Glomerular deposition of mannose-binding lectin (MBL) indicates a novel mechanism of complement activation in IgA nephropathy

Morito Endo1, Hiroyuki Ohi1, Isao Ohsawa1, Takayuki Fujita1, Misao Matsushita2 and Teizo Fujita2

1Second Department of Internal Medicine, Nihon University School of Medicine, Tokyo and 2Department of Biochemistry, Fukushima Medical College, Fukushima, Japan

Abstract

Background. IgA nephropathy (IgA-N) is considered the most common glomerular disease in the world and leads to renal failure in a substantial number of patients. Although many studies have looked at the pathogenesis of the disease, many points need to be clarified, including the mechanism of complement activation. Recent studies have shown that mannose-binding lectin (MBL or mannos binding protein, MBP) initiates activation of the complement cascade (lectin pathway) utilizing two types of MBP-associated serine protease, namely MASP-1 and MASP-2. The present study was undertaken to elucidate whether the lectin pathway was involved in the pathogenic mechanism of IgA-N.

Methods. Forty-five renal biopsy cases with IgA-N, 35 cases with other forms of glomerulonephritis (GN), and normal kidney tissues were collected and an immunohistochemical study was performed using monoclonal antibodies against MBL and MASP-1. Furthermore, clinicopathological and serological features were also analysed in the patients with IgA-N.

Results. Glomerular deposition of MBL, which was accompanied by MASP-1, was detected in 11 of 45 (24.4%) cases with IgA-N, while it was detected in only one case with other forms of GN. The deposited MBL/MA SP-1 was observed to associate with C3b/C3c and C5b–9 but not with IgG, IgM, C1q, C4c, or properdin. Compared with MBL/MA SP-1 negative cases with IgA-N, the positive cases with IgA-N were young and the renal biopsies had been performed at an early stage of the disease. No significant correlation was found between glomerular deposition of MBL/MA SP-1 and proteinuria, haematuria, creatinine clearance, and serum levels of IgA, C3, or C4 at the time of renal biopsy. There were also no significant differences between MBL/MA SP-1 positive cases and negative cases in the plasma levels of circulating immune complexes or soluble C5b–9.

Conclusion. The lectin pathway of complement activation, which is initiated by the MBL/MA SPs complex, evidently contributes to the development of glomerular injury in a significant number of cases with IgA-N. In addition, these findings will add insight to the pathogenesis of IgA-N, including its relation to infection, since MBL plays a crucial role in the host defense against various pathogens.

Key words: complement; IgA nephropathy; mannose-binding lectin; mannose-binding lectin associated serine protease

Introduction

IgA nephropathy (IgA-N) is considered the most common glomerular disease in the world [1–3] and leads to renal failure in a substantial number of patients [2–5]. Although a large number of studies have looked at the pathogenesis of this disease, many points need to be clarified, including the mechanism of complement activation. The mesangial IgA deposits are frequently accompanied by deposits of C3 [6,7], and it is suggested that the complement system is implicated in renal tissue damage. The complement cascade can be initiated either by the classical or the alternative pathway. While there is a general agreement that C3 deposits are caused predominantly by complement activation via the alternative pathway in IgA-N, several reports [7–9] suggest that in some cases the classical pathway may also be involved.

Recently, a novel pathway of complement activation, the lectin pathway, which is initiated by mannose/mannose-binding lectin (MBL or mannose binding protein, MBP) and two types of MBP associated serine protease (MASP-1 [10,11] and MASP-2 [12]) has been shown. Human MBL is a liver-derived, C-type serum lectin that binds to terminal mannose and N-acetylgalactosamine moieties present on the surfaces of various pathogens, including bacteria, mycobacteria, yeast, fungi, and viruses [13]. After binding to the carbohydrate ligands, MBL activates the complement cascade via the lectin pathway utilizing MASP's forming the
MBL/MASPs complex, which plays a critical role in the first line of host defense against these pathogens. Therefore, discerning the role and the mechanism of the lectin pathway in IgA-N will provide insights into the pathogenesis of this disorder.

In this study, we first demonstrated glomerular deposition of MBL/MASP-1 to prove that the lectin pathway played a significant role in the pathogenesis of IgA-N. Furthermore, the clinicopathological features of the cases with MBL/MASP-1 deposition were examined.

**Subjects and methods**

**Patients, clinical and histological data**

Forty-five patients with primary IgA-N, who had been referred to the Itabashi Hospital of Nihon University, were studied. Diagnosis was made by standard examination of the renal biopsy specimen by light microscopy and immunofluorescence (IF). None of the cases had clinical or serological evidence of systemic lupus erythematosus, Schönlein–Henoch purpura, or liver disease including liver cirrhosis. The age of the patients at the time of biopsy ranged from 15 to 48 years and the group included 21 males and 24 females (male/female ratio of 0.875). The relevant clinical parameters ascertained at the time of presentation included age, duration of the disease prior to biopsy, gender, recurrent macroscopic haematuria, nephrotic syndrome, persistent or intermittent proteinuria and/or macroscopic haematuria, and hypertension, characterized by the requirement of regular medication or a blood pressure $>140/90$ mmHg on at least three occasions. The laboratory assessment at the time of biopsy included routine urinalysis, 24 h urine protein excretion, and 24 h creatinine clearance. Serum immunoglobulin levels (IgG, IgA, and IgM) and serum complement levels (C3 and C4) were also measured. Blood samples were obtained on the day of biopsy and stored at $-80^\circ$C until analysis. All patients gave informed consent prior to entry into the study.

Findings by light microscopy were evaluated semi-quantitatively using a grading system previously described by Andreoli and Bergstein [14]. All biopsy specimens were scored for activity (percentage of glomeruli demonstrating crescent formation, degree of mesangial proliferation and interstitial infiltrate; maximum score $=9$) and chronicity (percentage of glomeruli demonstrating fibrous crescents, segmental sclerosis, global sclerosis, and degree of tubular atrophy and interstitial fibrosis; maximum score $=12$). Specimens for direct immunofluorescent microscopy were prepared by standard methods [5]. For the detection of IgG, IgA, IgM, C1q, C4c, C3c, properdin, and fibrinogen, sections of frozen kidney specimen were stained with fluorescein isothiocyanate-labelled rabbit or goat antibodies against each of these proteins (DAKO Japan Co. Ltd, Tokyo, Japan and Nordic Immunological Laboratories, Tilburg, Netherlands), and examined with a fluorescent microscope and evaluated.

**Immunohistochemical staining for components of the lectin pathway**

The following monoclonal mouse anti-human antibodies (mAb) were used in this study: mAbs against MBL (3E7) and MASP-1 (4C2) were obtained as previously described [15,16]. A mAb against C3b/C3c (C-5G), which reacts with C3b and C3c but not with native C3 nor with C3dg, was prepared as previously reported [17] and a mAb against C5b–9 (aE11) was purchased from DAKO. Immunochemical studies were performed using the avidin-biotin peroxidase method as previously described [17]. Briefly, frozen biopsy tissue sections were cut serially into 3 μm sections and fixed in cold acetone, air dried, and were washed in phosphate-buffered saline (PBS). The fixed sections were immersed in methanol containing 0.3% hydrogen peroxide $(v/v)$ to quench the endogenous peroxidase activity. Non-specific protein binding sites were blocked with 10% normal rabbit or goat serum in PBS. The sections were consecutively incubated with mAbs overnight at 4°C, washed in PBS, and incubated with biotin-conjugated F(ab’)2 fragments of rabbit antibody to mouse IgG (DAKO) for 30 min at room temperature. After washing in PBS, the sections were incubated with the avidin–biotin-peroxidase complex (ABC Vectastain Elite kit; Vector, Burlingame, CA), re-washed in PBS, and treated with diamobenzidine. Finally, the sections were counter-stained with haematoxylin, mounted, and observed using a conventional light microscope. In addition, normal portions of kidneys obtained as a result of nephrectomies performed in four patients due to renal tumours and renal biopsy specimens from 35 patients with various types of glomerulonephritis (GN), including 10 with mesangial proliferative GN (non-IgA), four with membranous nephropathy, three with focal glomerulosclerosis, two with mesangiocapillary GN, nine with minimal change nephrotic syndrome, one with thin-basement membrane disease, and six with lupus nephritis, were examined to be compared with IgA-N. Liver biopsy specimens from a patient with cirrhosis due to alcohol abuse were used as positive controls, while negative controls were established by substituting mAbs with an irrelevant mouse IgG [17].

**Circulating immune complex (CIC) detection and measurement of soluble C5b–9 (sC5b–9) by ELISA**

Commercially available ELISA kits were used for circulating immune complex (CIC) detection (FRELSA, C3d–CIC ELISA kit; QUIDEL, San Diego, CA) and for the measurement of sC5b–9 (SC5b–9 Enzyme Immunoassay kit; QUIDEL). For CIC detection, serum samples were added to wells coated with a mAb against C3d, and a peroxidase-conjugated mAb against a neo-epitope of human sC5b–9 and peroxidase-conjugated goat polyclonal antibodies to C6 and C7 were added. Amounts of IgG CICs were expressed as microgram equivalents of human IgG. IgA CICs were expressed as OD units, and sC5b–9 was expressed in ng/ml. Thirty-five healthy volunteers were utilized as controls.

**Statistical analysis**

Significant differences between groups were determined by the Mann–Whitney U-test and $P$ values $<0.05$ were considered significant. Data are presented as mean values ± SD.

**Results**

**Glomerular deposition of components of the lectin pathway**

In normal kidney tissues, neither MBL nor MASP-1 was detected (Figure 1a, b). Eleven of 45 cases (24.4%)
Fig. 1. Immunohistochemical demonstration of MBL (a) MASP-1 and (b) in normal kidney sections (×180); no staining is observed. Immunohistochemical staining of MBL (c), MASP-1 (d), C3b/C3c (e), and C5b–9 (f) in kidney biopsy sections from a patient with IgA nephropathy (No. 1 in Table 2) (×360); these show co-distribution predominantly in the mesangium.

with IgA-N demonstrated staining of glomeruli with mAbs against MBL and MASP-1 (Figure 1c,d). All of the 11 cases with MBL/MASP-1 were accompanied by glomerular deposition of C3b/C3c and C5b–9 with a similar distribution pattern in the same glomerulus (Figure 1e,f). In the 34 cases without MBL, although most of the cases (31 and 34 of 34, respectively) also demonstrated glomerular deposition of
C3b/C3c and C5b–9, none had deposition of MASP-1. In other forms of GN, only one case with lupus nephritis showed positive staining for MBL and MASP-1 (Table 1).

Comparison of glomerular MBL/MASP-1 deposition with IF and with histological study

Individual IF and histological parameters of IgA-N with glomerular MBL/MASP-1 deposition, as determined by the examination of renal biopsies, are summarized in Table 2. IgA was clearly the predominant immunoglobulin class deposited in the mesangial area of the glomeruli. In all 11 cases, C3c was distributed in a pattern similar to that of IgA. Similar but less intense staining was also observed for IgG in 4 of 11 cases (36.4%), for IgM in 3 of 11 cases (27.3%), for fibrinogen in 2 of 11 cases (18.2%), for C4q in 1 of 11 cases (9.1%), for C4c in 4 of 11 cases (36.4%), and for properdin in 6 of 11 cases (54.5%). Light microscopic studies revealed that the activity index was 3.4 ± 2.0 (range, 2–8) and that the chronicity index was 1.5 ± 1.9 (range, 0–6). These results demonstrate that glomerular deposition of MBL/MASP-1 is associated with histologic alterations at an early stage of the disease and not with the advanced stage.

Table 1. Glomerular deposition of components of the lectin pathway in IgA nephropathy and in various glomerular diseases (number of positive cases/number examined)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>MBL</th>
<th>MASP-1</th>
<th>C3b/c</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA nephropathy</td>
<td>11/45</td>
<td>11/45</td>
<td>42/45</td>
</tr>
<tr>
<td>Other glomerulonephritis</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>membranous nephropathy</td>
<td>0/4</td>
<td>0/4</td>
<td>4/4</td>
</tr>
<tr>
<td>focal glomerulonephrosis</td>
<td>0/3</td>
<td>0/3</td>
<td>2/3</td>
</tr>
<tr>
<td>mesangiocapillary glomerulonephritis</td>
<td>0/2</td>
<td>0/2</td>
<td>2/2</td>
</tr>
<tr>
<td>minimal-change nephrotic syndrome</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
</tr>
<tr>
<td>thin-basement membrane disease</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>lupus nephritis</td>
<td>1/6</td>
<td>1/6</td>
<td>6/6</td>
</tr>
</tbody>
</table>

Table 2. Immunofluorescent (IF) findings and histologic parameters in IgA nephropathy with glomerular MBL/MASP-1 deposition

<table>
<thead>
<tr>
<th>Number</th>
<th>IF findings</th>
<th>Histologic parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>IgA</td>
</tr>
<tr>
<td>1</td>
<td>(−)</td>
<td>(+ + +)</td>
</tr>
<tr>
<td>2</td>
<td>(−)</td>
<td>(+ + +)</td>
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<tr>
<td>3</td>
<td>(−)</td>
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<td>(−)</td>
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<tr>
<td>5</td>
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<td>(+ + +)</td>
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<tr>
<td>10</td>
<td>(−)</td>
<td>(+ + +)</td>
</tr>
<tr>
<td>11</td>
<td>(+)</td>
<td>(+ + +)</td>
</tr>
</tbody>
</table>

Abbreviations are: Fib, fibrinogen; Pr, properdin; Al, activity index; Cl, chronicity index.

Correlation to clinical and laboratory findings

Clinical and laboratory findings in IgA-N patients with and without MBL/MASP-1 deposition are shown in Table 3. Compared with the IgA-N patients without glomerular MBL/MASP-1 deposition, the IgA-N patients with glomerular MBL/MASP-1 deposition were young at the time of renal biopsy and their disease was a short duration. No other features were deduced in connection with MBL/MASP-1 deposition from these data (gender, clinical onset, urinary protein, haematuria, creatinine clearance, or serum levels of IgA, C3, or C4).

When the patients with IgA-N were divided into two groups according to the presence or absence of glomerular MBL deposition, the plasma levels of IgG–C1q, IgA–C1q, and C3b–9 were not significantly different between the groups (6.25 ± 2.44 μgEq/ml vs 5.58 ± 2.32 μgEq/ml, 0.37 ± 0.16 U vs 0.35 ± 0.12 U, and 329.0 ± 169.3 ng/ml vs 308.6 ± 104.1 ng/ml, respectively) (Figure 2).

Discussion

The pathogenesis of IgA-N has not been determined despite a great deal of research. A deeper understanding of the mechanism of complement activation in IgA-N may help to elucidate the pathogenesis, since it is apparent that the complement system participates in the development of the disease [7]. Some cases with IgA-N had glomerular deposition of MBL accompanied by MASP-1, which was not observed in normal kidney tissues, and the frequency was higher than that in other forms of GN (Table 1). These findings suggest that the lectin pathway, which is initiated by MBL/MASPs complex, is a certain mechanism of activating the complement cascade in IgA-N. The deposition of MBL/MASP-1, in addition, was concordant with the presence of C3b/C3c and C5b–9, which indicated the occurrence of on-going complement activation in situ (Table 1).
Although it has been accepted widely that complement activation in IgA-N occurs mainly via the alternative pathway [1], several reports [7–9] suggest that the classical pathway may also be involved. Miyazaki et al. [8] found glomerular deposition of C4 in 30% of patients with IgA-N and glomerular deposition of C4-binding protein (C4-bp), which was the sensitive indicator for the classical pathway activation, in 60% of patients with IgA-N. It, however, seems that IgG–IgA or IgM–IgA complexes are not always necessary for complement activation in IgA-N because mesangial C3 deposits were present even in the absence of IgG or IgM deposits (Table 2). In the lectin pathway, MBL/MASP complex activates the complement cascade by consuming C4 and C2 [11], and in addition, cleaves C3 directly, which subsequently activates the alternative pathway [18]. Consequently, the components of both the classical pathway and the alternative pathway are involved.

Table 3. Clinical and laboratory findings in IgA nephropathy patients with (MBL positive) or without MBL/MASP-1 deposition (MBL negative)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Clinical onset</th>
<th>Age at time of biopsy (years)</th>
<th>Duration of disease prior to biopsy (months)</th>
<th>Laboratory Values</th>
<th>Urinary Protein (g/24 h)</th>
<th>Haematuria (RBC/hpf)</th>
<th>CCr (ml/min)</th>
<th>IgA (g/l)</th>
<th>C3 (g/l)</th>
<th>C4 (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBL positive</td>
<td>F; 6</td>
<td>MiH; 8</td>
<td>20.7 ± 3.0*</td>
<td>12.6 ± 13.2*</td>
<td>0.88 ± 1.02</td>
<td>43.6 ± 31.3</td>
<td>98.6 ± 18.8</td>
<td>3.86 ± 1.76</td>
<td>0.54 ± 0.16</td>
<td>0.29 ± 0.10</td>
</tr>
<tr>
<td>(n=11)</td>
<td>M; 5</td>
<td>GH; 3</td>
<td>Pr; 6</td>
<td>NS; 1</td>
<td>Ht; 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBL negative</td>
<td>F; 18</td>
<td>MiH; 18</td>
<td>27.5 ± 8.1*</td>
<td>32.3 ± 26.1*</td>
<td>1.05 ± 1.40</td>
<td>49.6 ± 33.0</td>
<td>89.1 ± 19.3</td>
<td>3.12 ± 0.95</td>
<td>0.51 ± 0.12</td>
<td>0.28 ± 0.09</td>
</tr>
<tr>
<td>(n=34)</td>
<td>M; 16</td>
<td>GH; 10</td>
<td>Pr; 15</td>
<td>NS; 4</td>
<td>Ht; 6</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Data represent means ± SD. *Significantly different (P < 0.05).
 Abbreviations are: MiH, microscopic haematuria; GH, gross haematuria; Pr, proteinuria; NS, nephrotic syndrome; Ht, hypertension; CCr, creatinine clearance.
It should be noted that the cases with glomerular MBL/MASP-1 deposition were young and the duration of the disease prior to biopsy was short compared with that in cases without MBL/MASP-1 deposition (Table 3). These findings suggest that the lectin pathway participates in the development of the disease at an early stage in consideration of the result that none of the cases with MBL/MASP-1 deposition had advanced histologic alterations (Table 2). The finding in this study that there were no peculiar features in the clinical or laboratory data in any of the cases with MBL/MASP-1 deposition may result from the possibility that the time from the onset of the disease to the biopsy varied in each case. Taking the time of biopsy into account, the prevalence of glomerular C3 deposits may be caused by complement activation predominantly via the lectin pathway.

Another important point is to determine the mechanisms of glomerular deposition of MBL/MASP-1 in IgA-N. Since the deposition was not related to plasma levels of CIC or sC5b–9 (Figure 2), the complement cascade via the lectin pathway is likely to be activated by the MBL/MASPs complex locally in kidney tissues of patients with IgA-N. Several viral antigens (cytomegalovirus, Epstein–Barr virus, herpes simplex virus, adenovirus, and hepatitis B virus) [1,3,7] and bacterial antigens (Haemophilus parainfluenzae [19] and Escherichia coli) have been proposed to be responsible for the formation of mesangial IgA deposits in patients with IgA-N. Conversely, previous studies have shown that human MBL is able to bind a wide range of bacteria and viruses, as well as yeast, mycobacteria, and fungi [13]. Considering these factors together, glomerular deposition of MBL in IgA-N may be related to these antigens. Another possibility is that the glomerular deposition of abnormal glycosylated IgG or IgA triggers the lectin pathway activation, since a recent study by Malhotra and colleagues [20] demonstrated that IgG glycoforms, lacking galactose, bound to MBL directly and caused complement activation, which may be particularly important in rheumatoid arthritis (RA). An abnormal glycosylation pattern of IgA1 in patients with IgA-N was also previously reported by several investigators [21,22], although the aberrant pattern was described as a decreased galactosylation in O-linked glycans, in contrast with the pattern of IgG in patients with RA, which was considered to reflect decreased galactosylation in N-linked glycans [20].

MBL has been intensively investigated and these studies prove that a deficiency or a low level of MBL, which is caused by mutations in the MBL gene, is associated with increased risk of infection [23–25]. However, only a few studies have looked at tissue damage resulting from complement activation via the lectin pathway. In this respect, we first demonstrated that glomerular deposition of MBL/MASPs initiated complement activation via the lectin pathway in some cases of IgA-N. Based on the observations presented in this study, we hypothesize that the lectin pathway is initiated intermittently by MBL/MASPs complex and serves as a trigger for the activation of the amplification cycle via the alternative pathway and that this initiation is associated with repeated antigen exposures such as infection. This suggests a novel mechanism of complement activation in IgA nephropathy and may provide an insight into the aetiology of this disease.

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References
5. Ibels LS, Gysöry AZ. IgA nephropathy: analysis of the natural history, important factors in the progression of renal disease, and a review of the literature. Medicine 1994; 73: 79–102
7. Emancipator SN, Lamm ME. IgA nephropathy: pathogenesis of the most common form of glomerulonephritis. Lab Invest 1989; 60: 168–183


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