>
<td>eGFR, ml/min per 1.73 m²</td>
<td>82±30</td>
<td>82±28</td>
<td>81±33</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>33 (55)</td>
<td>20 (52.6)</td>
<td>13 (59.1)</td>
</tr>
<tr>
<td>Preceding infection, %</td>
<td>24 (40)</td>
<td>16 (42.1)</td>
<td>8 (36.4)</td>
</tr>
<tr>
<td>Gross hematuria, %</td>
<td>16 (27)</td>
<td>9 (24)</td>
<td>7 (32)</td>
</tr>
<tr>
<td>Treated with immunosuppressive agents or prednisone, %</td>
<td>10 (17)</td>
<td>6 (16)</td>
<td>4 (18)</td>
</tr>
<tr>
<td>Treated with ACEI/ARB, %</td>
<td>38 (63)</td>
<td>24 (63)</td>
<td>14 (64)</td>
</tr>
<tr>
<td>Accompanied with other comorbidities, %</td>
<td>31 (52)</td>
<td>21 (55)</td>
<td>10 (45)</td>
</tr>
<tr>
<td>Serum C3, g/L</td>
<td>1.03±0.23</td>
<td>0.97±0.21</td>
<td>1.15±0.24</td>
</tr>
<tr>
<td>Serum C4, g/L</td>
<td>0.23±0.06</td>
<td>0.22±0.06</td>
<td>0.26±0.05</td>
</tr>
<tr>
<td>Oxford classification, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>20 (33)</td>
<td>13 (34)</td>
<td>7 (32)</td>
</tr>
<tr>
<td>E1</td>
<td>16 (27)</td>
<td>9 (24)</td>
<td>7 (32)</td>
</tr>
<tr>
<td>S1</td>
<td>30 (50)</td>
<td>17 (45)</td>
<td>13 (59)</td>
</tr>
<tr>
<td>T1/2</td>
<td>17 (28)</td>
<td>10 (26)</td>
<td>7 (32)</td>
</tr>
<tr>
<td>C1/2</td>
<td>42 (70)</td>
<td>27 (71)</td>
<td>15 (68)</td>
</tr>
</tbody>
</table>

ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; M, mesangial hypercellularity; E, endocapillary cellularity; S, segmental sclerosis; T, interstitial fibrosis/tubular atrophy; C, crescents.

*At the time of diagnostic biopsy, 13 participants missed this part of data. Only cases with available data were analyzed.*

min per 1.73 m², and median (interquartile range) urine protein of 0.9 (0.4–2.1) g/24 h; 55% of participants were hypertensive at baseline. The distributions of M1, E1, S1, T1–T2, and C1–C2 were 33%, 27%, 50%, 28%, and 70%, respectively (Table 1). Local deposition of several complement proteins was found in participants with IgA nephropathy (Figure 1A, Supplemental Figure 1). The positive rate of each component is shown in Figure 1, B and C and Table 2. The positive rates of C3c and C5b-9 were both 97% (58 of 60); 87% of participants had deposition of Bb, indicating alternative pathway activation, and 67% of participants had the deposition of C4d, which is an intermediate product of the activation of both the classic and lectin pathways. However, C1q deposition was found in only 15% (nine of 60) of participants. These data are consistent with previous studies showing that the deposition of C4d in the glomeruli of participants with IgA nephropathy was mainly due to the activation of the lectin pathway (21,22). Sixty-three percent of participants in our study had the deposition of at least one initiator of the lectin pathway in the glomeruli, and there was a significant overlap among the three recognition molecules (Figure 1C). Among them, the positive rate of collectin11 was 37% (22 of 60). Coimmunostaining of collectin11 and IgA showed their colocalization in the glomeruli (Figure 1D). Among four participants with focal segmental sclerosis, two presented with glomerular C3 and C4d deposits, but none of them had glomerular deposition of IgA or collectin11 (Supplemental Figure 1).

To further elucidate the potential role of the lectin pathway in IgA nephropathy, 60 participants were separated into two groups: those with (65%) and without (37%) glomerular lectin pathway initiator deposits. The differences in serum C3 and C4 levels between two groups reached statistical significance, whereas no significant differences were observed in other clinical or pathologic manifestations (Table 1).

**Immunoglobulin A1–Immune Complexes from Participants with Immunoglobulin A1 Nephropathy Increased the Expression of Collectin11 in Mesangial Cells**

To assess whether the deposited collectin11 was locally produced by mesangial cells, primary mesangial cells were cultured under stimulation with IgA1-immune complexes purified from pooled plasma samples. IgA1-immune complexes obtained from pooled plasma showed high purity (＞90%), with little contamination of IgG (Supplemental Figure 2). As expected, compared with controls, IgA1-immune complexes from participants with IgA nephropathy significantly increased the release of the inflammatory factors IL-6 and MCP-1 (Figure 2, A and B), suggesting that IgA1-immune complexes from participants with IgA nephropathy are pathogenic. Next, we found that IgA1-immune complexes from participants with IgA nephropathy could significantly upregulate the expression and secretion of collectin11 in mesangial cells (Figure 2, C–F). The induced expression of collectin11 in mesangial cells was then confirmed by IgA1-immune complexes from an independent population of patients and controls, and contaminated IgG showed no effect on collectin11 expression (Supplemental Figure 3). Additionally, using IgA1-immune complexes purified from each individual, our results showed that IgA1-immune complexes from three of the five participants with glomerular collectin11 deposits upregulated collectin11 expression in cultured mesangial cells, whereas IgA1-immune complexes from the other six participants without glomerular collectin11 deposition and all four controls failed to increase
Our results suggested that IgA1-immune complexes derived from patients with glomerular collectin11 deposits were more likely to induce collectin11 expression. Moreover, when siRNA targeting collectin11 was used to knock down the expression of collectin11 (Figure 2G), IgA1-immune complexes from participants with IgA nephropathy failed to increase the expression of collectin11 (Figure 2, H and I).
Table 2. The deposition of complement proteins in glomeruli of participants with IgA nephropathy

<table>
<thead>
<tr>
<th>Complement Activation Pathways</th>
<th>Complement Protein</th>
<th>Proportion Positive, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternative pathway</td>
<td>Bb</td>
<td>87</td>
</tr>
<tr>
<td>Classic pathway</td>
<td>C1q</td>
<td>15</td>
</tr>
<tr>
<td>Lectin pathway</td>
<td>Mannose-binding lectin</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>L-ficolin</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Collectin11</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>MASP2</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>MASP1/3</td>
<td>48</td>
</tr>
<tr>
<td>Classic pathway and lectin pathway</td>
<td>C4d</td>
<td>67</td>
</tr>
<tr>
<td>Alternative pathway, classic pathway, and lectin pathway</td>
<td>C3c</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>CSb-9</td>
<td>97</td>
</tr>
</tbody>
</table>

MASP, mannose-binding lectin-associated serine protease.

Taken together, pathogenic IgA1-immune complexes from participants with IgA nephropathy could induce collectin11 expression in mesangial cells.

Collectin11 Codeposited with Immunoglobulin A1 on Mesangial Cells after Treated with Immunoglobulin A1–Immune Complexes from Participants with Immunoglobulin A1 Nephropathy

We then investigated whether collectin11 would deposit on mesangial cells after IgA1-immune complexes treatment. As shown in Figure 3A, deposited IgA1 and collectin11 both increased in a time-dependent manner after treated with IgA1-immune complexes from participants with IgA nephropathy. Collectin11 showed strong colocalization with IgA on the cell surface (Figure 3B). Although IgA deposited on mesangial cells treated with IgA1-immune complexes from controls also increased over time, we could only detect a tiny amount of collectin11 deposited on mesangial cells even 24 hours later (Figure 3, A, C, and D). A proximity ligation assay also confirmed the close proximity of collectin11 and IgA on mesangial cells (Figure 3, E and G). siRNA-mediated knockdown of collectin11 reduced the deposition of collectin11 on mesangial cells, and recombinant human collectin11 reconstituted the deposition of collectin11 (Figure 3, F and H), indicating that deposited collectin11 came from the release from mesangial cells but not from IgA1-immune complexes added.

Collectin11 Binds with Immunoglobulin A1–Immune Complexes in a Calcium-Independent Manner In Vitro

Next, we further confirmed that collectin11 and IgA1-immune complexes in a dose-dependent manner (Figure 4, A and D). Compared with monomeric IgA1, mannose-binding lectin and collectin11 both had stronger binding affinity to IgA1-immune complexes (Figure 4, B and E). Although we confirmed a previous report that the interaction between mannose-binding lectin and IgA1 was calcium dependent and could be inhibited by EGTA (21) (Figure 4C), we observed that the binding between collectin11 and IgA1-immune complexes was barely influenced in the presence of EGTA (Figure 4F), indicating that collectin11 interacted with IgA1-immune complexes in a calcium-independent manner. Moreover, preincubation with mannan—a ligand recognized through carbohydrate recognition domains by both mannose-binding lectin and collectin11—inhibited the binding of mannose-binding lectin to IgA1-immune complexes but not the binding of collectin11 to IgA1-immune complexes (Figure 4H), further demonstrating that collectin11 bound to IgA1-immune complexes via a structure other than the carbohydrate recognition domain with no need for calcium.

Compared with IgA1-immune complexes from controls, those derived from participants with IgA nephropathy showed higher binding capacity with collectin11 (Figure 4G). As Gd-IgA1 is the main component of IgA1-immune complexes from patients, we further examined the binding affinity of collectin11 to Gd-IgA1. Through digesting monomeric IgA1 with neuraminidase and galactosidase, we successfully prepared Gd-IgA1, as proven by the increased binding with HPA (Supplemental Figure 4). We found that galactose deficiency facilitated the binding of IgA1 with collectin11 and that collectin11 bound with Gd-IgA1 in calcium-independent manner (Figure 4D), suggesting that collectin11 potentially bound to IgA1-immune complexes directly by interacting with Gd-IgA1.

Collectin11 Induced Complement Activation on Mesangial Cells

We next assessed whether the deposition of collectin11 on mesangial cells could induce C3 deposition from factor-B-depleted serum, in which the alternative pathway could not be activated. After incubation with IgA1-immune complexes for 24 hours and 10% factor-B-depleted serum for another 1 hour, we observed obvious codeposition of collectin11 and C3c on mesangial cells treated with IgA1-immune complexes from participants with IgA nephropathy, but only tiny amounts of C3 and collectin11 deposition were detected on mesangial cells treated with PBS or IgA1-immune complexes from controls (Figure 5, A–C). A proximity ligation assay further confirmed the close proximity of collectin11 and C3 on mesangial cells (Figure 5, D and E). Knockdown of collectin11 using siRNA significantly reduced the deposition of collectin11 and C3 on mesangial cells, and recombinant collectin11 reconstituted the deposition of collectin11 and C3 (Figure 5, F and G). On the basis of the above results, it can be inferred that collectin11 deposited on mesangial cells induced complement activation through the lectin pathway.

Discussion

The important role of complement activation in IgA nephropathy has long been recognized. Additionally, alternative and lectin pathways were the focus of recent studies on this disease. In this study, we investigated the potential role of collectin11 in the pathogenesis of IgA nephropathy.

In our study, more than one-third of participants had collectin11 deposition in glomeruli. Moreover, 63% of participants had the deposition of at least one initiator of the
Figure 2. IgA1-immune complexes derived from participants with IgA nephropathy (IgAN) upregulated the expression of collectin11 in primary human mesangial cells. (A and B) Compared with IgA1-immune complexes derived from healthy controls, those from participants with IgAN increased the release of (A) MCP-1 and (B) IL-6 from mesangial cells. (C and D) IgA1-immune complexes derived from participants with IgAN, but not those from controls, significantly upregulated (C) collectin11 mRNA expression and (D) the concentration of collectin11 in the supernatant of mesangial cells. (E) Human mesangial cells were incubated with 100 μg/ml IgA1-immune complexes derived from participants with IgAN or controls for 4 hours; then, intracellular collectin11 expression was detected by immunofluorescence staining. Scale bars: 50 μm. (F) Fluorescence intensity quantification of intracellular collectin11 shown in (E). (G) Transflecting mesangial cells with siRNA targeting collectin11 effectively decreased collectin11 mRNA expression. (H) Human mesangial cells were first transfected with siRNA targeting collectin11 (si-Collectin11) or nontargeting control siRNA (si-Ctrl) for 24 hours and then treated with IgA1-immune complexes from participants with IgAN for 4 hours. Representative fluorescence microscopic images show that si-Collectin11 significantly decreased the production of collectin11 (green) induced by IgA1-immune complexes from participants with IgAN. Scale bars: 50 μm. (I) Fluorescence intensity quantification of intracellular collectin11 shown in (H). (J) IgA1-immune complexes were purified from each single person and were then used for cell stimulation. After treating mesangial cells with IgA1-immune complexes for 24 hours, the expression of collectin11 in mesangial cells was detected by immunofluorescence. Then, fluorescence intensity of intracellular collectin11 was quantified. DAPI was used to stain nuclei (blue). The total fluorescence intensity was adjusted for the area of the nuclei, and each dot represents an individual image. The results are representative of three independent experiments. HC, healthy control; IgAN-Collectin11(+), patients with IgA nephropathy and deposition of collectin11 in the kidney; IgAN-Collectin11(−), patients with IgA nephropathy and without deposition of collectin11 in the kidney; MCP1, monocyte chemoattractant protein-1; siRNA, small interfering RNA; DAPI, 4',6-diamidino-2-phenylindole; NS, not significant; InDen, integrated density. *P<0.05 by unpaired, two-tailed t test; **P<0.01 by unpaired, two-tailed t test; ***P<0.001 by unpaired, two-tailed t test.
Figure 3. Collectin11 deposited on mesangial cells through interacting with IgA1-immune complexes. (A) Representative fluorescence microscopic images of deposited collectin11 (green) and IgA1 (red) on mesangial cells after treatment with IgA1-immune complexes for different time intervals. Scale bars: 50 μm. (B) Representative confocal images show the codeposition of collectin11 (green) and IgA1 (red) on mesangial cells after treatment with IgA1-immune complexes from participants with IgAN for 24 hours. Scale bars: 20 μm. (C and D) Fluorescence intensity of deposited (C) IgA1 and (D) collectin11 on mesangial cells represented in (A) was quantified. (E) Representative fluorescence microscopic images of the proximity ligation assay show the close proximity of collectin11 and IgA1 on mesangial cells 24 hours after treated with IgA1-immune complexes from participants with IgAN. Scale bars: 50 μm. (F) Human mesangial cells were transfected with si-Collectin11 or si-Ctrl for 24 hours and then treated with IgA1-immune complexes from participants with IgAN for 4 hours with or without supplementation with recombinant collectin11 (2 μg/ml). Representative fluorescence microscopic images of deposited collectin11 (green) and IgA1 (red) on mesangial cells and a merged image of collectin11 and IgA1 demonstrate that the deposition of collectin11 on mesangial cells was reduced by transfection with si-Collectin11 and that supplementation with recombinant human collectin11 reconstituted the deposition of collectin11 on mesangial cells. Scale bars: 50 μm. (G) Fluorescence intensity quantification of positive signals shown in (E). (H) Fluorescence intensity quantification of deposited collectin11 and IgA1 shown in (F). DAPI was used for
lectin pathway accompanied with C4d deposition, indicating the activation of the lectin pathway in glomeruli. This was higher than the positive rates previously reported (4,7,23). As shown in a previous study, glomerular deposition of mannose-binding lectin and L-ficolin was associated with more severe histologic damage and higher levels of proteinuria (4). However, in this study, clinical and histologic data showed no significant difference between lectin pathway–positive and lectin pathway–negative participants, except for circulating levels of complement C3 and C4. This may be due to the relatively limited sample size in this study and the highly variable clinical and pathologic manifestations of IgA nephropathy. Further investigation is needed to clarify the accurate association between

Figure 3. | Continued. staining nuclei (blue). The total fluorescence intensity was quantified and adjusted for the area of the nuclei, and each dot represents an individual image. The results are representative of three independent experiments. *P=0.05 by unpaired, two-tailed t test; **P=0.01 by unpaired, two-tailed t test; ***P<0.001 by unpaired, two-tailed t test.

Figure 4. | Collectin11 binds with IgA1 in a calcium-independent manner. IgA1-immune complexes (0.15625 to 5 µg/ml) from participants with IgAN bound to a constant concentration of (A) MBL (2 µg/ml) and (D) collectin11 (2 µg/ml) in a dose-dependent manner. Compared with monomeric IgA1 (mIgA), IgA1-immune complexes showed higher binding ability to (B) MBL and (E) collectin11. The addition of BVB21/EGTA buffer abolished the binding of IgA1-immune complexes with (C) MBL but not (F) collectin11. (G) IgA1-immune complexes from participants with IgAN showed higher binding ability to collectin11 than IgA1-immune complexes derived from controls. (H) Incubation with mannan inhibited the binding of MBL to IgA1-immune complexes, but no effect was observed regarding the binding of collectin11 to IgA1-immune complexes. The percentage of binding of MBL or collectin11 to IgA was calculated as a percentage of the OD value read at Cmannan=0 mg/ml. (I) Galactose-deficient IgA1 (Gd-IgA1) showed higher binding with collectin11 than mIgA1. The addition of BVB21/EGTA buffer slightly increased the binding of collectin11 with Gd-IgA1, and no inhibitory effect was observed. The results are representative of three independent experiments. IgA1-IC, IgA1-immune complex; BVB21 buffer, the same buffer used in the study of Roos A et al. (VBS, 0.5 mM MgCl2, 1 mM CaCl2, 0.05% Tween 20, 1% BSA, pH 7.5). Adapted from ref. 20, with permission. *P=0.05 by unpaired, two-tailed t test; **P=0.01 by unpaired, two-tailed t test; ***P<0.001 by unpaired, two-tailed t test.
Figure 5. Deposited collectin11 induced C3 deposition on mesangial cells. (A) Human mesangial cells were treated with IgA1-immune complexes or PBS for 24 hours, followed by incubation with 10% factor-B–depleted serum (source of complement proteins) for an additional 1 hour. Then, cells were used for immunostaining of collectin11 and C3c. Representative fluorescence microscopic images of collectin11 (green) and C3 (red) show obvious codeposition of collectin11 and C3 on mesangial cells. Scale bars: 20 μm. (B and C) Fluorescence intensity of deposited collectin11 and C3 on mesangial cells represented in (A) was quantified. (D) Representative fluorescence microscopic images of the proximity ligation assay show the close proximity of collectin11 and C3 on mesangial cells after treated with IgA1-immune complexes from participants with IgAN for 4 hours and 10% factor-B–depleted serum for another 1 hour. Scale bars: 50 μm. (E) Fluorescence intensity quantification of positive signals shown in (D). (F) Mesangial cells were transfected with si-Collectin11 or si-Ctrl for 24 hours and then treated with IgA1-immune complexes from participants with IgAN for 4 hours with or without supplementation with recombinant collectin11 (2 μg/ml). Finally, factor-B–depleted serum was added for another 1 hour. Representative fluorescence microscopic images of deposited collectin11 (green) and C3 (red) on mesangial cells and a merged image of collectin11 and C3 demonstrated that the deposition of both collectin11 and C3 on mesangial cells was reduced by silencing collectin11 through
glomerular deposition of proteins of lectin pathway and phenotypes of IgA nephropathy.

Relatively low concentrations of collectin11 are present in the circulation (24,25), and it has been reported that locally produced collectin11 plays a crucial role in both AKI and chronic kidney inflammation (16,17,26). Hence, we speculated that deposited collectin11 in the glomeruli of participants with IgA nephropathy might be produced, at least in part, in situ. On the basis of a well-established injury model of mesangial cells, we found that IgA1-immune complexes purified from the plasma of participants with IgA nephropathy, but not healthy controls, could induce the expression of collectin11 in primary mesangial cells, and the secreted collectin11 subsequently deposited on the cell surface, indicating that glomerular-deposited collectin11 was probably produced by injured mesangial cells. Moreover, IgA1-immune complexes derived from participants with IgA nephropathy and glomerular CL11 deposits were more likely to induce CL11 expression. Our findings suggested that the protein compositions of IgA1-immune complexes were different among individual participants with IgA nephropathy and associated with mesangial cells activation. In addition, we could still not rule out the possibility that collectin11 from the circulation might play a role in vivo.

As deposited, collectin11 and IgA1 showed obvious colocalization not only in the glomeruli but also on mesangial cells in vitro. We further characterized the interaction between collectin11 and IgA1-immune complexes. Although mannose-binding lectin binds with polymeric IgA1 in a calcium-dependent manner (20), we found that collectin11 bound with IgA1-immune complexes in a calcium-independent manner, and this binding could not be inhibited by mannan, indicating that collectin11 binds to IgA1-immune complexes probably not via carbohydrate recognition domain. As IgA1-immune complexes consist of many other proteins in addition to Gd-IgA1 (27–29), it is difficult to determine whether collectin11 binds with IgA1-immune complexes by interacting with IgA1 or other proteins. Hence, we examined the binding of collectin11 with Gd-IgA1. Collectin11 also bound with Gd-IgA1 in a calcium-independent manner. However, the interaction of collectin11 with other components of IgA1-immune complexes could not be excluded. Furthermore, we found that the same amount of collectin11 captured more Gd-IgA1 and more IgA1-immune complexes in the presence of EGTA. We assumed that these changes in binding affinity may be due to a conformational change in interacting proteins. Because ionic interactions were reported to be involved in the interaction between collectin11 and nucleic acid (14), whether it would contribute to the interaction of collectin11 with IgA1-immune complexes remains to be further studied.

Because all participants with glomerular collectin11 deposition also had C4 and C3 deposition, we determined the ability of collectin11 to induce lectin pathway activation on mesangial cells in vitro. We could clearly see the colocalization of C3 and collectin11 on mesangial cells after incubating cells with 10% factor-B-depleted human serum. Although previous studies suggested that mesangial cells could produce C3 protein and that stimulation with polymeric IgA1 could increase its expression (30,31), we did not detect the secretion of C3a in the supernatant of mesangial cells under IgA1-immune complexes stimulation, suggesting that deposited C3 in the kidney was probably not in situ produced by mesangial cells.

Nevertheless, there are several limitations to this work. First, our study is a cross-sectional study, and our sample size is relatively small; therefore, we could not establish a causal link between collectin11 deposition and kidney injury. Second, because of the lack of a satisfactory animal model for IgA nephropathy, we did not illustrate our findings in vivo, and further studies on the basis of a good experimental animal model with typical pathologic manifestations are needed.

In summary, our study is the first to demonstrate that in situ–produced CL11 is one of the important mediators that leads to the activation of complement lectin pathway in patients with IgA nephropathy.

Disclosures

J.-c. Lv reports consultancy agreements with Chinoik Therapeutics. H. Zhang reports consultancy agreements with Calliditas, Janssen, Novartis, and OMEROS and serving as the vice-director of the nephrology committee of the Beijing Society of Medicine, a board committee member of nephrology of the Chinese Medical Doctor Association, a board committee member of the Chinese Society of Nephrology, a member of the International Society of Nephrology-Advancing Clinical Trials, a member of the International Society of Nephrology Global Outreach Program, and a member of the International Society of Nephrology Sister Renal Centers. All remaining authors have nothing to disclose.

Funding

This project was supported by National Science Foundation of China grants 81922013, 81970598, and 82070733; National Science Foundation of Beijing grant 7202206; National Key Research and Development Program of China grant 2020YFC2005003; Youth development project form Peking University Health Science Center grant BMU2021PY004; Beijing Science and Technology Plan Project of China grants D18110000118003 and Z16110000516005; and CAMS Innovation Fund for Medical Sciences grant 2019-I2M-5-046.

Acknowledgments

The authors thank the patients who participated in this study.

Supplemental Material

This article contains the following supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.04300321/-/DCSupplemental.
Supplemental Figure 1. Representative immunostaining images of complement proteins in kidney tissue.

Supplemental Figure 2. Purity and composition of IgA1-immune complexes analyzed by SDS-PAGE and western blot.

Supplemental Figure 3. Independent validation of the promoting effect of the IgA1-immune complexes from participants with IgA nephropathy on the expression of collectin11.

Supplemental Figure 4. Validation of the effective removal of terminal sialic acid and galactose from monomeric IgA1.

References
15. Kerkert-Moller N, Bayarri-Olmos R, Krogfelt KA, Garred P: C1q/TNF-related protein 6 is a pattern recognition molecule that recruits collectin-11 from the complement system to ligands. J Immunol 204: 1598–1606, 2020

Received: March 30, 2021 Accepted: September 18, 2021
Published online ahead of print. Publication date available at www.cjasn.org.