

## Mesangial factor V expression colocalized with fibrin deposition in IgA nephropathy

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### Mesangial factor V expression colocalized with fibrin deposition in IgA nephropathy.

**Background.** Factor V in its active form (Va) plays a key role at the termination of the intrinsic coagulation pathway, serving as a membrane-bound cofactor for the conversion of prothrombin to thrombin by factor Xa. Cross-linked fibrin (XFb) is often observed in mesangial areas in active types of human glomerulonephritis. In this study, to clarify contribution of factor V in intramesangial coagulation, mesangial factor V expression and its relationship to mesangial proliferation and fibrin deposition in IgA nephropathy (IgAN) were investigated.

**Methods.** Twenty-two patients with IgAN were studied. XFb was detected in renal biopsy specimens using anti-d-dimer antibody combined with plasmin exposure, and factor V was detected with rabbit antibody against human factor V. Double-labeling immunohistochemistry was used to investigate the relationship of the glomerular distribution of factor V to XFb. The relationship of factor V staining to the activity index or XFb deposition was evaluated. The expression of factor V mRNA was assessed by in situ hybridization in relationship to the antigen staining of  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA). The ultrastructural distribution of factor V in glomeruli was studied by immunoelectron microscopy.

**Results.** XFb and factor V were observed in the mesangium and along capillary loops in seven and nine specimens, respectively. Factor V had intense, frequent expression in the proliferating and necrotizing areas, showing a significant relationship to XFb ( $P < 0.05$ ). Furthermore, XFb deposition and factor V expression were markedly correlated with disease activity ( $P = 0.005$  and  $P = 0.008$ , respectively). By double-labeling experiments, XFb and factor V were often seen colocalized in mesangial areas of the glomeruli, which showed necrotizing lesions and/or intense cellular proliferation. By in situ hybridization, factor V mRNA was detected mainly in the mesangial cells, which were positive for  $\alpha$ -SMA, and partly in the endothelial cells. By immunoelectron microscopy, factor V presence was confirmed in the mesangium and endothelium.

**Conclusion.** The present findings suggest that factor V is

strongly expressed in mesangial cells in active IgAN accompanied with mesangial proliferation and may exert procoagulant activity, leading to intramesangial coagulation.

Coagulation is an important event in the development of severe mesangioproliferative or crescentic glomerulonephritis. We have reported that the intact form of cross-linked fibrin (XFb) is often observed in mesangial areas in active mesangioproliferative glomerulonephritis using the monoclonal antibody DD3B6/22 against d-dimer, combined with plasmin exposure [1, 2]. The intact form of XFb suggests that it is newly produced in the mesangial areas and that intramesangial coagulation proceeds in accordance with mesangial proliferation. In vivo, mesangial cells are supplied with various coagulation factors from blood circulation through fenestration of capillary endothelium [3]. In physiological states, fibrin deposition is scarcely observed in glomeruli. After inflammatory change, inactive forms of coagulation factors are converted to active forms, and finally, XFb may be produced in mesangial areas. Out of the various coagulation factors, tissue factor is known to be the initiator in the extrinsic coagulation cascade, the major cascade in the inflammatory diseases, and thereafter, to convert factor X to active form Xa. On the other hand, factor V in its active form (Va) serves as a membrane-bound cofactor to factor Xa, which facilitates activation of prothrombin to thrombin. Then thrombin immediately converts fibrinogen to fibrin. It is known that the rate of prothrombin activation by the prothrombinase complex, Xa/Va, is about 300,000 times higher than the rate of activation by factor Xa alone [4, 5]. Thus, factor Va plays a key role nearly in the ending of the coagulation pathway. As factor V is known to be expressed in various circulating and noncirculating cells such as platelets [6, 7], endothelial cells [8], monocytes [9], and hepatocytes [10], the expression of this factor in renal parenchymal cells, including mesangial cell, is highly suspected and plays a

**Key words:** glomerulonephritis, renal biopsy, cross-linked fibrin, inflammatory response,  $\alpha$ -smooth muscle actin.

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crucial role in local coagulation in various mesangium-involving glomerulonephritis, especially in IgA nephropathy (IgAN). In this study, we studied the mesangial factor V expression in relationship to XFb deposition and disease activity in IgAN.

## METHODS

### Patients

Three patients with minor glomerular abnormalities and 22 patients with IgAN were studied. The glomerular lesions of mesangial cell proliferation, necrotizing lesion, cellular crescent formation, and mononuclear interstitial infiltrate were scored to determine the activity index (AI) according to the modified criteria of Austin et al [11], which was originally developed for systemic lupus erythematosus (SLE), except for wire loops and leukocyte infiltrate in glomeruli. The presence of mesangial cell proliferation and interstitial mononuclear infiltrate was scored in a range of 0 to 3, with 0 indicating absence, 1 indicating mild (less than 30% of all glomeruli involved, or focal interstitial infiltrate), 2 indicating moderate (30% to 70% of all glomeruli involved or multiple foci of interstitial infiltrate), and 3 indicating severe (more than 70% of all glomeruli involved or diffuse interstitial infiltrate). The presence of necrotizing lesion and cellular crescent was scored in a range of 0 to 3, with 0 indicating absence, 1 indicating less than 10% of the glomeruli involved, 2 indicating 10 to 30%, and 3 indicating more than 30%. Scores of necrotizing lesion and cellular crescent were each multiplied by 2, according to the original criteria. The maximal AI was 18. The diagnoses were confirmed by renal biopsy by light microscopy, immunofluorescence microscopy, and electron microscopy, in addition to clinical findings.

### Immunoperoxidase staining for XFb, factor V, and proliferating cell nuclear antigen

Biopsy specimens were frozen in OCT compound (Miles Laboratories, Elkhart, IN, USA), cut into serial sections on a cryostat, fixed in acetone for five minutes at room temperature, and stained indirectly. XFb was detected indirectly before and after plasmin exposure using the monoclonal antibody specific for d-dimer, DD3B6/22 (AGEN, Brisbane, Australia). The method of plasmin treatment was described previously [1]. Briefly, sections were treated with the active form of plasmin (Nacalai Tesque, Kyoto, Japan) for 20 minutes at room temperature at a concentration of 0.25 U/mL in 0.01 mol/L phosphate-buffered saline (pH 7.4). The preparations were washed in Tris-buffered saline (pH 7.4) and incubated with antibody DD3B6/22 for one hour, rabbit anti-mouse immunoglobulins (Dako, Glostrup, Denmark), and mouse APAAP complex (Dako). Color was

then developed by incubation with fast red dye (Dako). The sections were counterstained with hematoxylin.

Factor V was detected by an indirect method using a monospecific rabbit antibody against human factor V, RAHu/FV (Nordic Immunological Laboratories, Tilburg, Netherlands), as described previously [12]. Briefly, the sections were incubated with antibody RAHu/FV followed by biotinylated antirabbit IgG (Vector Laboratories, Burlingame, CA, USA). The sections were then reacted with avidin-DH-biotinylated horseradish peroxidase complex (Vectastain ABC kit; Vector Laboratories). Color was then developed by incubation with an ImmunoPure Metal Enhanced DAB Substrate kit (Pierce, Rockford, IL, USA). Positivity of XFb and factor V was scored in a range of 0 to 3, with 0 indicating absence, 1 indicating less than 25% of glomerular tufts, 2 indicating 25 to 50%, and 3 indicating more than 50%.

Proliferating cell nuclear antigen (PCNA) was detected on sections that were Doubovsky-Brazil fixed, paraffin embedded, cut into 4  $\mu$ m, and deparaffinized using mouse monoclonal antibody against proliferating cell nuclear antigen (EPOS; Dako). Color was then developed by incubation with a DAB Substrate kit (Pierce).

### Double-labeling experiment

Fresh frozen tissue sections were fixed in acetone and stained indirectly according to a modified method previously reported [13]. After incubation with plasmin, the sites of antigen-antibody reaction were revealed by incubating each section with rabbit antibody against human factor V, RAHu/FV, followed by biotinylated antirabbit IgG. The preparations were washed twice in Tris-buffered saline (pH 7.4) and incubated with the monoclonal antibody DD3B6/22, rabbit anti-mouse immunoglobulins (Dako), and mouse APAAP complex (Dako). Color was then developed by incubation with fast red dye (Dako) and DAB Substrate kit (Pierce).

### In situ factor V mRNA hybridization

In situ hybridization was performed according to a modified method previously reported [14]. Briefly, 30-mer oligonucleotide probes, which corresponded to the antisense (5'-TGC ATC CCA GCT TGC AAA TGT TTT GGG GTG-3') and sense (5'-CAC CCC AAA ACA TTT GCA AGC TGG GAT GCA-3') of human factor V cDNA [5], labeled with a Digoxigenin Oligonucleotide Tailing kit (Boehringer Mannheim Biochemica, Mannheim, Germany), were used. The fresh frozen tissue sections were fixed in ice-cold 4% paraformaldehyde for 15 minutes. Sections were prehybridized for 1.5 hours at 37°C in prehybridization solution and 200 mg/mL sonicated denatured herring sperm DNA (Sigma, St. Louis, MO, USA). Hybridization was carried out at 37°C overnight with either sense or antisense probes. Sections were washed in 2  $\times$  standard saline citrate (SSC), 1  $\times$  SSC,

0.5 × SSC, and finally washed in 0.1 × SSC. Immunological detection of the hybridized probes was performed with monoclonal anti-DIG, rabbit anti-mouse immunoglobulins (Dako), and mouse APAAP complex (Dako). Color was then developed by incubation with fast red dye (Dako). Finally, sections were counterstained with hematoxylin.

#### Simultaneous in situ detection of factor V mRNA and antigen immunostaining of $\alpha$ -smooth muscle actin

To confirm the synthesis of factor V by mesangial cells, simultaneous in situ hybridization for factor V and antigen labeling for  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) was performed according to a modification of the method previously reported [15]. Fresh frozen tissue sections were fixed, incubated with the monoclonal antihuman  $\alpha$ -SMA (Dako) for one hour, and followed by biotinylated anti-mouse IgG (Vector Laboratories). The sections were then reacted with avidin-DH-biotinylated horseradish peroxidase complex (Vector Laboratories), and color was then developed by incubation with the DAB Substrate kit (Pierce). Slides were washed twice in distilled water and prepared for in situ hybridization by treatment with 0.2 N HCl for 10 minutes to remove immunoglobulins. Hybridization was carried out with either sense or antisense human factor V cDNA probes, and hybridized probes were detected as described previously in this article.

#### Immunoelectron microscopy

Part of the biopsy specimen was fixed in periodate-lysine-paraformaldehyde according to the method of McLean and Nakane [16]. After washing with different concentrations of sucrose solutions (10, 15, and 20%), the specimen was frozen in OCT compound and sectioned (6 mm) on a cryostat [17]. These sections were etched by 0.3% hydrogen peroxide for 15 minutes. After washing with phosphate-buffered saline (PBS), the sections were treated with 10% normal goat serum, followed by incubation with rabbit antibody against human factor V, RAHu/FV, at 4°C overnight. After washing, further incubation followed with biotinylated antirabbit IgG for three hours. The sections were then reacted with avidin-DH-biotinylated horseradish peroxidase complex (Vector Laboratories) for three hours, and color was developed as described previously in this article. Sections were postfixated in 2% osmic acid and embedded in Epon 812 (Oken Shoji), and ultrathin sections were cut. Observation was performed with a Hitachi 7100 electron microscope (Tokyo, Japan).

#### Statistics

Statistical analysis was performed using the  $\chi^2$  test and analysis of correlation. The XFb staining after plasmin exposure and the positivity of factor V staining were

**Table 1.** Clinical and laboratory data in patients with IgAN

Patient no.	Age years	Male/female	Urinary protein g/day	Urinary RBC <sup>a</sup> N/HPP <sup>b</sup> of sediment	Serum creatinine mg/dL
1	23	F	0.1	25	0.4
2	21	M	0.0	6	0.9
3	23	M	0.0	10	0.8
4	24	F	0.1	65	0.5
5	25	M	0.3	3	0.7
6	21	F	0.0	30	0.7
7	28	F	0.7	50	0.7
8	21	F	1.8	7	0.6
9	55	F	0.0	13	0.8
10	33	F	0.2	8	0.6
11	45	F	1.0	4	0.6
12	40	M	0.4	35	1.0
13	20	F	0.2	40	0.7
14	27	F	0.3	25	0.7
15	25	F	0.6	100	0.7
16	22	F	0.6	40	0.6
17	21	M	1.1	25	0.9
18	15	M	0.3	25	0.7
19	14	M	2.3	80	0.8
20	22	M	0.6	15	1.0
21	26	F	0.1	25	0.7
22	51	M	1.1	35	1.2
Mean ± SD	27 ± 10	M=9 F=13	0.5 ± 0.6	30 ± 25	0.7 ± 0.2

<sup>a</sup>RBC, red blood cell

<sup>b</sup>HPP, high-power field

compared according to the histologic parameters of disease activity. Factor V expression was also correlated with XFb deposition. The values were reported as mean ± SD, and  $P < 0.05$  was regarded as significant.

## RESULTS

Table 1 summarizes the clinical and laboratory data of IgAN patients. The distribution of disease activity presented two groups of inactive IgAN (AI 1 to 4; patients 1 through 11), and active IgAN (AI 7 to 15; patients 12 through 22), lacking AI 5 or 6 cases. Each group consisted of 11 cases, as shown in Table 2. Figure 1A shows microscopic findings in an inactive IgAN (AI 2) with slight mesangial widening. In an active IgAN case (AI 13), moderate diffuse mesangial proliferation was observed, as shown in Figure 1B. To determine the proliferation of glomerular cells, tissue sections were immunostained for PCNA. Figure 1C shows PCNA staining in an inactive IgAN, and Figure 1D shows PCNA staining in an active case. The number of PCNA-positive cells in glomeruli from active cases of IgAN (mean ± SD = 2.8 ± 1.2) was approximately ninefold greater than that from inactive cases (0.3 ± 0.4).

XFb staining was all negative in the three specimens from minor glomerular abnormalities. In IgAN, after plasmin treatment, XFb immunoreactivity turned posi-

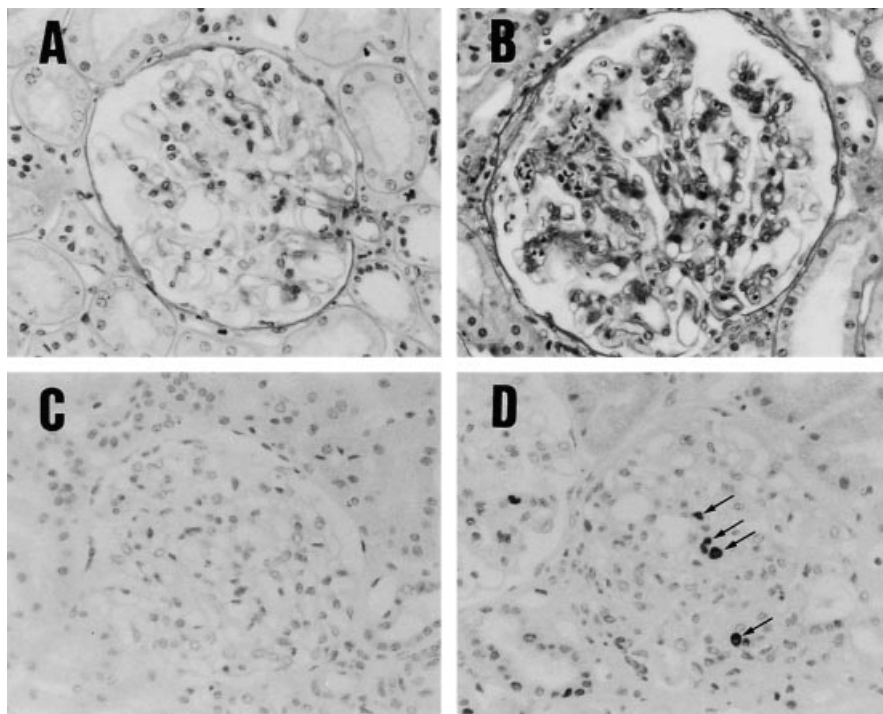
**Table 2.** Histological and immunohistochemical findings of glomeruli from patients with IgAN

Patient no.	Mesangial cell proliferation score <sup>a</sup>	Necrotizing lesion score <sup>b</sup>	Cellular crescent score <sup>c</sup>	Interstitial mononuclear infiltrate score <sup>d</sup>	Activity index <sup>h</sup>	PCNA-positive cells per glomerulus number	XFb staining		Factor V expression score <sup>e</sup>
							Before plasmin score <sup>e</sup>	After plasmin score <sup>f</sup>	
1	2	0	0	0	2	0.2	0	0	1
2	1	0	0	1	2	0.0	0	0	0
3	1	0	0	0	1	0.6	0	0	0
4	2	0	0	1	3	0.7	0	0	0
5	1	0	0	0	1	0.0	0	1	0
6	1	0	0	1	2	0.0	0	0	0
7	1	0	0	1	2	0.1	0	0	1
8	2	0	1	0	4	0.0	0	0	0
9	1	0	0	0	1	0.0	0	0	0
10	2	0	0	1	3	0.8	0	0	0
11	2	0	0	0	2	1.2	0	0	0
12	2	2	2	2	12	2.7	0	3	2
13	3	0	2	1	8	2.5	0	1	2
14	3	2	2	2	13	4.8	1	2	2
15	2	0	2	1	7	1.6	0	0	0
16	3	1	2	1	10	2.0	0	2	1
17	3	1	2	2	11	2.1	0	1	1
18	3	0	2	1	8	1.3	0	0	0
19	2	1	2	1	9	1.4	0	0	2
20	3	2	3	2	15	3.7	0	0	0
21	3	2	2	1	12	2.1	0	1	1
22	2	0	2	1	7	4.2	0	0	0
<i>P</i> -value vs. XFb after plasmin	0.02	0.02	0.02	0.04	0.005 <sup>i</sup>	0.02 <sup>i</sup>	0.13	—	0.02
Factor V	0.04	0.03	0.03	0.07	0.008 <sup>i</sup>	0.04 <sup>i</sup>	0.22	0.02	—

Scores of <sup>a</sup> to <sup>g</sup> are ranged 0-3.

<sup>h</sup>Activity index is amount of (a + 2xb + 2xc + d); maximal, 18

<sup>i</sup>*P*-value, using analysis of correlation; and others, chi-square test



**Fig. 1. Mesangial proliferation both in inactive and in active IgA nephropathy (IgAN).** Mesangial proliferation is slightly observed in a glomerulus in inactive type (A) and moderately detected in active type (B). With an antibody against PCNA, several proliferating cells in the mesangium (arrows) are detected by immunostaining in a glomerulus with active IgAN (D), while no positive cells are observed in a glomerulus with inactive IgAN (C; final magnification,  $\times 210$ ).

tive in 6 of 21 renal biopsy specimens except one case, in which XFB staining was already positive before plasmin treatment. The positivity of XFB after plasmin exposure was 7 out of 22 (32%) of total cases, 1 out of 11 (9%) of inactive cases, and 6 out of 11 (55%) of active cases. XFB staining was negative before and after plasmin exposure in inactive IgAN (Fig. 2 A and B). Positive XFB staining was observed mainly in proliferating and/or necrotizing lesions, especially after plasmin exposure. As shown in Figure 2 C and D, positive staining was detected diffusely in the mesangium and segmentally in capillary loops in the same specimen with Figure 1D.

Factor V staining was negative in all specimens from minor glomerular abnormalities. In the inactive IgAN, factor V staining was negative (Fig. 3A) except for two cases. In an active case, factor V staining was detected in mesangial cells and along capillary loops, and its expression was intense and frequent in the proliferating and/or necrotizing areas (Fig. 3B) in the same specimen with Figure 1D. Factor V staining was detected in 9 of the total 22 (41%), showing a significant relationship with that of XFB after plasmin exposure ( $P = 0.02$ ). As shown in Table 2, XFB deposition and factor V expression presented a significant correlation with mesangial cell proliferation, necrotizing lesion, cellular crescents formation, and PCNA-positive cells per glomerulus, especially with AI ( $P = 0.005$  and  $P = 0.008$ , respectively).

Factors V and XFB were often colocalized in glomeruli of highly active type IgAN, as shown in Figures 2D and 3B of the same glomerulus from patient 12. Intense XFB

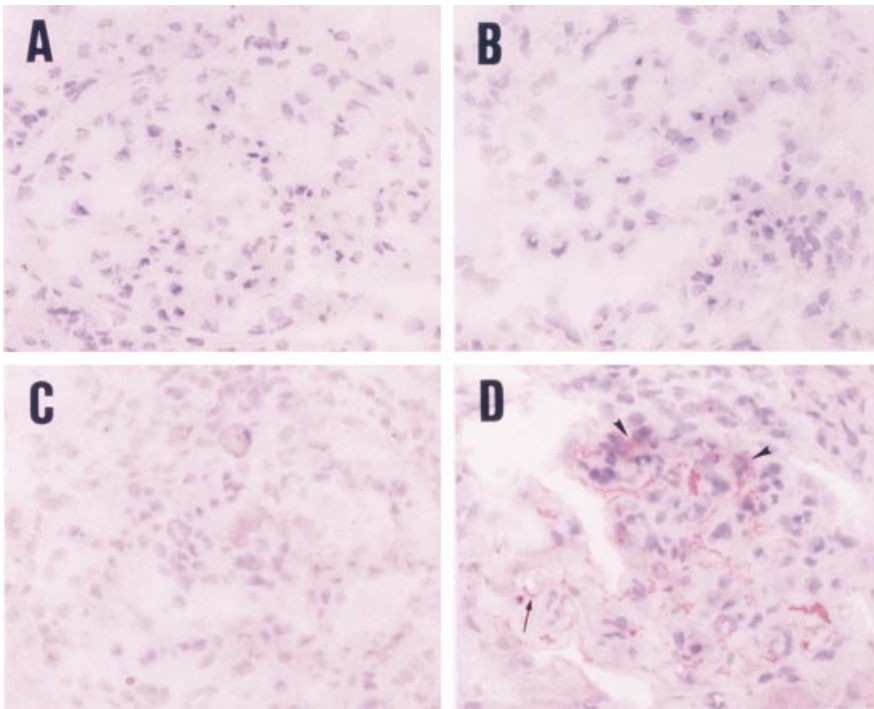
staining after plasmin exposure resembled the distribution of factor V staining. The double-immunolabeling immunohistochemistry to the same glomerulus with Figures 2D and 3B revealed the colocalization of XFB and factor V in proliferating and necrotic lesions (Fig. 4).

Expression of factor V mRNA was detected in the regions of cell proliferation in the specimen from patient 21 using the antisense probe (Fig. 5B), whereas the negative control with the sense probe resulted in no staining in any of these regions (Fig. 5A). This distribution was similar to that of the cell expressing factor V proteins. Furthermore, simultaneous immunohistochemistry for  $\alpha$ -SMA and in situ hybridization for factor V mRNA expression was performed in an active IgAN patient 14, as shown in Figure 5 C and D.  $\alpha$ -SMA immunostainings were discrete and localized to mesangial cells in the glomerulus. In the mesangium,  $\alpha$ -SMA antigen-positive cells also showed factor V mRNA expression, although there were other  $\alpha$ -SMA-negative cells that were positive for factor V mRNA observed along capillary walls. However, no hybridization with the sense probe was observed after  $\alpha$ -SMA antigen staining (results not shown).

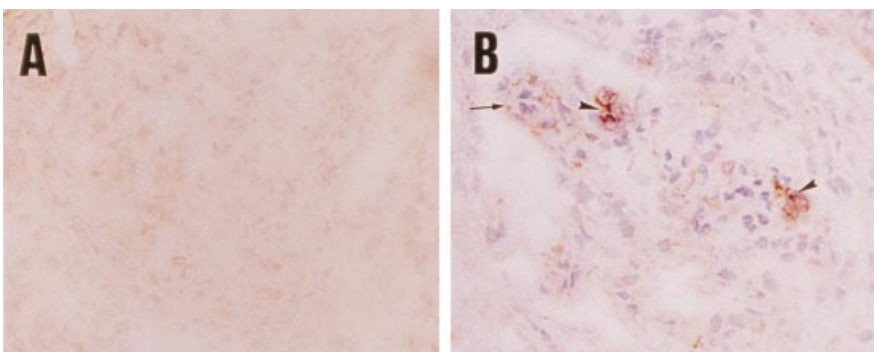
By immunoelectron microscopy, factor V presence was confirmed in the cytoplasm of both mesangial cells and endothelial cells in a glomerulus from an active IgAN (Fig. 6).

## DISCUSSION

Intraglomerular coagulation is thought to be involved in the development of glomerular injury [18, 19]. Various



**Fig. 2. Cross-linked fibrin (XFb) before and after plasmin exposure both in inactive and in active IgAN.** Staining for XFb is negative in the glomerulus in inactive type before (A) and after (B) plasmin exposure. Staining for XFb is negative before plasmin exposure (C), but positive after plasmin exposure, both in the mesangium (arrowheads) and along capillary loops (arrow; D) in active type (final magnification,  $\times 210$ ).



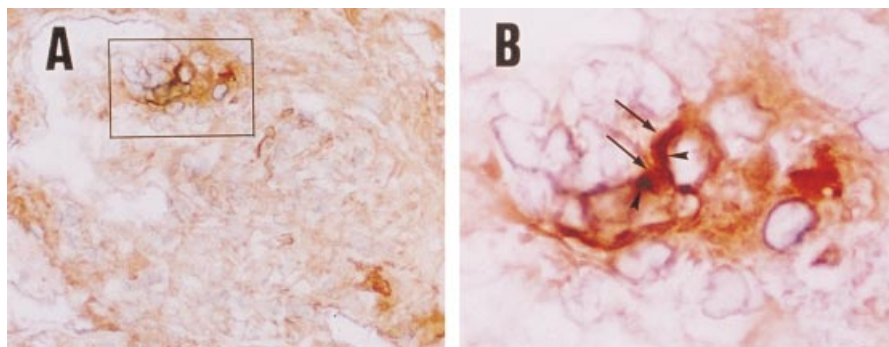
**Fig. 3. Factor V immunostaining both in inactive and in active IgAN.** Factor V is negative in inactive IgAN (A), and positive intensely in the mesangium (arrowheads), and weakly along capillary loops (arrow), in active IgAN (B; final magnification,  $\times 210$ ).

coagulation factors have been previously observed in the glomeruli of renal biopsy specimens by our group and other authors [17, 20–23]. In the recent study by Cunningham et al, tissue factor pathway inhibitor is shown to be dysregulated in the early stages of human crescentic glomerulonephritis when there is significant fibrin deposition [24].

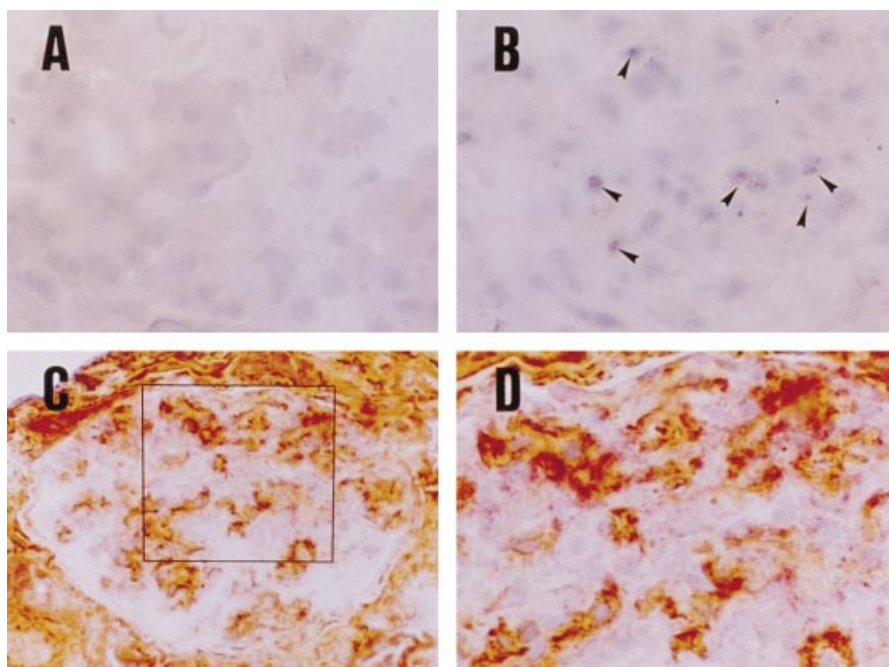
In IgAN, the major abnormality is mesangial proliferative glomerulonephritis accompanied by sclerosis and occasionally necrosis and crescents [25]. In our previous study, we observed an increase in immunoreactivity after plasmin exposure using the monoclonal antibody DD3B6/22, in renal biopsy specimens from both IgAN and Henoch-Schönlein purpura nephritis, which showed the presence of intact XFb in glomeruli. We presented our hypothesis that the intact XFb in the mesangium may be related to mesangial cell proliferation [2]. As for factor V expression, it is important to promote intraglomerular coagula-

tion, which may lead to glomerular sclerosis. The liver and megakaryocytes are known to synthesize factor V [26]. It is converted to its active cofactor, Va, from factor V by thrombin or factor Xa [27]. The Xa/Va complex, which is formed on membranes, is known as the prothrombinase complex, since it serves to convert prothrombin to thrombin. Subsequently, thrombin converts fibrinogen to fibrin [28].

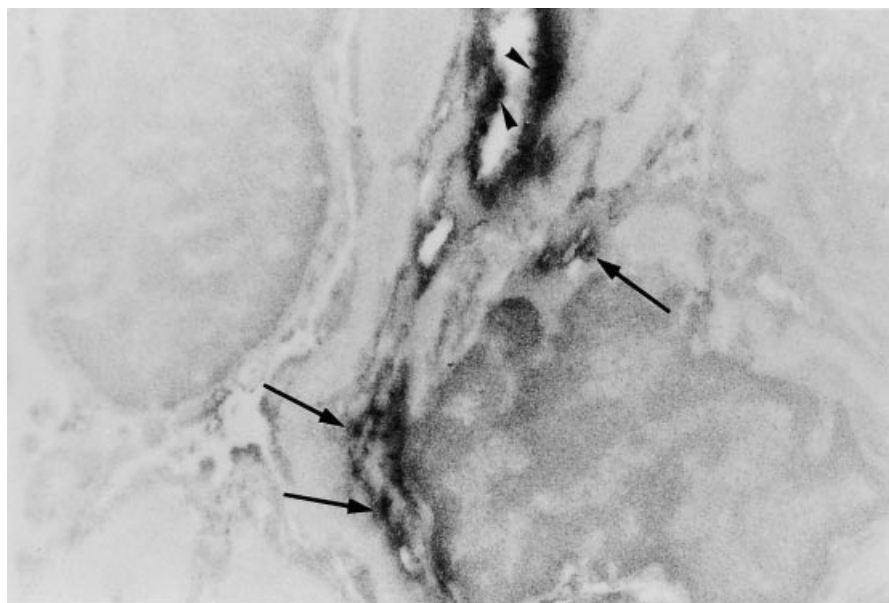
Therefore, in this study, we examined the mesangial expression of factor V in relationship to intact XFb deposition in the mesangium and level of disease activity. To determine the source of factor V and the mechanisms of XFb production in the glomeruli, we performed four experiments: (1) immunohistochemistry for factor V, XFb, and PCNA on biopsy sections; (2) double staining for factor V and XFb to determine their colocalization; (3) in situ hybridization for factor V mRNA in the mesangial cells in relationship to  $\alpha$ -SMA antigen expression; (4)



**Fig. 4. Double immunostaining of the glomerulus for XfB and factor V in the same glomerulus as shown in Figures 2D and 3B.** In the areas of the mesangial cell proliferations, XfB (red, arrows) and factor V (dark brown, arrowheads) are colocalized. The boxed area in A is shown at a higher power in B (final magnification, A  $\times 210$ ; B  $\times 800$ ).



**Fig. 5. Expression of factor V mRNA in the glomerulus from an active IgAN case.** The sections are hybridized with either sense (A) or antisense (B) factor V probes using serial sections. Localization of factor V mRNA (arrows, red) in a glomerulus shows expression of the factor V gene. Simultaneous in situ detection of factor V mRNA (red) and immunostaining for antigen of  $\alpha$ -SMA (dark brown) shows colocalization in mesangial cells (C). The boxed area is shown at a higher power in D (final magnification, A, B, and D  $\times 490$ ; C  $\times 210$ ).



**Fig. 6. Ultrastructural distribution of factor V viewed by immunoelectron microscopy in a glomerulus from the same case as Figure 4.** Factor V is observed in both a mesangial (arrows) and an endothelial cell (arrowheads; final magnification,  $\times 15,400$ ).

immunoelectron microscopy analysis using immunoperoxidase methods for confirmation of factor V localization.

The major finding of this study was that, as shown by immunostaining, intact XFb was frequently observed in the mesangium with active IgAN, and factor V was colocalized with XFb deposition. Increased XFb staining was observed mainly in the mesangial areas with intensely proliferating lesions and/or necrotizing lesions as well as segmentally along capillary loops. Factor V was also expressed in these regions. Furthermore, the deposition of factor V and the expression of factor V correlated markedly with the degree of disease activity. Based on the findings of these experiments, it is suggested that XFb production is promoted through factor V up-regulation in mesangial cells in active-type IgAN. In this context, urinary thrombin activity, which is thought to be the result of the prothrombinase complex Xa/Va activity, is increased in mesangial proliferative glomerulonephritis relative to nonproliferative glomerulonephritis [29]. From preliminary findings, factor V is synthesized in cultured mesangial cells via stimulation by tumor necrosis factor- $\alpha$  (abstract; Ono et al, *J Am Soc Nephrol* 9:483A, 1998). Because Alpers et al demonstrated a correlation between mesangial  $\alpha$ -SMA expression and proliferation of cell within the glomerulus [30], colocalization of factor V mRNA and  $\alpha$ -SMA antigen confirms that mesangial cells are the cell of major origin of factor V production in glomeruli after proliferative stimulation. As shown in our study, factor V expression in the mesangium in human glomerulonephritis may be linked to mesangial cell proliferation and inflammatory activity, which may induce various inflammatory cytokines such as tumor necrosis factor- $\alpha$  and interleukin-1, although the regulatory mechanism of factor V needs to be clarified.

In experimental glomerulonephritis, Malliaros et al reported that a reduction in the glomerular fibrinolytic activity might potentiate glomerular fibrin deposition [31]. Other authors reported that cultured mesangial cells induce antifibrinolytic activity because of the expression of plasminogen activator inhibitor-1 [32] and that extracellular matrix degradation was mediated by the plasmin/matrix metalloproteinase-2 cascade and ameliorated by the depletion of plasminogen activator inhibitor-1 in cultured human mesangial cells [33]. The expression of factor V in IgAN with active lesions in this study suggests that it may contribute to the progression of this glomerulonephritis.

In conclusion, our findings indicate that mesangial expression of factor V is accompanied by mesangial XFb deposition in active type IgAN. Present data suggest that after stimulation by inflammation, factor V is expressed in mesangial cells and may exert procoagulant activity, leading to intramesangial coagulation.

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## APPENDIX

Abbreviations used in this article are: AI, activity index;  $\alpha$ -SMA, alpha-smooth muscle actin; factor Va, factor V in its active form; IgAN, IgA nephropathy; PCNA, proliferating cell nuclear antigen; SLE, systemic lupus erythematosus; XFb, cross-linked fibrin.

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