Nephritis-Associated Plasmin Receptor and Acute Poststreptococcal Glomerulonephritis: Characterization of the Antigen and Associated Immune Response

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Abstract. The role of nephritis-associated antigen as a virulence factor for acute poststreptococcal glomerulonephritis (APSGN) remains to be fully clarified. Nephritis-associated plasmin receptor (NAPr) was previously isolated from group A streptococcus (GAS) and shown to bind plasmin(ogen). The nucleotide sequence of the napr gene from GAS isolates obtained from patients with APSGN was determined. The sequence of the putative open reading frame (1011 bp) showed 99.8% identity among isolated strains. Homology screen revealed an exact match with streptococcal glyceraldehyde-3-phosphate dehydrogenase (GAPDH). NAPr exhibited GAPDH activity in zymography, and it activated the complement pathway in vitro. In APSGN kidney biopsy specimens, NAPr was observed mainly in the early stage of the disease (1 to 14 d after onset) but was not colocalized with either C3 or IgG as assessed by double immunofluorescence staining. Sera of patients with APSGN, patients with GAS infection without renal involvement, nonrenal pediatric patients, and healthy adults as controls were assayed for anti-NAPr antibody titers. Anti-NAPr antibodies were present most frequently in APSGN sera, and antibody titers were also significantly higher than in patients with GAS infection alone or in other control patients. Moreover, antibody titers remained elevated during the entire 10-yr follow-up period.

Group A streptococcus (GAS) causes various levels of infection ranging from mild pharyngitis to severe streptococcal toxic shock syndrome. One sequela of GAS infection is acute poststreptococcal glomerulonephritis (APSGN), which is associated with long-term renal dysfunction in some patients (1). However, only certain strains appear to cause APSGN (2), and only these strains produce nephritis-associated antigens (3).

A number of streptococcal proteins, including nephritins, strain-associated protein, streptococcal pyrogenic exotoxin B (SPEB), preabsorbing antigen, and NAPr, are involved in the pathogenesis of APSGN (4–7). Nephritogenic antigens are expressed by so-called nephritis-associated serotypes, accumulate in the glomeruli of patients with APSGN, and induce high antibody titers in these patients. NAPr is such a nephritogenic antigen; it is expressed by streptococcal strains historically associated with APSGN, it is highly antigenic, and it is localized in affected glomeruli (7). However, we cannot exclude the possibility that NAPr is identical to previously described nephritogenic antigens because some other streptococcal proteins also exhibit plasmin(ogen)-binding activity (4,8,9). Only a partial amino acid sequence has been available for NAPr (7); however, the protein may be homologous to streptococcal glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (10,11) and may show GAPDH activity. Thus, additional analysis of NAPr is needed.

APSGN-related antibodies against putative nephritogenic antigens have been identified (12–14). High levels of anti-SPEB antibody were present in patients with APSGN (6), and subsets of APSGN kidney specimens were positive for anti-SPEB antibody. In addition, increased levels of anti-zymogen antibody appear to be a marker of APSGN (15,16). Glomeruli from patients with early APSGN can be stained with IgG obtained from the sera of convalescent patients (17). Reactivity is typically observed on the endothelial side of the glomerular basement membrane (GBM) and in the mesangial matrix (18). The streptococcal antigen, such as preabsorbing antigen, has also been detected in the glomeruli of patients in relatively early stages of APSGN (5).

In the study presented here, we determined the amino acid sequence of NAPr purified from GAS strains in patients with...
APSGN. We also examined GAPDH activity, complement activation, and immune responses to NAPrl in patients with APSGN.

**Materials and Methods**

**GAS and Preparation of NAPrl**

Strains of group A β-hemolytic streptococci belonging to T types 1, 4, and 12 and M types 12 and 49 were isolated from the pharynx of five patients with APSGN. Growth conditions and purification of NAPrl were as described previously (7). We had prepared NAPrl in 1, 4, and 12 and M types 12 and 49 were isolated from the pharynx of Clinical and laboratory features of patients with APSGN Table 1.

**DNA Sequencing**

Genomic DNA of GAS T types 1, 4, and 12 and M types 12 and 49 were purified with SepaGene (Sanko Junyaku, Tokyo, Japan) and used as templates to amplify a fragment of naprl by PCR. The following primers were used: forward primers, 5'-AAGTTAAA-GAAGGTGAT-3', 5'-AGCTGTCAAAACAGTAG-3', and 5'-TATATTTGGTGGTTTTG-3'; reverse primers, 5'-CAGCT-GAAGGTGGAT-3', 5'-AGCTGCTTCAAACGATAG-3', and 5'-CCCTCTCAATTCGCTTGGTTTA-3' at a concentration of 1 μM each. Primers were designed from the results of partial amino acid sequencing of purified NAPrl (7) and preliminary DNA sequence analysis. The PCR temperature profile was carried out as follows: consisting of an initial denaturation stop of 95°C for 5 min, followed by 99 cycles of a denaturation step of 95°C for 30 s, a primer annealing step at 55°C for 20 s, and an extension step at 60°C for 4 min. The amplified DNA fragments were sequenced with a BigDye Terminator Ready Reaction Kit and an ABI PRISM 377 XL DNA sequencer (Applied Biosystems, Foster City, CA).

**GAPDH Activity Assay**

Similarity of NAPrl to GAPDH was assessed by Western blot analysis (7). After SDS-PAGE and transfer of purified NAPrl to PVDF membranes (Millipore, Billerica, MA), proteins were reacted with mouse anti-Bacillus GAPDH antibody (1:1000 in PBS containing 0.1 M NaHPO₄ (pH 8.5) with physiologic saline, was incubated with 50 μl of human serum, diluted 1:40 with physiologic saline). In some samples, 10 μl of 0.1 M EGTA and/or 0.1 M EDTA was added before incubation to differentiate between the two complement activation pathways. The reactions were separated by electrophoresis on 1.1% agarose gels in veronal buffer (pH 8.6) with an ionic strength of 0.05 (Wako). Conversion of C3 was examined with anti-human C3 antibody (ICN, Costa Mesa, CA). Zymosan (Sigma) was used as a control for complement activation.

The product of NAPrl cleavage of C3, iC3b, was assayed by ELISA. For sample preparation, 50 μl of human serum, diluted 1:40 with physiologic saline, was incubated with 50 μl of NAPrl (ranging from 0.01 μg to 6.25 μg) at 37°C for 1 h. The level of iC3b in each sample was measured with a commercial iC3b EIA kit (Quidel, San Diego, CA) according to the manufacturer’s instructions.

**Complement Activation by NAPrl**

To analyze complement activation by NAPrl, we incubated normal human serum (50 μl) for 1 h at 37°C with NAPrl (10 μg/50 μl in physiologic saline). In some samples, 10 μl of 0.1 M EGTA and/or 0.1 M EDTA was added before incubation to differentiate between the two complement activation pathways. The reactions were separated by electrophoresis on 1.1% agarose gels in veronal buffer (pH 8.6) with an ionic strength of 0.05 (Wako). Conversion of C3 was examined with anti-human C3 antibody (ICN, Costa Mesa, CA). Zymosan (Sigma) was used as a control for complement activation.

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**Patients and Control Subjects**

Sera from 50 patients with APSGN (27 men and 23 women), diagnosed by renal biopsy, (Table 1) were tested for levels of anti-NAPrl antibody. Serum samples were obtained at the time of biopsy (1 to 90 d after disease onset); anti-NAPrl antibody levels were determined and taken as the initial antibody titers. Samples were collected over a 10-yr follow-up period and used to monitor antibody levels in each patient. The diagnosis of APSGN was confirmed by the

<table>
<thead>
<tr>
<th>From Onset to Biopsy</th>
<th>n</th>
<th>Mean Age in yr (range)</th>
<th>ASO&lt;sup&gt;a&lt;/sup&gt; (U)</th>
<th>ASK&lt;sup&gt;b&lt;/sup&gt; (U)</th>
<th>CH50&lt;sup&gt;c&lt;/sup&gt; (U)</th>
<th>C3&lt;sup&gt;d&lt;/sup&gt; (mg/dl)</th>
<th>Urine Protein</th>
<th>GFR (ml/min)</th>
<th>BUN (mg/dl)</th>
<th>S-cr (mg/dl)</th>
<th>BP (mmHg)</th>
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<tr>
<td>1–14d</td>
<td>25</td>
<td>30.8 (5–51)</td>
<td>584</td>
<td>4632</td>
<td>16.2</td>
<td>30.8</td>
<td>±~+++-</td>
<td>1~many</td>
<td>66</td>
<td>23.8</td>
<td>1.4</td>
</tr>
<tr>
<td>15–30d</td>
<td>18</td>
<td>26.1 (8–75)</td>
<td>550</td>
<td>8533</td>
<td>17.1</td>
<td>42.0</td>
<td>~++-+-</td>
<td>0~many</td>
<td>72</td>
<td>22.0</td>
<td>1.4</td>
</tr>
<tr>
<td>31–90d</td>
<td>7</td>
<td>31.4 (5–66)</td>
<td>865</td>
<td>4480</td>
<td>27.0</td>
<td>60.0</td>
<td>~+++-+</td>
<td>0~many</td>
<td>75</td>
<td>18.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>29.3 (5–75)</td>
<td>612</td>
<td>5952</td>
<td>18.1</td>
<td>44.3</td>
<td>~+++-+</td>
<td>0~many</td>
<td>70</td>
<td>22.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Per group.

<sup>b</sup> Normal values in our hospital:

<sup>c</sup> ASO-adults, < 240 U; children, < 320 U.

<sup>d</sup> ASK-adults, < 2560 U; children, < 3200 U.

<sup>e</sup> CH50, 30–40 U.

<sup>f</sup> C3, 55–120 mg/dl.
presence of proteinuria, hematuria, hypocomplementemia, history of antecedent streptococcal infection with titers of anti-streptolysin O (ASO) and/or anti-streptokinase (ASK), and renal biopsy. Fifty age-matched patients with GAS upper respiratory tract infection without detectable renal involvement (26 men and 24 women) (Table 2) were included as subjects. GAS upper respiratory tract infection was diagnosed on the basis of clinical sign with significant elevation of ASO and/or ASK titers. The control groups included 100 nonrenal pediatric patients and 100 healthy adults. Pediatric patients were categorized by age into two groups: pediatric I (age 0.2 to 10 yr, \( n = 50 \), 27 boys, 23 girls) and pediatric II (age 11 to 20 yr, \( n = 50 \), 23 boys and 27 girls). Healthy adults were also categorized by age into two groups: adult I (age 25 to 35 yr, \( n = 50 \), 25 men and 25 women, age matched with patients with APSGN), and adult II (age 52 to 59 yr, \( n = 50 \), 25 men and 25 women). These subjects showed no signs of recent streptococcal infection. Informed consent was obtained from all subjects in each group.

**Measurement of Serum Anti-NAPrlr Antibody**

Serum anti-NAPrlr antibody was measured by Western blot analysis as described previously (7). Affinity-purified NAPrlr (7) was separated

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**Table 2. Clinical and laboratory features of patients with group A streptococcal infection without renal involvement, children, and normal adults**

<table>
<thead>
<tr>
<th></th>
<th>( n )</th>
<th>Mean age in yr(^a) (range)</th>
<th>ASO (U)</th>
<th>ASK (U)</th>
<th>CH50 (U)</th>
<th>Urine Protein</th>
<th>Urine Occult Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcal infection without renal involvement</td>
<td>50</td>
<td>29.0 (8–64)</td>
<td>499</td>
<td>4764</td>
<td>37.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pediatric I</td>
<td>50</td>
<td>7.2 (0.2–10)</td>
<td>177</td>
<td>451</td>
<td>30.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pediatric II</td>
<td>50</td>
<td>14.1 (11–20)</td>
<td>187</td>
<td>554</td>
<td>28.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Normal adults I</td>
<td>50</td>
<td>30.0 (25–35)</td>
<td>186</td>
<td>576</td>
<td>36.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Normal adults II</td>
<td>50</td>
<td>53.2 (52–59)</td>
<td>80</td>
<td>401</td>
<td>37.8</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^a\) Per group.

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**Figure 1.** Nucleotide sequence and predicted amino acid sequence of the naprlr gene in group A streptococci T type 12. Putative conserved promoter sequences (−35 and −10) and ribosome-binding site sequence (RBS) are indicated by bars. The predicted transcription start site, +1, is denoted by an asterisk. The predicted ATG start codon and TAA stop codon are indicated by bars. Positions of the PCR primers and their orientations are indicated by arrows.
by SDS-PAGE (10% polyacrylamide gel) and the proteins were transferred to PVDF membranes (Millipore) at 0.8 mA/cm² for 50 min in a semidy transfer cell (Bio-Rad Laboratories, Hercules, CA). Membranes were blocked with 5% nonfat milk in 10 mM Tris-HCl (pH 7.2) containing 0.15 M NaCl and 0.1% Tween 20 (TBS-Tween) for 1 h. The membranes were incubated with each serum sample (1:500 to 1:2000 in nonfat milk/TBS-Tween) for 1 h. Membranes were washed with TBS-Tween and then incubated with horseradish peroxidase-conjugated anti-human IgG antibody (1:2000 in nonfat milk/TBS; American Qualex, San Clemente, CA) for 1 h. Immune complexes were visualized by development with ECL (Amersham). Pooled sera from convalescing patients were included as positive controls, and pooled sera from age-matched healthy donors were used as negative controls. NAPIr bands were quantified with a Densitometry System and Imaging Software (ATTO, Tokyo, Japan). The level of anti-NAPIr antibody was determined relative to the density of the positive control band (titer: 1000 units) and that of the age-matched healthy control band (titer: 60 to 140 units).

**Immunofluorescence Microscopy**

Direct and indirect immunofluorescence microscopy, FITC-conjugated rabbit anti-NAPIr antibody, and monoclonal antibody to recombinant Pfr were as described by Yamakami et al. (7). Briefly, direct immunofluorescence was used for the detection of NAPIr, complement components (C3, C1q, C4, P), immunoglobulins (IgG, IgA, IgM), fibrinogen, and plasminogen (ICN, Irvine, CA). Indirect immunofluorescence was used to detect other complement components (C5, C9, S, MAC) (ICN). As a negative control, sections were pretreated with either unlabeled rabbit anti-NAPIr antibody or serum from a convalescing patient with APSGN. NAPIr-C3 and NAPIr-IgG colocalization assays were performed with double staining for NAPIr and C3 or IgG in renal sections from several NAPIr-positive patients. To examine colocalization of NAPIr and C3, we labeled anti-NAPIr antibody (1 mg protein) with Alexa Fluor 594 (Molecular Probes, Eugene, OR), according to the manufacturer’s instructions and applied the labeled antibody with FITC-labeled anti-C3 antibody (ICN). For NAPIr-IgG colocalization experiments, Alexa Fluor 594-labeled goat anti-human IgG antibody (Molecular Probes) and FITC-labeled anti-NAPIr antibody were applied simultaneously to the sections.

**Statistical Analyses**

Statistical analyses of ASO titers and anti-NAPIr antibody titers in the present report were performed by unpaired t test. Two-tailed P values of less than 0.05 were considered statistically significant.
**Results**

**naplr Gene Sequences**

The full-length nucleotide sequence of the naplr gene of T type 12 is shown in Figure 1. Among sequences from the five-nephritogenic strains analyzed, only two nucleotides in the open-reading frame (ORF) differed; however, the predicted NAPlr amino acid sequence was identical among strains. The predicted naplr ORF is 1011 bp long, and the putative promoter contains a conserved TATA box at \(-\)11002 and a CAT box (TTGCAT) at \(-\)104. In addition, a potential ribosome binding site (TAAGGAGG) is located nine nucleotides upstream from the predicted ATG start codon. Guanine at position 1066 of naplr was substituted for thymine in comparison to the nucleotide sequence of the plr gene encoding plasmin receptor (Plr), which is identified as GAPDH of GAS strain 64/14 (10; GenBank Database accession number M95569). Thus, NAPlr and Plr showed 99.8% identity at nucleotide and 99.7% identity at amino acid levels.

The naplr ORF encodes a 336 amino acid polypeptide with a predicted isoelectric point of 5.2 and a predicted molecular mass of 35.8 kD. The predicted molecular mass was lower than that determined by SDS-PAGE (43 kD), which may reflect the amino acid compositions of NAPlr. The N-terminal amino acid sequence of purified NAPlr contained the following five residues: VVKVG. The N-terminal amino acid sequence of native NAPlr was homologous to the deduced N-terminal sequence of NAPlr, with the exception of an additional N-terminal methionine.

**Functional Analysis of NAPlr**

On the basis of the naplr nucleotide sequence, which was homologous to the GAPDH sequence (11), NAPlr was tested for reactivity with anti-GAPDH antibody and for GAPDH activity. Western blot analysis revealed that NAPlr reacted with anti-Bacillus GAPDH antibody (Figure 2, left). In addition, zymographic analysis showed that both purified NAPlr and crude extract each contained activity in single bands that had identical migration profiles (Figure 2, right).

The ability of NAPlr to activate complement was measured as conversion of C3 (Figure 3A). C3 conversion was observed in the presence or absence of chelating reagent. Thus, NAPlr activated the alternate complement pathway. In addition, we found that NAPlr induced the formation of iC3b in a dose-dependent manner (Figure 3B).

<table>
<thead>
<tr>
<th>Age in yr, range</th>
<th>Anti-NAPlr Antibody (positive rate)*</th>
<th>Anti-NAPlr antibody titersb</th>
</tr>
</thead>
<tbody>
<tr>
<td>APSGN 5–75, mean 29.3</td>
<td>46/50 (92%)</td>
<td>566.0 ± 106.1c</td>
</tr>
<tr>
<td>Streptococcal infection 8–64, mean 29.0</td>
<td>30/50 (60%)</td>
<td>227.1 ± 51.2</td>
</tr>
<tr>
<td>Pediatric I 0.2–10, mean 7.2</td>
<td>13/50 (26%)</td>
<td>138.9 ± 23.4</td>
</tr>
<tr>
<td>Pediatric II 11–20, mean 14.1</td>
<td>18/50 (36%)</td>
<td>166.0 ± 25.7</td>
</tr>
<tr>
<td>Normal adults I 25–35, mean 30.0</td>
<td>24/50 (48%)</td>
<td>100.1 ± 18</td>
</tr>
<tr>
<td>Normal adults II 52–59, mean 53.2</td>
<td>36/50 (72%)</td>
<td>186.0 ± 17.3</td>
</tr>
</tbody>
</table>

* The presence of anti-NAPlr antibody was determined by Western blot analysis.

b Values of anti-NAPlr antibody titers are expressed as mean ± SEM.

* P < 0.05 for APSGN versus streptococcal infection, pediatrics, and normal adults.

**Figure 4.** (A) Levels of anti-NAPlr antibody titers in acute poststreptococcal glomerulonephritis (APSGN), streptococcal infection without renal involvement (SI), nonrenal pediatric patients, and normal adults. Values are mean ± SEM. *P* < 0.05 for APSGN versus SI, pediatrics, and normal adults by *t* test. (B) Levels of ASO titers from the same group of patients compared with anti-NAPlr antibody titers. The APSGN and SI groups show significantly elevated ASO titers in comparison to those in nonrenal pediatric patients and normal adults. Values are mean ± SEM. **P** < 0.001 for titers in APSGN and SI versus those in other groups by *t* test.
Measurement of Serum Anti-NAPlr Antibody

Sera from patients with APSGN, pediatric and adult patients with streptococcal infection without renal involvement, and control subjects were tested to determine the titers of anti-NAPlr antibody. Anti-NAPlr antibody was detected more frequently in the sera of patients with APSGN than in sera from other subjects (Table 3), and significantly increased levels of anti-NAPlr antibody were found in the sera of patients with APSGN (Figure 4A). It is noteworthy that as much as 72% of the adult II group (mean age 53.2 yr) possessed anti-NAPlr antibody, whereas only 26% of the nonrenal pediatric patients (mean age 7.2 yr) possessed anti-NAPlr antibody.

ASO titers compared with the anti-NAPlr antibody titers are shown on Figure 4B. ASO titers were significantly higher in the APSGN and streptococcal infection groups than in the control groups, indicating a serologic response to a streptococcal product, regardless of renal involvement. Over the 10 yr that the sera of the 50 patients with documented APSGN were monitored, the anti-NAPlr antibody titers tended to increase during the acute phase of the disease. After the acute phase, titers decreased but remained significantly higher in patients with APSGN than in age-matched control adults (Figure 5). The rate of anti-NAPlr antibody positivity was also higher than that of controls during the 10-yr follow-up period.

Immunofluorescence Studies of Kidney Biopsy Specimens

Thirty-six (72%) of 50 APSGN renal biopsy specimens were positive for glomerular NAPlr with anti-NAPlr antibody (Table 4). All 25 renal biopsy specimens obtained in the early disease stage (1 to 14 d after APSGN onset) and 11 (61%) of 18 biopsy specimens obtained in the middle disease stage (15 to 30 d after onset) were positive for glomerular NAPlr. The antigen was localized mainly to the mesangium and part of the GBM, and infiltrating leukocytes were observed in a ringlike pattern (Figure 6A). However, no staining was observed 31 d after onset.
Pretreatment of sections with unlabeled anti-NAPlr antibody or with serum from a convalescing patient abolished the staining with FITC-labeled anti-NAPlr antibody. In addition, preabsorption of FITC-labeled anti-NAPlr antibody with recombinant streptococcal Plr abolished glomerular staining of NAPlr. All APSGN renal biopsy specimens obtained within 30 d of onset showed intense and extensive deposition of C3 along the GBM and/or in the mesangium (Figure 6B). IgG staining was present in the glomeruli of 64% and 61% of the sections representing the early and middle disease stages, respectively, and it was not always colocalized with C3. Staining of IgA and IgM ranged from blush to faint. Colocation studies of NAPlr with C3 or IgG revealed that the distribution of NAPlr differs from that of C3 or IgG (Figure 7).

Fibrinogen was stained intensely in the glomeruli in 15 (60%) of 25 patients 1 to 14 d after onset. Fibrinogen was observed mainly on the endothelial side of the GBM (Figure 6C), and the frequency of staining was relatively consistent throughout the course of the disease. In contrast, plasminogen was observed in the glomeruli in 10 (40%) of 25 patients in the early stage and less frequently in the later stage. The localization of plasminogen was predominantly in the mesangium and part of the GBM (Figure 6D), and when present, it was always colocalized with NAPlr. Most complement components, except C1q and C4, were detected frequently in glomeruli. We observed intense staining of C3, P, C5, C9, S, and MAC, particularly in the early stage (Table 4). Staining of C1q and C4 was weak and infrequent.

**Discussion**

Characterization of NAPlr has been incomplete (7). The available partial amino acid sequence for purified NAPlr was identical to that of streptococcal Plr (10) and similar to that of streptococcal GAPDH, suggesting that NAPlr has GAPDH activity (11). Thus, NAPlr is implicated as a virulence factor. However, the role of cytoplasmic GAPDH in APSGN was not clear. In the study presented here, we found that purified NAPlr has GAPDH activity on zymograms. Characteristics of NAPlr that are similar to characteristics of GAPDH include adhesion to fibronectin, myosin, and actin and plasmin receptor activity (10,19,20). Thus, NAPlr is expected to interact with these molecules in the pathogenesis of APSGN.

We previously detected anti-NAPlr antibody in sera at a relatively early stage of APSGN (7). In the study presented here, analysis of sera from patients with APSGN showed a significantly higher frequency of anti-NAPlr antibody than in...
other subjects, including those with streptococcal infection alone. In addition, anti-NAPlR antibody titers tended to be highest during the first week of infection and decreased thereafter. However, the titers did not decrease to the baseline levels of age-matched controls, and they remained significantly higher than those of the control subjects over the 10-yr follow-up period. These findings suggest that recurrence of APSGN is rare and that a single infection confers life-long immunity. Anti-NAPlR antibody was present in only 26% of subjects in the youngest control group (age 0.2 to 10 yr), and the rate increased to 72% (age 52 to 59 yr). This may explain why younger children have a greater tendency to suffer from this disease. Individuals appear to acquire immunity gradually through repeated streptococcal infection and thus, older people seldom develop APSGN (21).

As we reported previously, immunohistochemistry showed that NAPlR was present in glomeruli in APSGN renal biopsy specimens (7). In the study presented here, all specimens obtained 1 to 14 d after APSGN onset were positive for glomerular NAPlR, whereas no specimen obtained 31 to 90 d after onset was positive for the antigen. Thus, glomerular NAPlR tended to decrease over time in patients with APSGN. Furthermore, the difference in the localization of NAPlR in comparison to that of IgG or C3 indicated that NAPlR exists as a free antigen with or without plasmin(ogen). We suspect that during the early phase of APSGN, the antigenic sites are not fully saturated and can interact with anti-NAPlR antibody, whereas later in the course of the disease, the sites are saturated.

We previously reported that complement components were deposited in affected glomeruli 1 to 12 d after onset of APSGN (7). In the study presented here, the majority of biopsy specimens showed frequent and intense staining for C3, P, C5, C9, S, and MAC, particularly at 1 to 90 d after onset. NAPlR was deposited in 100% of the specimens obtained from early in the disease course (1 to 14 d after onset). Thus, NAPlR as well as complement components are associated with APSGN (22–24).

In the study presented here, the deposition of C3 without IgG in glomeruli in 9 of 25 patients 1 to 14 d after APSGN onset and the lack of circulating anti-NAPlR antibody in 4 of 50 patients suggest that complement components are associated with the initial inflammatory reaction (25–27). In addition, NAPlR cleaved C3 to C3b in human serum in vitro. Thus, NAPlR may activate the complement cascade in circulation (28,29).

NAPlR was detected in glomeruli of all early APSGN biopsy specimens, and anti-NAPlR antibody was detected in the majority of serum samples from patients with APSGN. Because NAPlR has plasminogen-binding activity (7,19), NAPlR on the mesangial matrix and GBM is expected to interact with plasmin(ogen). Plasmin may induce glomerular damage by degrading the GBM through activation of matrix metalloproteinase precursors. In fact, we recently observed significant glomerular plasmin activity that reflected the distribution of NAPlR deposition in the early phase of APSGN (T. Oda et al., unpublished data). Circulating immune complexes may readily pass through the altered GBM and accumulate in the subepithelial space (30). Taken together, our findings suggest that NAPlR is a virulence factor for APSGN and that the presence of a high titer of anti-NAPlR antibody should prevent autoimmune sequelae. Further studies regarding the role of NAPlR will allow us to better understand the pathology of APSGN.

Acknowledgment

We thank Dr. Takayuki Fujita (Second Department of Internal Medicine, Nihon University School of Medicine) for discussion of the complement analysis.
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