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Immunofluorescent studies on alpha 2-plasmin inhibitor (α2-PI) in glomeruli from patients with IgA nephropathy

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SUMMARY

Detection of alpha 2-plasmin inhibitor (α2-PI) and/or fibrinogen in glomeruli by immunofluorescence in 26 patients with IgA nephropathy was described. The present study showed that glomerular injuries such as glomerular adhesion to Bowman's capsule and the cellular and/or fibrous crescent were predominantly observed in glomeruli with α2-PI and/or fibrinogen deposits in patients with IgA nephropathy. Alpha 2-PI coexisted with fibrinogen in glomeruli from patients with IgA nephropathy. It was postulated that the deposition of α2-PI *in vivo* might lead to the accumulation of glomerular fibrinogen deposits in patients with IgA nephropathy. It was suggested that the depositions of α2-PI and/or fibrinogen in glomeruli may be one of the exacerbative factors in glomeruli from patients with IgA nephropathy.

Keywords α2-plasmin inhibitor fibrinogen IgA nephropathy

INTRODUCTION

IgA nephropathy is presumed to be an immune-complex-mediated glomerulonephritis although the antigenic substances of this disease are still unknown (Berger *et al.*, 1975; Clarkson *et al.*, 1984; McCoy, Abramowsky & Tisher, 1974). It has been postulated that activation of blood coagulation and/or inhibition of plasmin activities may play some role in either the development or exacerbation of chronic glomerulonephritis including IgA nephropathy (Bennett & Ogston, 1970; Dixon, 1967). It has been demonstrated that fibrinolytic activities are closely correlated with activities of their inhibitory factors such as alpha 2-plasmin inhibitor (α2-PI), alpha 2-macroglobulin (α2-MG), alpha 1-antitrypsin (α1-AT), antithrombin III (AT III) and C1 inactivator *in vivo* (Moroi & Aoki, 1976), and with activities of coagulation factors such as platelet, fibrinogen and Hageman factor (Cameron, 1983; Dixon, 1967).

The purpose of the present study was to elucidate whether the deposition of α2-PI and/or fibrinogen acts as one of the exacerbative factors in glomeruli from patients with IgA nephropathy. The results of this study indicated that there is a significant correlation between the deposition of α2-PI and/or fibrinogen and the degree of histopathological changes in patients with IgA nephropathy.

MATERIALS AND METHODS

Patients. Twenty-six patients with IgA nephropathy (IgAN) were examined. Twenty-nine

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patients with other glomerular diseases and seven healthy adults were also examined for measurement of plasma levels of α2-PI, plasmin and plasminogen. All these patients were diagnosed by light microscopic, electron microscopic and immunofluorescent analysis as described previously (Tomino *et al.*, 1982). Patients with IgA nephropathy whose biopsy specimens were stained predominantly for IgA in mesangial areas were included in this study after exclusion of patients with systemic lupus erythematosus, Henoch-Schoenlein purpura, liver cirrhosis or other systemic diseases.

Immunofluorescent staining and histopathological evaluation. Renal biopsy specimens embedded in a gelatin matrix (Tissue-Tek II, Lab-Tek Products, USA), using dry ice and acetone, were sectioned 2–4 μ with a rotary microtome in a cryostat at about −25°C and air dried. Direct immunofluorescence was performed using FITC-conjugated rabbit antisera to heavy-chain specific anti-human IgG, IgA, IgM, C3, fibrinogen (Behringwerke AG, Marburg-Lahn, West Germany) (F/P molar ratios ranged from 1·8 to 3·0), α2-PI (Lot No. 019, Mochida Pharmaceuticals Co., Ltd, Tokyo, Japan) (F/P molar ratios ranged from 2·0 to 3·0) (Moroi & Aoki, 1976) and α2-MG, α1-AT, AT III and C1-inactivator (Behringwerke AG, Marburg-Lahn, West Germany) (F/P molar ratios ranged from 2·0 to 3·0). These antisera were absorbed three times with mouse liver acetone powder. Specificities of these antisera were determined by immunodiffusion, immunoelectrophoresis and blocking tests (Kawamura, 1977) prior to use. FITC-conjugated anti-human α2-PI antisera did not contain 'non-immune reactants' such as albumin, α1-AT, haptoglobin, ceruloplasmin and α2-MG. Dilution of these antisera was performed at 1:10 in phosphate-buffered isotonic saline (PBS, pH 7·2) unless mentioned otherwise. Renal cryostat sections were stained with these FITC-conjugated antisera in a moist chamber at 4°C overnight. The sections were washed with PBS and then examined with a Zeiss Orthoflux fluorescent microscope (Model 9902; Carl Zeiss, Inc., New York, USA). The intensity of the fluorescence was graded as none (−), trace (±), 1 (+), 2 (+) and 3 (+). All samples were examined independently by two observers. The reproducibility of the detection of a 'positive reaction' was evaluated by duplicate tests.

Renal biopsy specimens stained with hematoxylin and eosin (H & E), periodic acid-Schiff (PAS) and periodic acid methenamine silver Masson (PAM) were examined by light microscopy. Specimens which contained more than 100 glomeruli per section were used for histopathological examination. Percentages of glomerular adhesion to Bowman's capsule, glomerular sclerosis and crescent formation were calculated in the renal biopsy specimens as described by Shirai *et al.* (1978).

Measurement of plasma levels of a2-PI, plasmin and plasminogen. 10 ml of fresh blood samples containing 3·8% of citrate were obtained from the patients and healthy adults. Plasma samples were separated at room temperature. Activities of α2-PI were examined using a Testzym APL kit® (Daiichikagaku, Tokyo, Japan) before renal biopsy. Quantification of serum α1-AT and α2-MG was performed using a single radial immunodiffusion method. For quantification of plasmin, 0·4 ml of plasma obtained from the patients was mixed with 0·1 ml of 2,000 IU/ml of urokinase and then incubated at 37°C for 25 min. These mixture were added to a fibrin plate containing 0·1 ml/dl of fibrin (Lot No. 8210, Kitasato Institute, Tokyo, Japan), and then incubated at 37°C for 4 h in a moist chamber. Quantification of plasminogen in plasma was performed using a Tri-Partigen plate® (Lot No. 05137, Behring Diagnostics, Summerville, New Jersy, USA). Stored plasma or serum samples were not used in any part of this study. The reproducibility of the measurement of α2-PI, α1-AT, α2-MG, plasmin and plasminogen was evaluated by the serial follow-up of a patient.

Statistical analysis. The Mann-Whitney U test was used for statistical analysis of clinical and pathological data.

RESULTS

Immunofluorescent staining and histopathological changes

IgA was a prominent immunoglobulin detected in glomeruli of all patients with IgA nephropathy (Fig. 1). Although IgG was observed in 17 out of 26 patients (65·4%) with IgA nephropathy, IgM or C3 was observed in all patients examined. α2-PI was observed in eight out of 26 patients (30·8%) with IgA nephropathy (Fig. 2). Fibrinogen was observed in 19 out of 26 patients (73·1%) (Fig. 3).

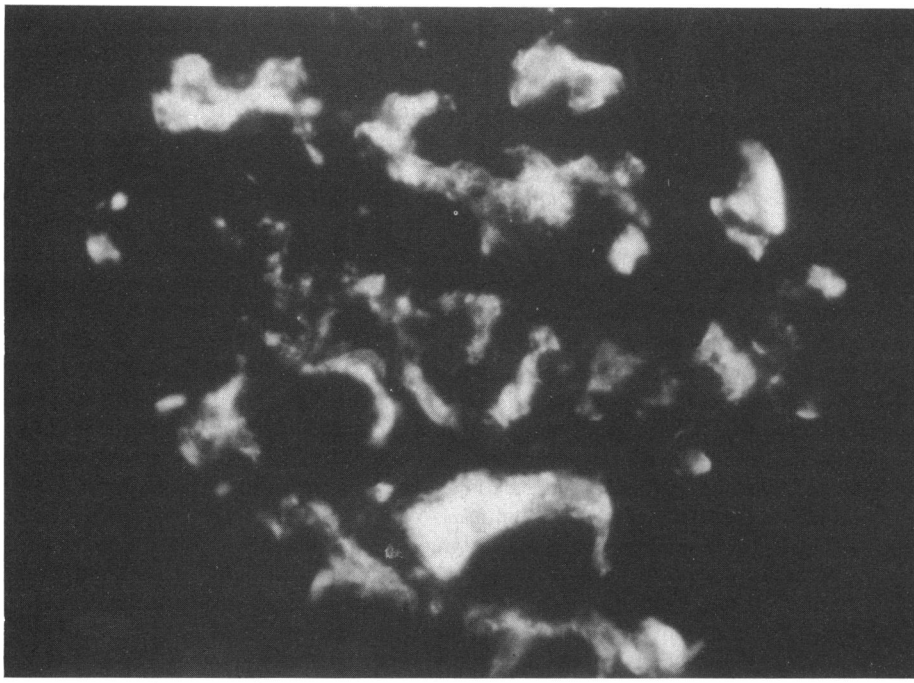

Fig. 1. Immunofluorescent findings of IgA in glomeruli from a patient with IgA nephropathy (× 400).

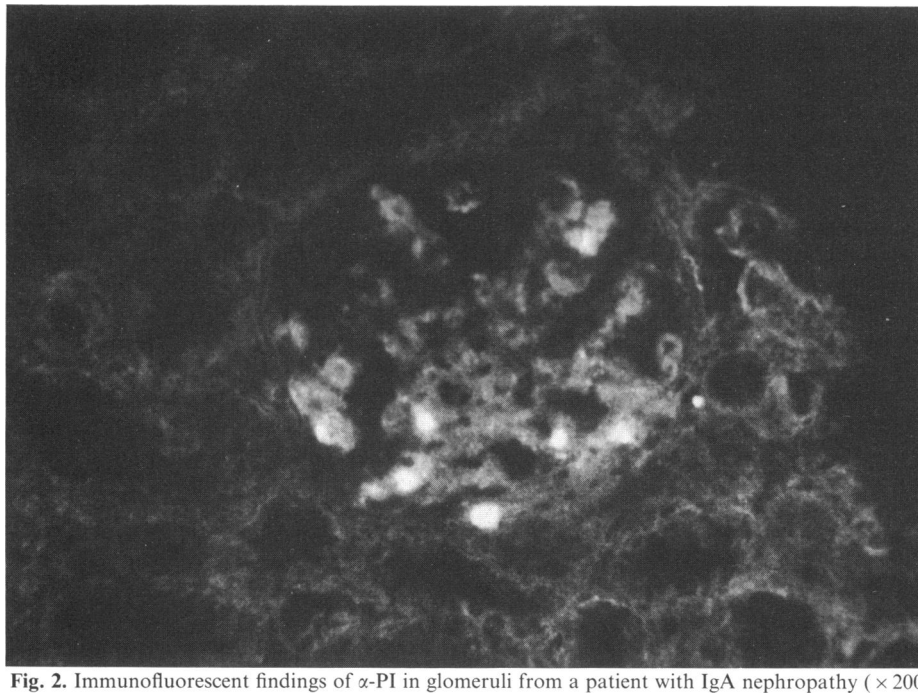

Fig. 2. Immunofluorescent findings of α-PI in glomeruli from a patient with IgA nephropathy (× 200).

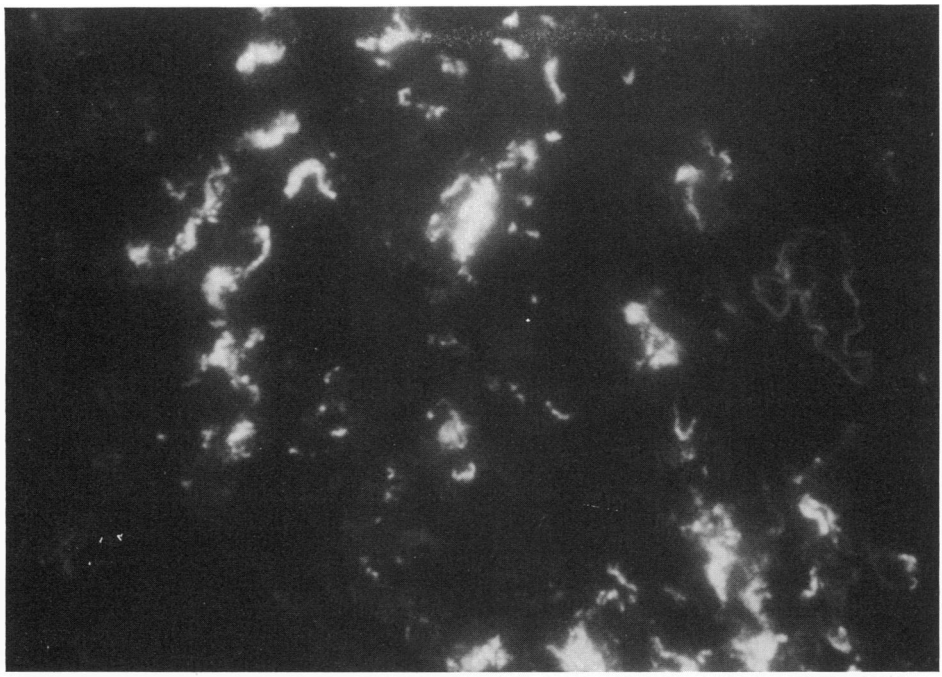

Fig. 3. Immunofluorescent findings of fibrinogen in glomeruli from a patient with IgA nephropathy ($\times 400$).

α2-PI coexisted with fibrinogen in eight out of 26 patients (30·8%) with IgA nephropathy. α2-MG, α1-AT, AT III and C1-inactivator were not observed in all patients examined.

The distribution of α2-PI and fibrinogen was similar to that of IgA, although that of α2-PI was focal and/or segmental in some patients with IgA nephropathy. The intensity of IgG, IgM, C3, α2-PI or fibrinogen in glomeruli was always less than that of IgA. There was a significant correlation between the incidence of α2-PI deposition and that of fibrinogen in glomeruli from patients with IgA nephropathy ($P < 0{\cdot}05$).

The degree of glomerular adhesion to Bowman's capsule in patients with positive deposition of α2-PI in glomeruli was significantly greater than that in patients with negative deposition of α2-PI ($P < 0{\cdot}01$). The degree of the cellular and/or fibrous crescent in patients with positive deposition of α2-PI was also significantly greater than that in patients with negative deposition of α2-PI ($P < 0{\cdot}05$). There was no significant correlation between the degree of glomerular sclerosis and the intensity of deposition of α2-PI in glomeruli from patients with IgA nephropathy ($P > 0{\cdot}05$) (Fig. 4). The degree of glomerular adhesion to Bowman's capsule in patients with positive deposition of fibrinogen in glomeruli was significantly greater than in patients with negative deposition of fibrinogen ($P < \cdot 0{\cdot}01$). The degree of cellular and/or fibrous crescent in patients with positive deposition of fibrinogen was significantly greater than that in patients with negative deposition of fibrinogen ($P < 0{\cdot}01$). There was no significant correlation between the degree of glomerular sclerosis and the intensity of deposition of fibrinogen in glomeruli from patients with IgA nephropathy ($P > 0{\cdot}05$) (Fig. 5).

The degree of glomerular adhesion to Bowman's capsule and cellular and/or fibrous crescent in patients with positive depositions of both α2-PI and fibrinogen were significantly greater than with those in patients with negative depositions of both α2-PI and fibrinogen ($P < 0{\cdot}01$, $P < 0{\cdot}01$, respectively). There was no significant correlation between the degrees of glomerular sclerosis and the intensity of both depositions of α2-PI and fibrinogen ($P > 0{\cdot}05$). There was no significant correlation between histopathological changes and the intensity of depostion of IgG, IgA, IgM or C3 in glomeruli from patients with IgA nephropathy.

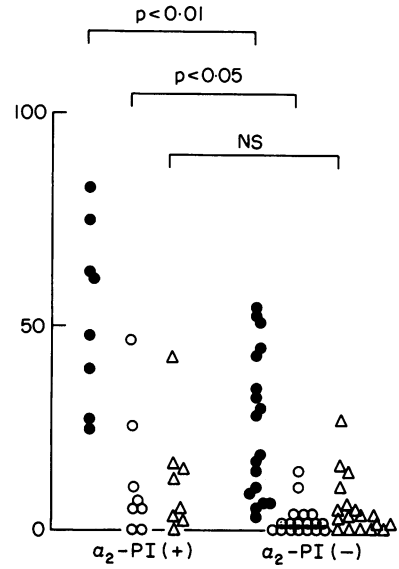

Fig. 4. Correlation between the percentages of histopathological injuries and the deposition of α2-PI in glomeruli from patients with IgA nephropathy. (●) Adhesion; (○) crescent; (△) sclerosis.

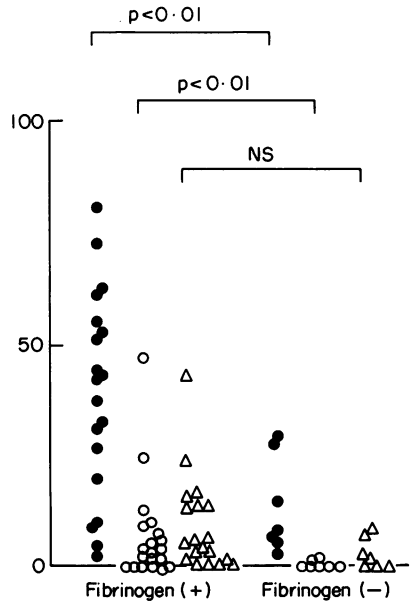

Fig. 5. Correlation between the percentages of histopathological injuries and the deposition of fibrinogen in glomeruli from patients with IgA nephropathy. (●) Adhesion; (○) crescent; (△) sclerosis.

Quantitation of plasminogen, plasmin and α2-PI in plasma from patients with IgA nephropathy and other glomerular diseases

The levels of plasminogen in plasma ranged from 7·8 to 14·7 mg/dl in patients with IgA nephropathy. There was no significant difference in plasminogen levels in plasma among patients with IgA nephropathy; other glomerular diseases such as diffuse proliferative glomerulonephritis (PGN), membranous nephropathy (MN), membranoproliferative glomerulonephritis (MPGN) and minimal change nephrotic syndrome (MCNS); and healthy adults. The levels of plasmin in

Table 1. Laboratory findings of serum levels of plasminogen, plasmin and α2-PI activities in patients with IgA nephropathy, other glomerular diseases and healthy adults

Disease	Number of cases	Plasminogen (mg/dl)	Plasmin (casein units)	α2-PI (%)
IgA nephropathy	26	11·2±1·8	106·1±8·7	111·2±12·5
Other glomerular diseases	29	10·9±2·3	109·5±7·4	107·5±7·8
Healthy adults	7	11·3±1·6	10·2±5·9	106·8±16·2

plasma ranged from 92 to 116 casein units in patients with IgA nephropathy. The levels of plasmin in plasma obtained from patients with IgA nephropathy were slightly higher than those of healthy adults, but the difference was not statistically significant ($P > 0{\cdot}1$). The activities of α2-PI in plasma ranged from 96% to 129% in patients with IgA nephropathy (Table 1). There was no significant difference in α2-PI activities among patients with IgA nephropathy, other glomerular diseases and healthy adults. There was no significant correlation between the intensity of α2-PI deposition in glomeruli and the serum levels of α2-PI in the patients and healthy adults. There was no significant correlation between the disease activities such as serum levels of creatinine, IgA and complement components and the histopathological changes in patients with IgA nephroapathy.

DISCUSSION

The results from the present study showed that the detection of α2-PI and/or fibrinogen in glomeruli was related significantly to the renal histopathological injuries such as glomerular adhesion to Bowman's capsule in glomeruli from patients with IgA nephropathy. The deposition of α2-PI was predominantly observed in glomeruli with capsular adhesion, and cellular and/or fibrous crescent in patients with IgA nephropathy.

α2-PI is a primary inhibitor of plasmin-catalysed fibrinolysis which is a glycoprotein with an estimated mol. wt. of 63,000–67,000 (Sakata & Aoki, 1982). Normal concentration of α2-PI in plasma is 6·9 mg/dl, which is produced by liver parenchymal cells (Sakata & Aoki, 1982). α2-PI was immediately bound to plasmin and fibrin in the presence of active fibrin-stabilizing factor in plasma *in vivo* (Sakata & Aoki, 1982; Wiman & Collen, 1977). Although several plasmin inhibitors have been recognized *in vivo*, α2-PI is the most intensive factor for fibrinolysis in human plasma (Sakata & Aoki, 1982). It was suggested that the plasmin-α2-PI complex and/or plasmin-fibrin complex in plasma reduced fibrinolysis in the hypercoagulable state of peripheral blood. There was no significant correlation between the activity of α2-PI in plasma and intensity of α2-PI deposition in glomeruli obtained from patients with IgA nephropathy in the present study. Although the activity of α2-PI in plasma was available, the deposition of α2-PI was immunohistopathologically observed in renal tissues of patients with IgA nephropathy. It appeared that the deposition of α2-PI persisted for a long time in glomeruli which had developed renal injuries.

IgA nephropathy is a well-recognized clinicopathologic entity which was first described by Berger & Hinglais (1968). Although the clinical course of IgA nephropathy is considered to be benign, hypertension and progress to renal failure are not as rare as originally thought (Clarkson *et al.*, 1984). Vassalli & McClusky (1964) reported that repeated intraglomerular coagulation might lead to the formation of lesions similar to those in some patients with chronic glomerulonephritis. Although IgA nephropathy is characterized by mesangial deposition of IgA, the deposition of fibrinogen was also observed in some patients with IgA nephropathy as described by Shirai *et al.* (1978). It appeared that the intraglomerular coagulation and/or their regulating systems may play a role in the development of inflammatory changes in glomeruli from patients with IgA nephropathy. α2-PI coexisted with fibrinogen in glomeruli from some patients with IgA nephropathy in the present study. Moreover, the deposition of α2-PI and/or fibrinogen was observed in glomeruli with

advanced histopathological changes such as capsular adhesion or crescent formation. It was suggested that the deposition of α2-PI might have inhibited fibrinolysis in glomeruli from patients with IgA nephropathy. It is feasible that the deposition of α2-PI *in vivo* might lead to the accumulation of glomerular fibrinogen deposits in patients with IgA nephropathy, and vice versa. However, it was suggested that fibrinolysis may not occur in renal glomerular sclerosis because no deposition of α2-PI and/or fibrinogen was observed in the glomerular sclerosis of patients with IgA nephropathy. It is premature to conclude whether or not the deposition of α2-PI and fibrinogen in the glomeruli is the sole reducing factor of fibrinolysis and these findings are specific for IgA nephropathy. Further examinations are warranted in various glomerular diseases other than IgA nephropathy.

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