Glomerular Localization of Interleukin-6 Suppressed by Steroid Mini-pulse Therapy in an IgA Nephropathy Patient

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A 16-year-old female with IgA nephropathy harboring histologically active lesions was treated with steroid mini-pulse therapy. Immunohistochemical examination revealed a diffuse distribution of interleukin-6 (IL-6) in the renal biopsy tissue. After treatment, her clinical factors and renal function improved, and renal biopsy showed reduced histological lesions and disappearance of the IL-6 distribution. Immunohistological studies of cytokines, such as IL-6, may be useful for evaluating the therapeutic effects in IgA nephropathy.
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Key words: immunohistochemistry, cytokines, steroid therapy

Introduction

IgA nephropathy (1) does not have the good long-term prognosis, as previously thought (2). Anticoagulants, antiplatelet agents (3), steroids, and immunosuppressants (4) have been used extensively to treat IgA nephropathy, but no consensus on their efficacy has been reached.

The present study found that steroid mini-pulse therapy had excellent efficacy in a patient with IgA nephropathy as shown by histological improvement and the disappearance of interleukin-6 (IL-6) in the glomeruli. Localized observation of IL-6 was useful for the evaluation of therapeutic effects in IgA nephropathy.

Case Report

A 16-year-old Japanese female was discovered to have occult hematuria during a school health examination in April 1991. She was subsequently examined by a local physician, but no abnormalities were found in the urine and consequently no treatment was given. A school health examination in April 1993 noted occult hematuria with proteinuria. She underwent an examination at the Department of Urology of Asa Municipal Hospital in July 1993. Her urinary protein was 2+ and many erythrocytes were found in the urinary sediment. Since no other urological abnormalities were found, IgA nephropathy was suspected. She was admitted to our hospital for a renal biopsy

on August 18. Nothing noteworthy was found in her medical history or family history.

Physical examination on admission found she was 160.8 cm tall and weighed 57.6 kg. Her blood pressure was normal (120/80 mmHg). There were no external abnormalities such as purpura, and no edema was evident. There were no abnormal neurological findings. Laboratory tests revealed albumin to be $\pm$ and occult hematuria was 3+, and many erythrocytes were found in the urinary sediment. Her total daily protein excretion was 0.14 g/day. No abnormalities were found by peripheral blood tests, coagulation tests or blood chemistry. Serological examinations showed elevated antinuclear antibody and serum IgA levels, but the complement showed no decrease. Renal function tests revealed no particular abnormalities: BUN was 11 mg/dl, serum creatinine 0.67 mg/dl, and 24-hour creatinine clearance 111.3 ml/min (Table 1). Biopsy of the renal tissue showed segmental active proliferative changes in the mesangial area, and segmental sclerosis and fusion with Bowman's capsule. Small cellular crescents were present in 3 of 35 glomeruli, and fibro-cellular crescents were present in 4 of 35 glomeruli (Fig. 1A). Fluorescent antibody staining mainly showed IgA deposits in the mesangial region (Fig. 2A). Deposits of C3 and fibrinogen were also observed. Electron microscopy showed electron dense deposits in the mesangial area (Fig. 3A). Based on these findings, histologically active, moderate IgA nephropathy was diagnosed. An immunohistochemical study using frozen sections revealed IL-6 to be diffusely distributed in the

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Table 1. Laboratory Data on First Admission

Urinalysis		Blood chemical exam.			
Colour	Yellow	Total bilirubin	1.0 mg/dl	Immunoglobulin G	1,420 mg/dl
Turbidity	Clear	Direct bilirubin	0.3 mg/dl	Immunoglobulin A	405 mg/dl
pH	7.0	Aspartate aminotransferase	15 U/l	Immunoglobulin M	301 mg/dl
Urobilinogen	N+	Alanine aminotransferase	8 U/l	Complement 3	61 mg/dl
Albumin	±	Lactate dehydrogenase	375 U/l	Complement 4	24 mg/dl
Occult	3+	Cholinesterase	343 U/l	Serum complement	35 U/ml
Sugar	—	Alkaline phosphatase	192 U/l	Renal function	
Ketone	—	Leucine aminopeptidase	39 U/l	Blood urea nitrogen	11 mg/dl
Sediment		γ-Glutamyltranspeptidase	5 U/l	Creatinine	0.67 mg/dl
Red blood cell	Many/HPF	Total protein	7.6 g/dl	Uric acid	4.0 mg/dl
White blood cell	0–1/HPF	Albumin	4.7 g/dl (66.9%)	Serum – β ₂ microglobulin	1.36 μg/ml
Cast	—	α ₁ -globulin	2.2%	Urinary – β ₂ microglobulin	0.29 μg/ml
Crystal	—	α ₂ -globulin	9.4%	Creatinine clearance (2 hours)	112.3 ml/min
Total daily urinary protein	0.14 g/day	β-globulin	7.9%	Creatinine clearance (24 hours)	111.3 ml/min
		γ-globulin	13.6%	Glomerular filtration rate	186 ml/min
Peripheral blood exam.		Zinc turbidity test	7 U	Renal plasma flow	637 ml/min
White blood cell count	6,500/μl	Total cholesterol	140 mg/dl	Renal blood flow	1,076 ml/min
Red blood cell count	466×10 ⁴ /μl	Triglycerides	76 mg/dl	Phenolsulfonphthalein test 15 minute	40%
Hemoglobin	14.5g/dl	Na	143 mEq/l	Fishberg concentration test	1.030
Hematocrit	40.8%	K	3.9 mEq/l	Chest X-P	
Platelet	19.2×10 ⁴ /μl	Cl	106 mEq/l	CTR=44.4%	
		Ca	4.6 mEq/l	Intravenous pyelography	
Coagulation exam.		P	3.3 mg/dl	Within normal limit	
Prothrombin activity	105%	Serum exam.			
aPTT	42.1 sec	C reactive protein	0.3> mg/dl		
Fibrinogen	286 mg/dl	Antistreptolysin O	56 IU/ml		
s-FDP	1.16 μg/ml	Antistreptokinase	×80		
u-FDP	0.1 >μg/ml	Rheumatoid factor	6.0> IU/ml		
		DNA test	×0		
		Anti nuclear antibody	×80		
		Anti DNA antibody	3.1 U/ml		

s-FDP: serum-Fibrin/Fibrinogen degradation products, u-FDP: urinary-Fibrin/Fibrinogen degradation products, aPTT: Activated partial thromboplastin time.

mesangium area (Fig. 4A). However, platelet derived growth factor (PDGF)-A and -B were not observed.

The treatment regimen consisted of oral administration of 30 mg/day of prednisolone and 300 mg/day of dilazep dihydrochloride, started immediately after the first renal biopsy. Steroid mini-pulse therapy was also performed, using 500 mg/day of methylprednisolone, because of the lower risk of various side effects such as infection and the same efficacy as 1.0 g/day of methylprednisolone. Complete consent was obtained from the patient and her parents prior to the start of the therapy. The active lesions had disappeared at the second renal biopsy on November 17, 1993, and the dose of prednisolone was gradually reduced. Clinical factors and BUN and serum creatinine improved slightly during treatment, and the proteinuria and occult hematuria showed improvement. Therefore, a third renal biopsy was performed on July 27, 1994 to further evaluate the treatment effects. The renal tissue contained only very slight proliferation of mesangial cells and matrix, crescents in only one of 17 glomeruli, and marked histological improvement was observed (Fig. 1B). Fluorescent antibody staining showed a

few IgA deposits in the mesangial region (Fig. 2B), and electron microscopy identified a few electron dense deposits in the mesangial area (Fig. 3B). The diffuse distribution of IL-6 seen at the first renal biopsy had disappeared (Fig. 4B).

Discussion

In recent years, steroid therapy has been widely used for patients with histologically active, progressive IgA nephropathy and moderate or severe proteinuria, and has been reported to be effective (5–8). However, steroid pulse therapy may cause rapid progression of renal dysfunction in some patients, because of adverse reactions such as increased blood pressure and exacerbated blood coagulation (9–11). Kobayashi et al (8) investigated this point in groups of patients given steroid therapy or untreated, and reported that steroid therapy is likely effective in progressive IgA nephropathy patients with proteinuria of 1–2 g/day and in renal function maintained with a creatinine clearance of at least 70 ml/min. The present patient had relatively mild proteinuria and her renal functions were

Figure 1. Light micrograph of renal biopsy tissue (PAS stain, $\times 280$). A) At the first renal biopsy, segmental sclerosis and fusion with Bowman's capsule, and fibro-cellular crescents are present. B) At the third renal biopsy after steroid mini-pulse therapy, only slight proliferation of mesangial cells and matrix are present.

Figure 2. Fluorescent antibody staining ($\times 100$). A) At the first renal biopsy, IgA deposits are present, mainly in the mesangial region. B) After steroid mini-pulse therapy, IgA deposits are few and weakly stained.

within the normal range, but histological examination had found small crescents in 20% of the glomeruli which appeared to be active (12). The small crescents may be associated with an unfavorable prognosis (13). The immunohistochemical study, using frozen specimens, found a distribution of IL-6 in the glomeruli, but no PDGF-A or -B. Recent immunohistochemical and other studies have suggested that cytokines such as IL-6 and PDGF are involved in the progression and exacerbation of IgA nephropathy (14–16). Our findings in the present patient indicated that progression and exacerbation of glomerular lesions had occurred. Therefore, early steroid therapy was undertaken, resulting in no increases in proteinuria, aggravation of renal function or progression of histological lesions, and the apparent improvement in her condition. Following steroid therapy, the distribution of IL-6 in the glomeruli disappeared, suggesting that steroid therapy is indicated for patients with IgA nephropathy harboring active lesions. The expression of IL-6 mRNA is inhibited by glucocorticoid (17), thus studies on the localization of cytokines in the glomeruli during renal biopsy may be useful as an index of the therapeutic effect. Recently,

Dohi et al (18) suggested that the measurement of urinary IL-6 activity is a helpful method for monitoring the progression of IgA nephropathy and it can be used as an alternative to renal biopsy. Further study is required in more patients to evaluate the potential of measurement of urinary IL-6 activity as a clinical indicator.

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Figure 3. Electron micrographs (A. $\times 3,000$; B. $\times 2,500$). A) At the first admission, electron dense deposits were present in the mesangial region. B) At the third admission, only mild electron dense deposits were present in the mesangial region.

Figure 4. Immunohistochemical findings (×300). A) At the first admission, diffuse distribution of IL-6 was found in the mesangial region. B) After steroid mini-pulse therapy, almost all the IL-6 distribution had disappeared.

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