Light-Chain Ratio of Serum IgA1 in IgA Nephropathy

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Patients with primary IgA nephropathy have deposits of IgA1 in their kidneys and increased IgA1 in circulation. We had previously shown that IgA nephritic patients displayed a unique immunological response characterized by a predominance of IgA with lambda chain in glomerular deposits and in circulation. We have now studied the kappa/lambda (κ/λ) ratio of serum IgA1 in 21 IgA nephritic patients at quiescence, with 11 patients investigated during exacerbation as well. A novel enzyme-linked immunosorbent assay was used with monoclonal mouse anti-human IgA1 as the solid-phase capture antibody and peroxidase-labeled anti-human kappa and lambda antibodies as tracers. The ratio of serum IgA1 to total IgA (mean ± SD) was significantly higher in patients (90.1 ± 8.2% at quiescence, P < 0.01; 88.7 ± 8.1% during exacerbation, P < 0.02) than in 20 healthy age- and sex-matched controls (80.0 ± 9.8%). Furthermore, serum IgA1 kappa/lambda ratios were significantly lower in patients (1.02 ± 0.27 at quiescence, P < 0.01; 0.93 ± 0.16 during exacerbation, P < 0.01) than in controls (1.31 ± 0.30). These findings indicate a predominance of lambda light-chain IgA1 in the serum of IgA nephritic patients. However, no difference in IgA1 kappa/lambda ratio was observed in these patients at quiescence and during exacerbation.

KEY WORDS: IgA nephropathy; enzyme-linked immunosorbent assay (ELISA); IgA1; κ/λ ratio.

INTRODUCTION

Primary IgA nephropathy (IgAN) is characterized by deposition of IgA in the mesangium (1). Despite the earlier conflicting results related to the subclasses of mesangial IgA, it has now been confirmed

that the glomerular IgA deposits consist mainly of monomeric IgA1, with a minor share of IgA2 (2-4). In fact, normal serum IgA contains approximately 90% IgA1 and 10% IgA2 (5, 6). Recently, it has been demonstrated in peripheral blood mononuclear-cell culture that IgAN patients preferentially produced antibodies of the IgA1 subclass (7). It was further suggested that the disturbance in immune regulation in such patients resulted in an increased production of IgA1 in the bone marrow, leading to abnormally elevated levels in the circulation and secondary deposition in the kidney (8, 9).

We had previously (10, 11) observed that a predominance of λ light-chain immunofluorescence staining occurred in the mesangial immunoglobulin A deposits in IgAN, despite the normal ratio of κ and λ light-chain immunoglobulin in human sera being approximately two to one. We have also demonstrated that IgAN patients displayed a unique immunological response characterized by a predominance of IgA with λ chain in the glomerular deposits and in circulation (12). Here we report the study of the κ/λ ratio of serum IgA1 in IgAN patients, using a newly developed enzyme-linked immunosorbent assay (ELISA).

METHODS

Patients

Twenty-one patients with a clinical and renal immunopathological diagnosis of primary IgAN were studied. They had been symptomatic for 12 months or more, with proteinuria ranging from 0.5 to 2.0 g/day, and were between 16 and 60 years of age (mean, 32.5 ± 8.4 years). IgAN was diagnosed by the presence of predominant granular IgA deposits, mainly in the glomerular mesangium and occasionally along the peripheral capillary basement.
membrane in immunofluorescent studies, as well as mesangial electron-dense deposits in ultrastructural examination. Systemic lupus erythematosus, Henoch–Schonlein purpura, and hepatic disease were excluded by detailed clinical history, examination, and negative laboratory tests for hypocomplementemia, anti-DNA antibody, or hepatitis B virus surface antigen. No significant renal impairment was documented in these patients and their endogenous creatinine clearances were greater than 65 ml/min/1.73 m².

Blood samples were collected from each patient at clinical quiescence. An additional blood sample was taken from 11 patients during exacerbation with synpharyngitic macroscopic hematuria.

Controls

Twenty-one age-matched healthy subjects with normal renal function were used as controls.

Determination of Serum IgA Concentration

Serum IgA concentration was determined by a double-sandwich ELISA method modified from Hale et al. (13). Rabbit anti-human α-chain antiserum (Dakopatts Laboratory, Copenhagen, Denmark) was diluted 1/2000 in 50 mM sodium carbonate–bicarbonate buffer, pH 9.6 (coating buffer). Microtiter plates (Lab System OY, Finland) were coated by adding 100 μl (approximately 0.5 μg) of the diluted antiserum to each well. The plates were incubated overnight at 4°C and then washed 10 times with 10 mM phosphate buffer, pH 7.5, containing 150 mM sodium chloride and 0.5 ml/L Tween 20 (PBS-Tween). Nonspecific binding sites of each coated well were then blocked by the addition of 100 μl of PBS containing 10 g/L bovine serum albumin (BSA), with further incubation at 37°C for 1 hr. The plates were again washed 10 times. Test samples and standard human serum (Behringwerke AG, Marburg, France) appropriately diluted (from 1 to 200 ng/ml of IgA) in assay buffer were introduced, 100 μl/well in duplicate. After incubation at 37°C for 3 hr, the plates were washed 10 times with PBS-Tween. The same antiserum as used for coating, but conjugated with horseradish peroxidase (Dakopatts) and diluted 1/6000 in PBS-Tween, was then added, 100 μl/well. Incubation at 37°C was continued for another 2 hr and the washing repeated. A freshly prepared peroxidase sub-

strate solution containing 20 mg of urea hydrogen peroxide and 70 mg of o-phenylenediamine dihydrochloride in 100 ml of 20 mM phosphate-citrate buffer, pH 5.0, was then added, 100 μl/well, and the plates were incubated at 37°C for 15 min. Finally, the reaction was terminated by the addition of 50 μl/well of 4 M sulfuric acid, and the absorbances were measured at 450-nm wavelength using a Bio-Rad Model 2550 EIA Reader (Bio-Rad, Richmond, CA). A dose–response curve was constructed by plotting the absorbance values against the IgA concentration of standards, and the IgA concentration of samples was read out from the curve.

Determination of Serum IgA1 Concentration

Serum IgA1 concentration of patients and controls was determined by a similar ELISA as for serum IgA concentration, except that microtiter plates were coated with mouse anti-human IgA1 monoclonal antibody (Becton Dickinson Immunocytometry Systems, Mountain View, CA) diluted 1/50 (3 μg) in coating buffer at 4°C overnight. Before use, the plates were washed 10 times with PBS-Tween, followed by blocking with 10 g/L BSA in PBS at 37°C for 1 hr. The plates were washed again. Samples and IgA1 standards (Calbiochem Corporation, San Diego, CA) appropriately diluted (from 0 to 200 ng/ml) in assay buffer were introduced, 100 μl/well in duplicate. After incubation at 47°C overnight, the plates were washed and developed in the same way as the IgA ELISA.

Standardization of IgA1κ and IgA1λ

IgA1κ and IgA1λ standards were derived from the IgA1 standard (Calbiochem), using ELISA methods previously described (14). Briefly, microtiter plates were coated by overnight exposure to 100 μl (approximately 0.5 μg) of rabbit antiserum to human IgA (Dakopatts) diluted 2000-fold in coating buffer at 4°C. Before use, the plates were washed 10 times with PBS-Tween, followed by blocking with 10 g/L BSA in PBS at 37°C for 1 hr. The plates were washed again. Standard human serum (Behringwerke, AG, Marburg, France) appropriately diluted (from 0 to 200 ng/ml of IgA) in PBS containing 5 g/L BSA (assay buffer) were then introduced, 100 μl/well in duplicate. After incubation at 37°C for 3 hr, the plates were again washed 10 times with PBS-Tween. The same antiserum as used for coating, but conjugated with horseradish peroxidase (Dakopatts) and diluted 1/6000 in PBS-Tween, was then added, 100 μl/well. Incubation at 37°C was continued for another 2 hr and the washing repeated. A freshly prepared peroxidase sub-

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