C4B Deficiency Associated with Membranoproliferative Glomerulonephritis

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Abstract

A 10-year-old girl was noted to have microscopic hematuria and proteinuria in 1986. As her urinary abnormalities were persistent, she underwent a renal biopsy on 4 occasions until 2003. Although the appearances of the renal biopsies were strongly suspicious of systemic lupus erythematosus, she never exhibited specific autoantibodies or distinctive symptoms. She received corticosteroid therapy and the urinary findings responded. The 4th component of complement remained low during the period of the observation. Both genotyping and allotyping analysis revealed complete C4B deficiency. Some case reports have mentioned renal disease associated with C4B deficiency and we consider the nephropathy in this case to be related to the C4B deficiency.

Key words: C4B deficiency, membranoproliferative glomerulonephritis, systemic lupus erythematosus

Introduction

The complement system is an essential element of the innate immune system such that the lack of complement components may cause a severe disturbance in host defense. The 4th component of complement consists of C4A and C4B isozymes, and plays an important role in the activation of the classical pathway. Although C4A deficiency is well described as a cause of systemic lupus erythematosus (SLE) (1), there are few case reports regarding C4B deficiency. In this report we present a case of complete deficiency of C4B associated with long-term nephropathy and discuss the relationships between complement deficiency and renal disease.

Case Report

A 10-year-old girl was noted to have microscopic hematuria (2+~3+) on elementary school urinary screening in 1986. A 24-hour urine collection revealed a urine protein excretion of 0.3 g/day whilst renal function was normal. She was asymptomatic and had no significant medical history except for mild allergic dermatitis. There was a family history of rheumatic fever (grandfather), elevation of serum amylase of unknown cause (father) and Kawasaki disease (sister). A renal biopsy was performed in 1987 in order to determine whether there was serious underlying renal disease. This demonstrated a mild excess of mesangial matrix and a few hyalinized arterioles. Electron micrographs showed myxovirus-like particles that raised the possibility of a nephropathy related to SLE. She did not receive any specific treatment and was followed up in the pediatric clinic.

In 1991, her urine protein excretion increased to 0.7~1.0 g/day and a 2nd renal biopsy was performed. This biopsy exhibited paramesangial deposits, a double contour of basement membranes and subendothelial deposits (Fig. 1-A, Fig. 1-B). Subendothelial deposits were confirmed on electron microscopy (Fig. 2-A). Immunofluorescent staining was positive for IgG and C3, borderline for IgM and C4 and negative for IgA. These findings supported the diagnosis of...
membranoproliferative glomerulonephritis. She was kept under observation without medication for a further 6 years.

At the age of 18 years, she was referred to us by her pediatrician. Although she remained asymptomatic, laboratory findings revealed that she was positive for rheumatoid factor and immune complexes with mildly elevated ESR and IgA and IgM levels. Urine analysis revealed microscopic hematuria (1+) and the urine protein excretion was 1.0 g/day. The serum creatinine and BUN were normal.

When she suffered from herpes zoster in April 1997, urine analysis revealed increased proteinuria and hematuria (both 3+). Two months later, she consulted us because of fever caused by acute bronchitis. Her urine protein excretion increased markedly with levels up to 4.9 g/day. In addition, her fever persisted and she was therefore admitted for further investigations in September 1997. The WBC count, ESR, BUN and IgA levels were mildly elevated whilst the total protein, albumin, IgM, hemoglobin and complement levels were decreased. Although she remained positive for rheumatoid factor and immune complexes, the anti-nuclear antibody and anti-DNA antibody levels remained negative.

She underwent a 3rd renal biopsy. Light microscopy revealed mesangial proliferation and capillary wall thickening of the glomerular basement membrane (Fig. 1-C, Fig. 1-D). Electron microscopy revealed dense deposits in the paramesangial area (Fig. 2-B) and myxo-virus-like particles in the endothelial cells (Fig. 2-C). Immunofluorescent staining was positive for C3, C1q and immunoglobulin but negative for C4. The pathological diagnosis was membranoproliferative glomerulonephritis compatible with lupus nephritis. However, as there was no evidence for extra-renal disease and she had no clinical symptoms except for nephropathy, she was not clinically diagnosed with SLE.

She received corticosteroid therapy between January 1998 to October 2001 and this resulted in the disappearance of the proteinuria. In March 2003, however, the urine protein excretion increased again (Table 1) in the absence of clinical symptoms such as fever, arthralgia and skin rash. She was admitted in July 2003 for a 4th renal biopsy. Although the thickening of the capillary wall and mesangial proliferation were decreased in comparison with the 3rd renal biopsy there was evidence of new crescent formation (Fig. 1-E, Fig. 1-F). Electron micrographs showed mesangial interposition (Fig. 2-D). Immunofluorescent staining was positive for immunoglobulin and C3 but still negative for C4 (Fig. 3). A diagnosis of crescentic glomerulonephritis was made and she received methylprednisolone pulse therapy (0.5 g per day) followed by oral corticosteroid therapy.
Figure 2. Electron micrographs of the renal biopsies. (A) In the second biopsy, subepithelial deposits are seen (arrowhead). In the third biopsy (B, C), dense deposits in the paramesangial area (star) and myxo-virus like particle (arrowhead) are observed. (D) In the fourth biopsy, mesangial interposition is evident (asterisk).

Table 1. Laboratory Findings Just before the Fourth Renal Biopsy

<table>
<thead>
<tr>
<th>Urine</th>
<th>&lt;blood chemistry&gt;</th>
<th>&lt;Complements&gt;</th>
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<tbody>
<tr>
<td>Occult blood</td>
<td>BUN 19 mg/dl</td>
<td>C1q 7.4 mg/dl (8.8-15.3)</td>
</tr>
<tr>
<td>Sugar (-)</td>
<td>Cr 1.19 mg/dl</td>
<td>C3 58 mg/dl (65-135)</td>
</tr>
<tr>
<td>Protein 2098 mg/day</td>
<td>TP 5.9 g/dl</td>
<td>C4 5 mg/dl (13-35)</td>
</tr>
<tr>
<td>NAG 250 IU/L</td>
<td>Alb 3.6 g/dl</td>
<td>C4a 1550 ng/ml (50-250)</td>
</tr>
<tr>
<td>GFR 62.1 ml/min.</td>
<td>LDH 201 IU/L</td>
<td>C5 12.2 mg/dl (8.0-15.0)</td>
</tr>
<tr>
<td>RBC &gt;=100/HPF</td>
<td>GOT 16 IU/L</td>
<td>C6 5.6 mg/dl (2.5-4.5)</td>
</tr>
<tr>
<td>WBC 10-15/HPF</td>
<td>GPT 10 IU/L</td>
<td>C8 9.6 mg/dl (5.5-8.9)</td>
</tr>
<tr>
<td>Granular cast 10-15/WF</td>
<td>&lt;serology&gt;</td>
<td></td>
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<tr>
<td>Fatty cast 1-2/WF</td>
<td>CRP &lt;0.2 mg/dl</td>
<td></td>
</tr>
<tr>
<td>RBC cast 5-6/WF</td>
<td>IgA 423 mg/dl</td>
<td></td>
</tr>
<tr>
<td>ESR 70 mm/hr.</td>
<td>IgG 673 mg/dl</td>
<td></td>
</tr>
<tr>
<td>Hematology</td>
<td>IgM 415 mg/dl</td>
<td></td>
</tr>
<tr>
<td>Hb 9.5 g/dl</td>
<td>anti nuclear antibody (-)</td>
<td></td>
</tr>
<tr>
<td>PLT 29.4 x 10^4 /μl</td>
<td>anti DNA antibody (-)</td>
<td></td>
</tr>
<tr>
<td>WBC 13250 /μl</td>
<td>anti Sm antibody (-)</td>
<td></td>
</tr>
<tr>
<td>(Lym. 18.0 %)</td>
<td>anti RNP antibody (-)</td>
<td></td>
</tr>
<tr>
<td>N-Seg. 76.0 %</td>
<td>anti SS-A antibody (-)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MPO-ANCA (-)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>anti cardiolipin antibody (-)</td>
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The urinary findings responded to corticosteroid therapy but the low serum level of C4 persisted (Fig. 4). We suspected an underlying complement deficiency and therefore performed gene analysis and allotyping according to previously reported methods (2, 3).

Polymerase chain reaction (PCR) amplification of C4A and C4B genes was based on the method of Barba et al (4). Genomic DNA was extracted from peripheral blood leukocytes after informed consent was obtained from the patient. Three sets of isotype-specific primers were prepared according to previously described sequences (2). A set of primers (C4AI) for the C4A gene, and two sets of primers (C4BI
and C4BII) for the C4B gene were as follows: 5'-GCA TGC TCC TGT CTA ACA CTG GAC-3' and 5'-TGC GGA TCC AGC AGT TTC GGA AG-3' (C4AI set); 5'-TGC TCC TAT GTA TCA CTG GAG AGA -3' and 5'-TGC GGA TCC AGC AGT TTC GGA AG-3'(C4BI set), and 5'-AGG ACC TCT CTC CAG TGA TAC AT -3' and 5'-ATA GGA TCC TAA GGT CCC CTG GGC CT -3' (C4BII set). PCR amplification was performed according to the protocol of X-Y Man et al (2). The PCR products, including C4AI (377bp), C4BI (377bp) and C4BII (578bp) were then applied on a 5% polyacrylamide gel and stained with ethidium bromide. The samples of this patient could not be amplified by primers for isotypes of C4B (Fig. 5).

The western blotting assay to reveal the haplotypes of C4 was performed according to the method of Sim and Cross (3). Samples of plasma (7.5 μl) were treated with 1 μl of carboxypeptidase B (type I) and 1 μl of neuraminidase (type VI) and incubated at 4°C overnight. A 0.8% Seakem agarose gel was made with tris-glycine-veronal buffer at pH 8.0 and electrophoresis was carried out under the following conditions: 30 minutes at 300V, 30 minutes at 400V, three hours at 500V and 30 minutes at 600V. The gel was then exposed to rabbit anti-human C4 antibodies (Dako, High Wycombe, Bucks, U.K.) and immersed in saline overnight in order to eliminate excessive protein and antibodies. Finally, the gel was dried on a gel bond film and was stained with Coomassie Brilliant Blue R250. The haplotypes of this patient were C4A3, BQ0/C4A3, 2 BQ0 thereby confirming a diagnosis of complete C4B deficiency (Fig. 6).

**Discussion**

In this case, the patient underwent a renal biopsy on 4 occasions and we obtained findings suspicious of lupus nephritis each time. However, she had no symptoms of arthritis, stomatitis or skin rash typical of lupus such that her symptoms and serological profile never satisfied the classification criteria for SLE (5). In addition, she exhibited a low C4 level during the extended period of observation. Finally, the genetic phenotyping of complement revealed that she was homozygous for C4B deficiency.

The 4th component of complement belongs to the classi-
Figure 5. Specific PCR analysis of C4AI with primers Aup/L3 (A), C4BI with primers Bup/L3 (B) and C4BII with primers Bdo/L4 (C). Lane 1 is the DNA size marker (BioLabs, 100 bp DNA ladder N3231L). The arrows indicate 400 bps bands (A, B). The arrowhead indicates 500 bps band (C). Lanes 2 and 3 are samples of other SLE patients. Lane 4 is a sample of this case. No bands of C4B are seen in lane 4.

Figure 6. Typing of C4 by western blotting (Agarose slab gel electrophoresis and immunostaining). Lane 1 and 8: the sample of this case. Lane 2: A5,3, B2,2. Lane 3: A3,3 B5,2. Lane 4: A3,3 B1,1. Lane 5: A3,3,2 B1,Q0. Lane 6: A3,3 B1,1 Lane 7: A4,3 B5,2 In lanes 1 and 8, the A3 band is denser than the A2 one, but no B-loci bands are seen. This result is interpreted as duplication of C4A3 and depletion of C4B (C4BQ0). Thus, the haplotypes of this case are C4A3, BQ0/C4A3, 2 BQ0.

cal pathway of complement activation and plays an important role in host defense. C4 consists of two isozymes that are functionally distinct: C4A and C4B. C4A is 100 times more reactive with target containing free amino groups than C4B (6). The amide bonds formed between activated C4A and target containing amino groups facilitate the clearance of the immune complexes (7). C4A deficiency causes autoimmune disease, especially systemic lupus erythematosus (8). Indeed, approximately 15% of Caucasian SLE patients exhibit homozygous C4A deficiency whilst more than 50% of Caucasian patients with SLE have heterozygous C4A deficiency (6).

In contrast, C4B is 10 times more reactive than C4A with targets containing hydroxyl groups (6). Activated C4B results in transesterification of the target and this is important for the propagation of the classical and the mannose-binding lectin pathway of complement activation. This reaction finally leads to formation of the critically important membrane attack complex against microbes (7).

C4B deficiency is considered to be involved in various diseases (9) including severe infections (10). Although several reports have mentioned renal diseases associated with C4B deficiency (11, 12), the exact nature of the relationship between complement deficiency and nephropathy is unclear. An insidious infection resulting from the failure of classical pathway activation may affect the nature of the renal lesion. The resultant dysfunction of the membrane attack component may adversely affect the phagocytosis of apoptotic cells and cause impaired negative selection of autoreactive B cells. The residual debris from apoptotic cells and autoreactive B cells may account for the development of autoimmune diseases. Although Moulds et al reported that the mean C4A or C4B levels are reduced when partial deficiencies of C4A or C4B are present, total C4 levels show considerable overlap with and do not differ significantly from the non-C4 null groups in either patients or controls (13). This fact indicates that heterozygous individuals with C4B deficiency have an increased level of C4A and vice versa. In the present case, the total C4 was almost always present at a low level except when high-dose corticosteroids were administered. Whereas the production of C4A might be increased as a compensatory response instead of C4B, much C4A should be consumed by continuous activation of the classical pathway. This speculation was based on the fact that her level of the C4 fragment C4a was markedly increased (1550 ng/mL, normal range: 50-250 ng/mL) thereby
indicating the existence of massive consumption of C4 in this case. The increased C4A may cause glomerulonephritis because C4A has a higher affinity to immune complexes than C4B and may deposit in glomeruli (14). But this hypothesis cannot explain the fact that it is C4A deficiency and not C4B deficiency that correlates with the development of SLE.

An alternative hypothesis is that C4B deficiency may result in some kinds of recurrent infection such that the activation of complement possibly induces an immune complex-mediated glomerulonephritis. The diversity of pathogens and/or diverse pathway of complement activation might account for the difference of the histological types of glomerulonephritis evident in this patient over time.

In Japan, the allele frequency of C4AQ0 and C4BQ0, whose phenotypes are deficiencies of C4A and C4B, are 7% and 16%, respectively (15). The calculated frequencies of complete deficiency of C4A and C4B are 0.49% and 2.56%, respectively. This seems to be too high to consider that all patients with C4 deficiency would be affected by the development of renal disease. It is possible, however, that asymptomatic hematuria of unknown cause may include some cases of defective expression of complement. Such cases are usually noted during school urinary screening and diagnosed as IgA nephropathy either clinically or pathologically. Indeed, since several studies have indicated that C4B deficiency may possibly be related to IgA nephropathy (16, 17), we believe that it is necessary to consider complement deficiency in the differential diagnosis of microscopic hematuria.

**Conclusion**

We report a case of C4B deficiency accompanied with nephropathy which presented various pathological findings compatible to lupus nephritis over a period of 17 years. Further studies are needed to clarify the relationship of C4B deficiency and renal disease.

**References**


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