Alport Syndrome Diagnosed by Immunofluorescence Using a New Monoclonal Antibody

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A 14-year-old female with microscopic hematuria was admitted for a renal biopsy. She had a family history of renal disease without deafness. The findings of light microscopy and conventional immunofluorescence were normal. Electron microscopy showed a diffuse thinning of the glomerular basement membrane (GBM) with its mild splitting. Irregular thickening of GBM and glomerular small dense particles was not observed. Thin basement membrane syndrome was suspected from these findings. However, it was difficult to differentiate from Alport syndrome. Immunofluorescence analysis using the monoclonal antibody to the 28-kilodalton monomers of the noncollagenous domain of type IV collagen verified the diagnosis of heterozygous Alport syndrome.

Key words: NC-1 domain of type IV collagen, HLA examination, thin basement membrane syndrome

Introduction

Alport syndrome is a hereditary disease characterized by the onset of hematuria in early childhood and later progression to renal failure, accompanied by the development of sensorineural hearing loss and ocular abnormality (1). Recently, the pathogenesis of Alport syndrome has been suggested to be a lack of reactivity with Goodpasture syndrome antigen which might alter the structure of the glomerular basement membrane (GBM) (2). In 1987, Kleppel et al (3) revealed that Goodpasture antigen is present in the 28-kilodalton (kDa) monomers of the noncollagenous terminal C (NC-1) domain of type IV collagen and prepared monoclonal antibodies to these monomers. We report herein a case of Alport syndrome diagnosed by immunofluorescence analysis using the monoclonal antibody to the NC-1 domain of type IV collagen.

Case Report

A 14-year-old female with microscopic hematuria since 6 years of age was admitted to Juntendo University Hospital for a renal biopsy. She had a family history of renal diseases as shown in Fig. 1. Her grandfather had chronic renal failure treated by hemodialysis. Her father and uncle showed mild or severe proteinuria and microscopic hematuria with red blood cell (RBC) casts and oval fat bodies. Two elder brothers and a younger brother also had mild proteinuria and hematuria without casts. No deafness or abnormality of the eyes was found in her relatives. HLA examination revealed that DR4 was common in the patient, her father and her brothers.

On admission, blood pressure was 124/74 mmHg and pulse rate 56 beats per minute. Physical examination showed no abnormalities in the chest, abdomen and extremities. No sensory hearing disturbance on an audiogram or abnormality of the eyes was observed. Laboratory data are shown in Table 1. Peripheral blood cell count and the erythrocyte sedimentation rate (ESR) were within the normal ranges. Microscopic hematuria of 20–25 RBC per high power field (HPF) without casts was observed. Biochemical data were within the normal ranges. BUN was 15 mg/dl (normal values: 8–17 mg/dl) and serum creatinine 0.9 mg/dl (normal values: 0.5–1.3 mg/dl). The levels of serum IgA were 99 mg/dl (normal values: 90–400 mg/dl). IgG, IgM and complement levels in sera were also within the normal ranges. Antinuclear antibody and anti-DNA antibody were negative. No impairment of renal function was observed.

Renal biopsy was performed on the 4th hospital day. By light microscopy, global sclerosis was observed in one out of 15 glomeruli. Glomerular adhesion to Bowman’s...
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Capsules, crescent formation and inflammatory cell infiltration in the interstitium were not observed in the renal tissues. Accumulation of foam cells in the interstitium was not observed. No deposition of immunoglobulins, complements or fibrinogen in three glomeruli was found by immunofluorescence. Electron microscopy in two glomeruli showed a diffuse thinning of the glomerular basement membrane (GBM) with mild splitting. Irregularity of the GBM and gap formation were not observed. The GBM thickness was determined by measuring with a ruler the distance between the epithelial and endothelial membranes facing the GBM on vertical section profiles of filtration barrier at a magnification of ×11,000. Average thickness of the GBM was 167 nm (normal values: 200–300 nm). No small dense particles, or electron dense deposits in the glomeruli and tubular epithelial cells were observed (Fig. 2). These renal biopsy findings led to suspicion of
Fig. 2. Diffuse thinning of the glomerular basement membrane (GBM) with mild splitting in this patient (×1,500).

Discussion

Alport syndrome is clinically characterized by the development of sensorineural hearing loss and ocular abnormalities (1). This syndrome is a hereditary renal disease that can be inherited in an autosomal dominant, autosomal recessive or X-linked dominant fashion. Diagnosis of Alport syndrome is generally determined by the family history, extrarenal symptoms and electron microscopic findings of renal tissues. The most characteristic electron microscopy changes of renal tissues in patients with Alport syndrome are segmental thickening and/or splitting of the GBM with small dense particles between its parallel layers (2, 5).

The thin basement membrane syndrome, similarly recognized as benign recurrent hematuria (BRH), is characterized by isolated hematuria. No hearing loss and progression to renal failure were observed. The thin basement membrane syndrome is generally inherited in an autosomal dominant fashion (6). Renal biopsy specimens of the thin basement membrane syndrome show normal kidneys except for the presence of erythrocytes in Bowman’s capsules and/or tubular lumen by light microscopy. Diffuse attenuation of the lamina densa in the GBM without deposits is also demonstrated by electron microscopy in this disease (7).

On the other hand, Piel et al (8) reported some degree of lamination of the GBM in patients with benign familial hematuria. Yoshikawa et al (9) reported uniform attenuation of the lamina densa in some patients in the early stage of Alport syndrome, in some BRH and in some sporadic cases of benign hematuria. In our patient, the chief complaint for 6 years was only persistent hematuria without proteinuria or renal dysfunction. Diffuse thinning and mild splitting of the GBM were observed on electron microscopy. Thickening of the GBM, glomerular small dense particles and interstitial foam cells were not observed by light and electron microscopy. Therefore, in this patient, it was difficult to distinguish between Alport syndrome and thin basement membrane syndrome. Recently, regarding the pathogenesis of Alport syndrome, it has been suggested that the lack of reactivity with Goodpasture antigen might cause a defect in the biosynthesis or turnover of the

the thin basement membrane syndrome. However, it was difficult to completely differentiate from Alport syndrome because of the histological findings. Thus, we carried out indirect immunofluorescence using monoclonal antibody to the 28-kilodalton (kDa) monomers of the noncollagenous terminal C (NC-1) domain of type IV collagen provided by Prof. A.F. Michael, University of Minnesota, MN, USA. The NC-1 domain of type IV collagen in the GBM was weakly stained in a segmental pattern as shown in Fig. 3a. However, the 7S domain of type IV collagen was markedly stained in the glomeruli, tubular basement membranes and vascular walls (Fig. 3b). The NC-1 domain of type IV collagen in the GBM was not observed in a patient (9-year-old, male) with homozygous Alport syndrome (Fig. 4a) (4), but was observed in some patients with benign recurrent hematuria (BRH), IgA nephropathy and normal kidneys as controls (Fig. 4b). Thus, we finally diagnosed this patient as heterozygous Alport syndrome.
Fig. 3a. Segmental pattern of the noncollagenous terminal C (NC-1) domain of type IV collagen in a glomerulus of this patient (x400). 3b. Diffuse staining of the 7S domain of type IV collagen in the renal tissues of this patient (x400).

Fig. 4a. No staining of the noncollagenous terminal C (NC-1) domain of type IV collagen in a glomerulus of a patient with heterozygous Alport syndrome (9-year-old, male). 4b. Positive staining of the NC-1 domain of type IV collagen in a normal kidney as positive control (x400).

In conclusion, immunofluorescence using monoclonal antibody to the 28-kDa monomers of type IV collagen appears useful in discriminating Alport syndrome from the thin basement membrane syndrome.

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References