Complement System in IgA Nephropathy

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A study on the histopathological findings and analysis of complement systems in patients with IgA nephropathy is described. A close association between complement deposition and destruction of glomerular tissues was observed. The prominent complement components related to the glomerular deposition of IgA were those of the alternative pathway of complement activation. However, components of the classical complement pathway might be related to the deposition of IgG and IgM in the glomerulus. It is suggested that the analysis of the complement system in patients with IgA nephropathy is useful for evaluation of tissue damage and thus the prognosis of such patients.

(Key Words: Alternative Pathway, Complement Components, Properdin, GBG)

INTRODUCTION

IgA nephropathy is characterized by mesangial localization of IgA with less intense localization of IgG and C3 in patients without evidence of systemic diseases.

A variety of histological alterations in glomeruli have been reported, but focal segmental proliferative glomerulonephritis is the most common finding.

The pathogenesis of IgA nephropathy, including the mechanism of complement activation, is obscure. Since the localization of IgA in glomeruli is frequently associated with IgG as well as IgM, it is difficult to determine whether the complement system is activated by IgA or other classes of immunoglobulins.

The present study reports the results of histopathological findings and analysis of complement systems in patients with IgA nephropathy.

A close relationship between the histopathological changes and the pattern of complement deposition in glomeruli was demonstrated.

It is suggested that the analysis of complement deposition in glomeruli is useful for estimation of histopathological alterations and thus the prognosis of patients with IgA nephropathy.

MATERIALS AND METHODS

Renal biopsy specimens were obtained from 335 patients with clinical signs of primary glomerular diseases in the Sapporo City General Hospital. One hundred and thirty four of these patients (40%) were diagnosed as IgA nephropathy. All specimens were examined by light microscopic and immunofluorescent techniques.

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(1) Light microscopic studies

Specimens were fixed for 6-24 hours in Dubosq-Brazil fluid and subsequently in 10% neutral buffered formalin for 24 hours. These specimens were sectioned to 3 to 4μ and stained with hematoxylin-eosin (H-E), periodic acid Schiff (PAS), massontrichrome light green or periodic acid methenamine (PAM).

(2) Immunofluorescent studies

Renal biopsy specimens were embedded and rapidly frozen in liquid nitrogen sectioned to 2 to 3μ with a rotary microtome in a cryostat at about -25°C, and air-dried.

Immediately before staining, sections were washed three times in phosphate buffered isotonic saline (pH7.2) for 15 min.

Fluorescein-labelled antisera to polyvalant human immunoglobulins (IgG, IgM, IgA, kappa and lambda), as well as heavy chain-specific anti human IgG, IgM, IgA, IgD, IgE and anti human Clq, C3, C4, fibrinogen and albumin were obtained from the Behringwerke AG, Marburg-Lahn, West Germany (F/P molar ratios ranged from 1.8 to 2.9).

Fluorescein-labelled antisera to IgE were obtained from Kallestad, USA.

Specificities of these antisera were determined by immunodiffusion and immunoelectrophoresis. These fluorescein-labelled antisera were diluted 1:10 to 1:20 in PBS.

Cryostat sections were stained with the fluorescein-labelled antisera in a moist chamber at room temperature for 30 min.

Indirect immunofluorescent studies were performed using rabbit antisera to human Clq, C3, C4 and C9 obtained from the Behringwerke AG, Marburg-Lahn, West Germany. Rabbit antisera to human properdin, C5 and glycine-rich beta glycoprotein (GBG) were kindly provided by Drs. Kobayashi, Konno and Fujita, Department of Biochemistry and Internal Medicine, Hokkaido University School of Medicine, Sapporo, Japan. Specificity of antisera to human Clq, C3, C4, C5, C9, properdin and GBG was determined by immunodiffusion. The degree of dilution of these antisera to complement components and complement activating factors was 1:10 to 1:20.

FITC-labelled goat antirabbit Ig sera were obtained from the Behringwerke AG (F/P ratio 4.0), and were absorbed by human liver and kidney homogenates, and used at dilutions of 1:20 or 1:30. The sections washed with PBS were then incubated with antisera to Clq, C3, C4, C5, C9, properdin and GBG in a moist chamber at room temperature for 30 min.

The sections washed with PBS were then incubated with the FITC labelled goat antirabbit Ig sera at room temperature for 30 min, and washed with PBS.

The sections were then covered with buffered glycerol and a cover slip and examined with a Nikon and Leitz Ortholux fluorescent microscope.

The intensity of the fluorescence was graded as none (-), trace (+), 1 (+), 2 (+) and 3 (+).

RESULTS

1) Histopathological findings

Histopathological findings of the renal biopsy specimens from patients
with IgA nephropathy were classified into three groups. In Group I (29 cases),
the changes were found mainly around the arteriolar walls and mesangial
matrix at the base of the glomerular tuft. The afferent arteriolar walls were
thickened in all cases. These changes were recognized easily in the sections
stained by PAS. Tubulus, extraglomerular arterioles and interstitium showed
no changes. In Group II (56 cases), the main alterations in the glomeruli
were mesangial hypertrophy and slight mesangial cell proliferation. These
changes were found mainly at the axial and peripheral mesangial matrix.
The distribution of glomerular changes was mainly focal and/or segmental.

In Group III (49 cases), mesangial hypertrophy was also observed.
Capsular adhesion was present in more than 50% of the glomeruli. Crescent
formation and glomerular sclerosis were also found in more than 30% of the
glomeruli.

Segmental and/or diffuse sclerosis of glomerular lobules was present in
some cases. Changes in the glomerular basement membrane (GBM) were
minimal, although mild to moderate irregular thickening of the GBM was
noted. Mononuclear cell infiltration, fibrosis of the interstitium and tubular
atrophy were frequently observed.

In addition to these findings, thickening of the extraglomerular arteriolar
walls was observed (Fig. 1).

![Fig. 1 Diffuse mesangial hypertrophy with an increase of matrix, × 200, PAS-stain.](image)

2) Immunofluorescent studies
(a) Immunoglobulins

The results of immunofluorescent studies are summarized in Table I. Immunoglobulin-A was the predominant class of immunoglobulin noted in
the glomeruli. Four types of IgA nephropathy were classified with regards to
the classes of immunoglobulins deposited in the glomeruli, i.e. deposits
composed of IgA alone, IgA and IgM, IgA and IgG, and IgA, IgM and IgG.

In 49 cases (37%), IgA was the only immunoglobulin detected in the
glomeruli. In 39 cases (29%), IgA was detected in association with IgM.
In 23 cases (17%), IgA was detected with IgG, while in 23 cases (17%), IgA was detected with IgG and IgM. The intensity of IgG and IgM deposition was always less than that of IgA deposition. The distribution of IgG and IgM was similar to that of IgA, although the distribution of IgM was focal and/or segmental in some cases.

None of the cases examined for IgD and IgE showed positive stainings (Fig. 2).

Table 1 Incidence of positive fluorescence of immunoglobulins in glomeruli

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Incidence of positive fluorescence of immunoglobulins in glomeruli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgA Alone</td>
</tr>
<tr>
<td>Group I</td>
<td>15 (45%)</td>
</tr>
<tr>
<td>Group II</td>
<td>20 (56%)</td>
</tr>
<tr>
<td>Group III</td>
<td>16 (33%)</td>
</tr>
</tbody>
</table>

Fig. 2 Immunofluorescent localization of IgA in mesangial areas, segmentally involving capillary walls

(b) Complement components and complement activating factors

Table II summarizes the results of glomerular localization of complement components and complement activating factors.

Clq was observed in eight out of 62 cases (13%). C4 was observed in six out of 116 cases (5%). Positive staining of Clq and C4 were found in cases with combined deposits of immunoglobulins. Clq and C4 were not found in cases with sole deposition of IgA. Strong deposition of C3 was observed in 113 out of 134 cases (84%).

C3 was observed in 41 cases (85%) in which IgA occurred as the sole immunoglobulin. In cases with combined deposits of immunoglobulins, C3 was also observed in 72 out of 86 cases (84%) (Fig. 3).

C5 was observed in 37 out of 50 cases (74%) (Fig. 4). C9 was observed
in 46 out of 50 cases (92%). In the cases with sole deposition of IgA, C5 was found in 11 out of 14 cases (79%) and C9 was found in 13 out of 14 cases (93%).

GBG was observed in only one out of 53 cases. In contrast, 29 out of 46 cases (63%) were positive for properdin (Fig. 5). The distribution of properdin was identical to that of IgA, C3, C5 and C9. A distinct pattern of staining for IgA, complement components and properdin was the localization of granular deposits in mesangial areas which were comparable to the strong PAS positive areas.

Table 2. Incidence of complement components in glomeruli

<table>
<thead>
<tr>
<th>Complement components</th>
<th>Immunoglobulins in the glomeruli</th>
<th>Incidence of positive fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgA Alone</td>
<td>IgA + IgG</td>
</tr>
<tr>
<td>C1q</td>
<td>0/15</td>
<td>1/7</td>
</tr>
<tr>
<td>(0%)</td>
<td>(14%)</td>
<td>(8%)</td>
</tr>
<tr>
<td>C4</td>
<td>0/35</td>
<td>3/23</td>
</tr>
<tr>
<td>(0%)</td>
<td>(13%)</td>
<td>(3%)</td>
</tr>
<tr>
<td>C5</td>
<td>41/48</td>
<td>20/24</td>
</tr>
<tr>
<td>(85%)</td>
<td>(85%)</td>
<td>(82%)</td>
</tr>
<tr>
<td>C5</td>
<td>11/14</td>
<td>6/7</td>
</tr>
<tr>
<td>(79%)</td>
<td>(86%)</td>
<td>(69%)</td>
</tr>
<tr>
<td>C9</td>
<td>13/14</td>
<td>7/7</td>
</tr>
<tr>
<td>(95%)</td>
<td>(100%)</td>
<td>(94%)</td>
</tr>
<tr>
<td>Properdin (P)</td>
<td>7/13</td>
<td>7/7</td>
</tr>
<tr>
<td>(54%)</td>
<td>(100%)</td>
<td>(46%)</td>
</tr>
<tr>
<td>GBG (B)</td>
<td>1/13</td>
<td>0/11</td>
</tr>
<tr>
<td>(8%)</td>
<td>(0%)</td>
<td>(0%)</td>
</tr>
</tbody>
</table>

Fig. 3  Immunofluorescent localization of C3 in mesangial areas and extraglomerular arteriolar wall
(c) The relation between histopathological changes and the pattern of IgA and complement depositions

Table III shows the relation between the histopathological changes in glomeruli and the localization of complement components.

In Group I, fine granular deposits of IgA and complement components were often detected in walls of arterioles and mesangial areas at the base of glomerular tufts.

In Groups II and III, fine or coarse granular deposits of IgA and complement components were detected in the axial and peripheral mesangial areas. In some cases of Groups II and III, IgA was segmentally involved in the capillary walls. The intensity of IgA in Group II was identical to that in Group III.

Intensity and incidence of C3, C5, C9 and properdin deposits were mild
in Group I, moderate in Group II, and severe in Group III.

C3, C5 and C9 were frequently observed in the basement membrane of Bowman's capsules and extraglomerular arteriolar walls, in addition to localization in the mesangial regions.

<p>| Table 3. Incidence of positive fluorescence of complement components in glomeruli |
|---------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|</p>
<table>
<thead>
<tr>
<th>Histopathologic changes</th>
<th>Clq</th>
<th>C4</th>
<th>C3</th>
<th>C5</th>
<th>C9</th>
<th>Properdin</th>
<th>GBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0/8</td>
<td>2/27</td>
<td>20/29</td>
<td>4/8</td>
<td>6/8</td>
<td>2/7</td>
<td>1/11</td>
</tr>
<tr>
<td></td>
<td>(0%)</td>
<td>(7%)</td>
<td>(69%)</td>
<td>(50%)</td>
<td>(75%)</td>
<td>(29%)</td>
<td>(9%)</td>
</tr>
<tr>
<td>Group II</td>
<td>1/30</td>
<td>1/46</td>
<td>47/56</td>
<td>17/24</td>
<td>22/24</td>
<td>14/21</td>
<td>0/23</td>
</tr>
<tr>
<td></td>
<td>(3%)</td>
<td>(2%)</td>
<td>(84%)</td>
<td>(71%)</td>
<td>(92%)</td>
<td>(67%)</td>
<td>(0%)</td>
</tr>
<tr>
<td>Group III</td>
<td>7/24</td>
<td>3/43</td>
<td>46/49</td>
<td>16/18</td>
<td>18/18</td>
<td>13/18</td>
<td>0/19</td>
</tr>
<tr>
<td></td>
<td>(29%)</td>
<td>(7%)</td>
<td>(94%)</td>
<td>(89%)</td>
<td>(100%)</td>
<td>(73%)</td>
<td>(0%)</td>
</tr>
</tbody>
</table>

DISCUSSION

IgA nephropathy is characterized by mesangial localization of IgA in renal biopsy specimens by immunofluorescence staining (1, 2). Recently IgA has been found to fix complement mainly through the alternative pathway (5), and, to some extent, by the classical pathway (6). Since the localization of IgA in the glomeruli in IgA nephropathy is frequently associated with IgG as well as IgM, it is difficult to determine whether the complement system is activated by IgA or other classes of immunoglobulins. In cases with sole deposition of IgA, early complement components (Clq and C4) were absolutely absent in the glomeruli, although late complement components (C3, C5 and C9) were present in a pattern similar to that of IgA deposition. These observations suggest that the complement system in IgA nephropathy is activated mainly at C3 via the alternative pathway by IgA. This assumption was supported by the fact that glomerular deposition of properdin was demonstrated in most cases: this finding was consistent with that of Evans et al. (5) and McCoy et al. (7). Denatured IgA deposited in glomeruli might be responsible for this activation of alternative pathways (8). Fearon et al. (4) reported that GBG might be released from the site of complement activation in vitro. However, GBG was observed in only one of the 53 cases examined in this study. Similar findings were observed in cases with glomerular deposits of other classes of immunoglobulins although certain cases showed Clq and/or C4.

Histopathological findings in IgA nephropathy were classified into three groups, Group I: minimal lesion, Group II: mesangial hyperplasia with an increase in matrix and Group III: Group II with severe capsular adhesion, fibroepithelial crescent, glomerular hyalnosis and sclerosis. In Group I, IgA was mainly detected in the axial portion of the mesangial matrix. There was basically no difference between Group II and Group III, with regard to the localization of IgA and other immunoglobulins. The difference
between these two groups was the pattern of the glomerular localization of complement components: a higher incidence and a greater intensity of glomerular deposition of complement components was observed in Group III than in Group II. These observations suggest that the inflammatory changes in IgA nephropathy such as capsular adhesion, crescent formation, hyalinosis and sclerosis are correlated with complement activation.

The histopathological classification employed in this study correlates well with the immunohistological, clinical and laboratory findings in patients with IgA nephropathy. It is concluded that the determination of the glomerular deposition of complement, in association with histopathological classification, is useful for evaluation of tissue damage and thus the prognosis of patients with IgA nephropathy.

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