Coagulation in the mesangial area promotes ECM accumulation through factor V expression in MsPGN in rats

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Liu, Ning, Toshiaki Makino, Fumiaki Nagaki, Hitoshi Kusano, Katsuo Suyama, Eri Muso, Gisho Honda, Toru Kita, and Takahiko Ono. Coagulation in the mesangial area promotes ECM accumulation through factor V expression in MsPGN in rats. Am J Physiol Renal Physiol 287: F612–F620, 2004. First published June 1, 2004; 10.1152/ajprenal.00322.2003.—It is well known that tissue factor starts the extrinsic coagulation pathway, which activates factor X to Xa, and factor V is a membrane-bound potent cofactor for the terminating stage of prothrombin activation by factor Xa. In a previous in vitro study, factor V was induced in cultured mesangial cells by inflammatory stimulation and increased expression of factor V promoted fibrin generation on the cultured mesangial cell surface. We report that extracellular matrix (ECM) accumulation is increased in association with coagulation in the mesangial area through factor V expression in mesangio proliferative glomerulonephritis (MsPGN). Wistar rats were intravenously injected with rabbit anti-rat thymocyte serum accompanied with or without simultaneous injection of rabbit anti-factor V antibody. Time course study in immunohistochemistry revealed that factor V expression was prominent on day 3 and fibrin-related antigen (FRA) deposition, then ECM accumulation, followed from day 3 to day 8. Massive fibronectin depositions and transforming growth factor (TGF)-β expression were also noted in glomeruli from the disease control group, markedly higher than those in the normal group, and these depositions and expressions were significantly decreased in the anti-factor V neutralizing antibody-injected group. Northern blot analysis revealed that factor V mRNA expression was prominent on day 3 and was weak on day 8. Double-labeling experiments revealed the frequent colocalization of α-smooth muscle actin with factor V, FRA, and fibronectin in the same mesangial areas of glomeruli. TGF-β, connective tissue growth factor (CTGF), collagen type IV, and fibronectin mRNA were upregulated in the disease control group, and anti-factor V-neutralizing antibody injection suppressed these mRNA expressions in glomeruli. The present results suggest that ECM components accumulation may progress in accordance with coagulation in the mesangial area through mesangial factor V expression and upregulated expression of TGF-β and CTGF in MsPGN.

Experimental glomerulonephritis; extracellular matrix components accumulation; transforming growth factor-β; connective tissue growth factor

The pathogenic role of locally accelerated coagulation accompanying fibrin deposition was reported in various active mesangio proliferative glomerulonephritis (MsPGN), including IgA nephropathy or Henoch-Schönlein purpura nephritis (32). This suggests factor (TF) is the principal initiator of the extrinsic coagulation pathway, and glomerular expression of TF is upregulated in human and rabbit crescentic glomerulonephritis (35, 36). We previously reported that mesangial factor V expression accompanied by the intact form of cross-linked fibrin deposition was often detected in the active type of IgA nephropathy (21). In a recent in vitro study, we observed procoagulant activity in cultured mesangial cells via expression of factor V (31). Factor V in its active form (Va) plays a key role at the termination both of intrinsic and of extrinsic coagulation pathways, serving as a membrane-bound potent cofactor for the conversion of prothrombin to thrombin by factor Xa (16, 22).

Anti-thymocyte serum (ATS)-induced rat nephritis is known as an experimental model for MsPGN, referred to as Thy-1 nephritis (12). ATS contains antibodies against Thy-1, originally an antigen of thymocytes, and also appears in rat glomerular mesangial cells. ATS selectively stimulates mesangial cells to proliferate and causes symptoms similar to human MsPGN. Thy-1 nephritis is characterized by the process of necrosis (27). The features of both mesangial cell proliferation and mesangial matrix expansion are similar in ATS nephritis and human MsPGN. Intraglomerular accumulation of extracellular matrix (ECM) proteins and mesangial cell proliferation are important components of the pathophysiological changes in various glomerular diseases, often resulting in end-stage renal disease with development of severe glomerulosclerosis (3, 18). It was reported that heparin could inhibit mesangial cell proliferation and ECM expansion in Thy-1 nephritis and that treatment with conventional heparin was effective in experimental MsPGN (9). However, heparin is known to have various effects other than anti-coagulation. For example, hepatocyte growth factor has anti-fibrotic effects in a mouse model of chronic renal disease and increased its plasma levels after heparin treatment (23, 25). In this context, non-anti-coagulant heparin was also effective in Thy-1 nephritis (4). Therefore, we conducted a pure anti-coagulant in vivo study on the inhibition of factor V activity by neutralizing antibody. To clarify the contribution of intramesangial coagulation on ECM accumulation through mesangial factor V activity in MsPGN, the expression of transforming growth

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factor (TGF)-β and connective tissue growth factor (CTGF), which were situated downstream of thrombin activation, was also investigated in an experimental model.

MATERIALS AND METHODS

Experimental design. MsPGN was induced in 6-wk-old male Wistar rats (Shimizu Laboratory Materials, Kyoto, Japan) by the intravenous injection of ATS, which was prepared as reported previously (12) and provided by Nippon Shinyaku (Kyoto, Japan). For the intervention study, 24 rats were divided into four experimental groups, injection of ATS and with simultaneous injection of rabbit anti-human factor V antibody, RAHu/FV, against rat plasma factor V, a goat anti-rabbit fibrinogen (ICN, Aurora, OH), a goat anti-human collagen IV (Southern Biotech, Birmingham, AL), or a goat anti-rabbit fibronectin (Calbiochem-Novabiochem, La Jolla, CA), as described previously (21). Briefly, the deparaffinized sections were incubated with each primary antibody for 1 h, followed by biotinylated IgG of secondary antibody (Vector Laboratories, Burlingame, CA). 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 diaminobenzidine (DAB) Substrate kit (Pierce, Rockford, IL).

Factor V was detected on fresh frozen sections in optimum cutting temperature (OCT) compound, fixed in acetone for 5 min at room temperature, and stained by an indirect method using a sheep antibody against human factor V (Cedarlane Laboratories, Ontario, Canada) to avoid a cross-reaction between secondary antibodies with the injected rabbit antibody, RAHu/FV. Color was then developed by incubation with a DAB substrate kit (Pierce). These sections were counterstained with hematoxylin. The areas of deposition were evaluated quantitatively by measuring the intensity of the positive areas in 20 selected glomerular sections by evaluation using NIH Image software.

Double-labeling experiment between α-smooth muscle actin and other components. Fresh frozen tissue sections were fixed in acetone and stained indirectly according to a modified method previously reported (21). The sites of the antigen-antibody reaction were revealed by incubating each section with a sheep antibody against human factor V, a goat anti-rabbit fibrinogen, or a goat anti-rabbit fibronectin antibody followed by biotinylated IgG of secondary antibody. The preparations were washed two times in Tris-buffered saline (pH 7.4) and incubated with a monoclonal anti-human α-smooth muscle actin (α-SMA; DAKO, Glostrup, Denmark), rabbit anti-mouse immunoglobulins (DAKO), and mouse APAAP complex (DAKO). Color was then developed by incubation with a DAB Substrate kit (Pierce) and a fast red dye (DAKO).

Immunofluorescence staining for TGF-β and CTGF. Tissue sections frozen in OCT compound (Miles Laboratories, Elkhart, IN) were cut into serial sections on a cryostat and fixed in acetone for 5 min at room temperature. The detection of TGF-β was performed by an indirect method using a goat anti-human TGF-β antibody (Cortex Biochem, San Leandro, CA) or a goat anti-human CTGF antibody (GT, Minneapolis, MN). Next, FITC-labeled rabbit anti-goat IgG (ICN) was used as the second antibody. The grades of deposition were evaluated quantitatively by measuring the intensity of the fluorescence in glomerular areas with Photoshop 6.0 (Adobe, San Jose, CA) and graded from 0 to 2.55.

Expression of factor V, collagen type IV, fibronectin, TGF-β, and CTGF mRNA for Northern blotting. Immediately after excision, kidneys were placed in 1.0 M vanadate ribonuclease complex-added PBS. Glomeruli were isolated by graded sieving, as mentioned earlier. The method of Northern blotting was described previously (28). Briefly, total RNA was extracted from a pellet of glomeruli that was homogenized with a Polytron (Kinematica) by the acid guanidinium thiocyanate-phenol-chloroform method (5). The pool of RNA from isolated glomeruli of all rats in each group was used in one lane. RNA (10 μg) was denatured and electrophoresed in a 1% agarose-formaldehyde gel and transferred to nylon membranes (Biolyne PALL, Glen Cove, NY) by capillary action.
The cDNA probes used were factor V (a human 1.8-kb cDNA fragment, from Dr. W. H. Kane of the Duke University Medical Center), TGF-β (a mouse 0.96-kb cDNA fragment, from Dr. C. Shiota of the Wakayama Medical University, Wakayama, Japan), CTGF (a mouse 1.0-kb cDNA fragment, from Dr. G. R. Grotendorst of the Miami School of Medicine), type IV collagen (a mouse 0.83-kb cDNA fragment, from Dr. Y. Yamada of the National Institutes of Health), fibronectin (a mouse 2.3-kb cDNA fragment, a plasmid from Japanese Collection of Research Bioresources), and GAPDH (a human 1.2-kb cDNA fragment; ATCC). Hybridization of the RNA-transferred nylon membranes was carried out at 65°C overnight, with each cDNA probe radiolabeled by the random primer method (Boehringer Mannheim Biochemica) with [α-32P]dCTP (DuPont, Boston, MA). Autoradiography films were scanned using the BAS 2000 system (Fuji Film, Tokyo, Japan). For quantitative densitometric measurements of Northern blots, all the signals were normalized in comparison with the signal for GAPDH.

Immunoelectron microscopy. Part of the biopsy specimen was fixed in periodate-lysine-paraformaldehyde according to the method of McLean and Nakane (24). After being washed with different concentrations of sucrose solutions (10, 15, and 20%), the specimen was frozen in OCT compound and sectioned (6 µm) on a cryostat (29). These sections were etched by 0.3% hydrogen peroxide for 15 min. After being washed with PBS, the sections were treated with 10% normal serum, followed by incubation with sheep antibody against human factor V (Cedarlane Laboratories) or a goat anti-rat fibrinogen (ICN) at 4°C overnight. After washing, further incubation followed with biotinylated IgG for 3 h. The sections were then reacted with avidin-DH-biotinylated horseradish peroxidase complex (Vector Laboratories) for 3 h, and color was developed as described above. Sections were postfixed in 2% osmic acid and embedded in Epon 812 (Oken Shoji), and ultrathin sections were cut. Observations were made using a HITACHI 7100 electron microscope (Tokyo, Japan).

Statistical analysis. Values are represented as means ± SE. Statistical analysis of differences between the groups was performed by ANOVA and subsequent t-testing with Bonferroni correction for multiple comparisons. Differences at P < 0.05 were considered statistically significant.

RESULTS

Influence of RAHu/FV on prothrombin time. In the evaluation of the neutralizing effect of RAHu/FV against procoagulant activity of factor V, significantly prolonged prothrombin time was observed in RAHu/FV addition compared with control in PBS addition (RAHu/FV, 19.0 ± 1.5; control, 13.8 ± 1.6 s; P < 0.05).

Reaction of human and rat plasma for RAHu/FV and iNOS antigen expression in glomeruli. To confirm the reactivity of RAHu/FV with rat plasma factor V, both human and rat samples of plasma were analyzed by Western immunoblotting (Fig. 1A). Changes in glomerular iNOS expression were detected on day 1 after the injection of RAHu/FV. The specific bands could be clearly detected by immunoblotting both from the disease control group and the RAHu/FV-injected high-dose group after 1 day of intravenous injection with ATS (Fig. 1B). In contrast, very low levels of iNOS protein could be detected from the normal group. By densitometric analysis, markedly elevated levels of iNOS expression were confirmed both in disease control and the treatment groups, reaching 5.7- and 5.4-fold of normal levels (each P < 0.01), respectively, although no significant difference was observed between the former two groups on day 1 (Fig. 1C).

Time course of factor V, FRA, and total ECM deposition in rat MsPGN. Figure 2 shows that glomerular factor V, FRA, and ECM deposition increases after ATS injection. From day 1 until at least day 15, factor V, FRA, and ECM stainings were significantly increased compared with the normal group (each P < 0.001). Mesangial factor V expression was prominently observed on day 3, and FRA deposition was massive on day 3 to day 5; next, ECM accumulation followed on day 3 to day 8. The glomerular factor V staining was detected intensely mainly in the mesangium and partially along capillary loops and was observed intensely in the disease control group on day 3 but was negative in the normal group (Fig. 3). As shown in Fig. 2.
3D, the factor V mRNA expression by Northern blotting showed a marked increase in the disease control group on day 3 compared with the normal control (day 0); on the other hand, the mRNA expression of factor V was decreased on day 8. By immunoelectron microscopy, intense factor V presence was confirmed in the cytoplasm of mesangial cells on day 3; FRA deposition was seen mainly in the mesangium and partially in the capillary lumen on day 8 (Fig. 4).

**PCNA expression and macrophage infiltration after RAHu/FV injection.** PCNA- or ED-1-positive cells were significantly augmented in glomeruli in the disease control group compared with those of normal rats (*P* < 0.01). RAHu/FV treatment did not decrease the number of PCNA-positive cells (normal, 0.08 ± 0.02; control, 0.22 ± 0.03; low dose, 0.19 ± 0.01; and high dose of RAHu/FV, 0.17 ± 0.04; cells/glomerular cross section) or ED-1-positive cells (normal, 0.14 ± 0.07; control, 0.92 ± 0.19; low dose, 0.70 ± 0.12; and high dose of RAHu/FV, 0.90 ± 0.16; cells/glomerular cross section).

**FRA deposition after RAHu/FV injection.** Inhibitory effects of RAHu/FV on FRA deposition are shown in Fig. 5. Immunohistochemical study revealed massive FRA depositions in

Fig. 4. Ultrastructural distribution of factor V and FRA viewed by immunoelectron microscopy in glomeruli. A: factor V was observed in the cytoplasm of mesangial cells (arrows). B: FRA deposition mainly in the mesangium (arrows) and partially in the capillary lumen (arrowheads). Final magnification ×2,500.

Fig. 5. Inhibitory effects of RAHu/FV on FRA deposition on day 8. Compared with normal (A), FRA deposition was detected intensely in the disease control group (B) and RAHu/FV group [low dose (C) and high dose (D)] at day 8. Magnification ×270. E: significant effects of the treatment of RAHu/FV injection in the suppression of FRA deposition were observed in a dose-dependent manner. ***P < 0.001 vs. disease control group (*n* = 6 rats/group).
the mesangium, and segmentally along capillary loops in glomeruli in the disease control group, markedly higher than that in the normal group, increased to 16.7-fold \((P < 0.001)\). The staining intensity was decreased to 86 and 44\% \((P < 0.001)\) of the disease control level in the dose of 0.1 and 0.5 mg IgG/kg, respectively.

**Effects of RAHu/FV injection on total matrix accumulation.**

Figure 6 shows total ECM accumulation in glomeruli from the ATS-injected control group; the levels were markedly higher than that in the normal group and increased to 2.2-fold \((P < 0.001)\). The area of ECM staining was decreased to 81 and 63\% of the control at the dose of 0.1 and 0.5 mg IgG/kg RAHu/FV \((P < 0.01 \text{ and } P < 0.001, \text{ respectively})\).

**Inhibitory effects of RAHu/FV on fibronectin and collagen IV deposition.**

Fibronectin and collagen IV depositions were detected intensely in the mesangium in the disease control group; the levels were markedly higher than that in the normal group and increased to 3.9- and 11.9-fold \((P < 0.001)\), respectively. They were suppressed by the RAHu/FV injection. Results of the quantitative image analysis of immunoperoxidase staining for fibronectin and collagen IV are shown in Fig. 7. Compared with the disease control group, the staining areas of fibronectin and collagen IV were significantly smaller in the RAHu/FV-injected group \([\text{control, } 1,056.6 \pm 63.9, 390.5 \pm 22.1 (100\%); \text{low dose of } 0.1 \text{ mg/kg, } 790.8 \pm 55.4 (75\%, P < 0.05), 370.9 \pm 16.9 (94\%); \text{high dose of } 0.5 \text{ mg/kg, } 486.3 \pm 36.1 (46\%, P < 0.001), 197.4 \pm 9.3 \mu \text{m}^2 (50\%, P < 0.001), \text{ respectively}]\).

**Urinary findings.**

The amount of urinary protein was decreased significantly in the RAHu/FV-injected group at a dose of 0.5 mg/kg IgG compared with the disease control group \([\text{normal, } 13.8 \pm 2.0; \text{disease control, } 59.1 \pm 14.5; \text{RAHu/FV injected, } 23.4 \pm 3.8 \text{ mg/day}, P < 0.05 \text{ vs. disease control; Fig. 8}]\).

**Double-labeling experiments between α-SMA and other components.**

Double-labeling experiments between α-SMA and factor V (Fig. 9A), between α-SMA and FRA (Fig. 9B), or between α-SMA and fibronectin (Fig. 9C) revealed their frequent colocalization in the same mesangial areas of glomeruli.

**Glomerular TGF-β and CTGF staining.**

The glomerular TGF-β and CTGF staining was detected intensely, mainly in the mesangial areas and partially along capillary loops in the
disease control group and weakly in the normal group. As shown in Fig. 10, RAHu/FV injection significantly decreased glomerular TGF-β deposits obtained by quantitative analysis density (disease controls, 20.6 ± 3.0; and high dose of 0.5 mg/kg, 13.3 ± 1.3; P < 0.05). Figure 11 shows CTGF deposition in glomeruli from the ATS-injected control group; the levels were markedly higher than that in the normal group (disease controls, 26.3 ± 0.7; and high dose, 17.3 ± 1.0; P < 0.01).

Northern blot analysis of collagen type IV, fibronectin, TGF-β, and CTGF mRNAs in glomeruli. The collagen type IV fibronectin mRNAs apparently appeared in the disease control group in isolated glomeruli. These expressions were suppressed markedly in the RAHu/FV-injected high-dose group. The TGF-β and CTGF mRNA expressions were also upregulated in the disease control group and markedly suppressed those expressions in the RAHu/FV-injected high-dose group (Fig. 12).

DISCUSSION

Human MsPGN, which progresses to end-stage renal failure, is histologically characterized by a marked mesangial increase and expansion of ECM in the glomeruli (33). Intraglomerular coagulation was suggested to be involved in the development of glomerular injury (17, 37). Various coagulation factors were previously observed in the glomeruli of renal biopsy specimens (11, 19, 29, 30). Glomerular expression of TF, the principal initiator of the extrinsic coagulation pathway, is known to be upregulated in human and rabbit crescentic glomerulonephritis (35, 36). Previously, we observed the close relationship between factor V expression and mesangial cell proliferation in IgA nephropathy (21). In that study, using human renal biopsy specimens from nearly normal or diseased tissue, we showed that, in the physiological state, factor V expression was weak or absent in glomeruli and was upregulated in the active type of IgA nephropathy. Because factor V staining was intense and frequent in the proliferating and/or necrotizing areas in IgA nephropathy, factor V expression is likely to be cytokine induced, and infiltrating macrophages are likely to be the cytokine source. In our recent in vitro study, we observed that the coagulation process proceeds on the surface of cultured mesangial cells via expression of factor V after the stimulation of inflammatory cytokine tumor necrosis factor-α with exogenous factor Xa (31).

In the present study using ATS-induced rat MsPGN, we found that fibrin deposition was advanced mainly in the glomerular mesangium, and partially along the capillary loops, together with PCNA expression and macrophage infiltration. Although the glomerular factor V staining was negative in the normal group, it was intensely detected in the mesangium early in the disease control group; next, fibrin was increased, and then ECM was accumulated. In addition, Northern blotting in glomeruli revealed that factor V mRNA expression was upregulated in the disease control groups, in contrast to the scarce expression in the normal group. Double-labeling experiments

Fig. 8. Effect of RAHu/FV on 24-h urinary protein excretion from day 3 to day 4. Urinary protein excretion was significantly lower in the RAHu/FV high-dose group. *P < 0.05 vs. disease control (n = 6 rats/group).

Fig. 9. Double-labeling experiments between α-smooth muscle actin (SMA) and factor V, α-SMA and FRA, or α-SMA and fibronectin in the same glomerulus on day 8. In the same mesangial areas, α-SMA (red) is colocalized together with factor V (A), FRA (B), and fibronectin (C; dark brown). The boxed areas are shown at a higher power in B, D, and F. Magnification: A, C, and E, ×280; B, D, and F, ×600.
between α-SMA with factor V, FRA, or fibronecin in the section from the disease control group revealed their frequent colocalization in the same mesangial areas of glomeruli. Because Alpers et al. (1) demonstrated a correlation between mesangial α-SMA expression and cell proliferation in glomeruli, colocalization of these components with α-SMA antigen confirms that mesangial cells are the cell of origin of factor V production in glomeruli, that fibrin production proceeded on mesangial cell surface, and that ECM proteins coexisted with fibrin. These results show that, after factor V expression and ATS injection, fibrin deposition and ECM accumulation proceed. Mesangial cells are known to express TF by inflammatory cytokines (38). TF was also detected in the mesangium in the present MsPGN in vivo model (data not shown). In active MsPGN, factor X activation to Xa may occur through TF expression in mesangial cells. Mesangial cells, which are adjacent to fenestrated endothelium in vivo and are supplied by plasma flow from blood circulation through fenestration of glomerular capillary endothelium (20), can activate prothrombin through endogenous factor V production together with fibrin.

Fig. 10. Glomerular transforming growth factor (TGF)-β staining on day 8. Mesangial TGF-β expression was detected intensely in the disease control group (B) and decreased by 0.5 mg IgG/kg in the RAHu/FV-injected group (C), in contrast to the weak expression in the normal group (A). Magnification ×300. D: *P < 0.05 vs. disease group (n = 6 rats/group; 15 glomeruli/rat).

Fig. 11. Glomerular connective tissue growth factor (CTGF) staining on day 8. Mesangial CTGF expression was detected intensely in the disease control group (B) and decreased by 0.5 mg IgG/kg in the RAHu/FV-injected group (C) in contrast to the weak expression in the normal group (A). Magnification ×300. D: **P < 0.01 vs. disease group (n = 6 rats/group; 15 glomeruli/rat).
exogenous factor Xa. Therefore, we have described our findings as “coagulation in the mesangial area,” which is often noted in MsPGN.

In the present study, characteristics of RAHu/FV as neutralizing antibody against rat plasma were confirmed both on the procoagulant activity by prothrombin time measurement and on the antigenicity by Western blotting. Therefore, it is obvious that RAHu/FV may act as a neutralizing antibody in vivo when it is administered intravenously to rats. Intra-venous administration of the anti-factor V antibody, RAHu/FV, induced a decrease in fibrin deposition together with a decrease in ECM proteins in the present study. Factor V, the cofactor for factor Xa, is abundantly available in circulation. It is assumed that blockade of mesangial factor V expression is important, but blockade of circulating factor V is also likely to be relevant. With regard to the role of factor V in mesangial cells, it is comparable to that of TF, which also acts on their membrane surface. The active site of factor V is situated in the final stage of prothrombin activation, in contrast to TF, which initiates the cascade. Erlich et al. (7) reported that infusion of recombinant human TF pathway inhibitor significantly reduced the development of glomerular fibrin deposition, even when administered intravenously. Recently, Dale et al. (6) reported that stimulated platelets use serotonin conjugation to augment the retention of exogenous factor V in vitro.

The sequence of events from the decreased fibrin deposition through factor V inhibition to the decreased matrix accumulation should be clarified. Yamabe et al. (39) reported that thrombin stimulates TGF-β production by human mesangial cells, and TGF-β was reported to increase various ECM proteins (2). CTGF is a profibrotic growth factor and is a member of the CCN family of immediate early genes, which is upregulated in renal fibrosis (14). CTGF expression is upregulated by TGF-β, as a downstream mediator of TGF-β (13). It is tempting to speculate that production of ECM components is upregulated through TGF-β and CTGF expression because of thrombin activation. In contrast to the efficacy on ECM accumulation, RAHu/FV did not suppress cell proliferation. Block of thrombin generation using anti-factor V antibody could inhibit further coagulation cascade, but may not be efficient in suppressing mesangial cell proliferation, because factor Xa alone could initiate the mitogenic process (26). As another reason, platelets are rich in growth factors, such as platelet-derived growth factor, which may promote mesangial cell proliferation in Thy-1 nephritis (15), and anti-factor V treatment might have a weak effect on platelet influx in this model.

In ATS-induced glomerulonephritis, the maximum proteinuria value varies from rat strain to rat strain. The Goto-Kakizaki rat is known to be protected from proteinuria after the administration of anti-Thy-1 antibody (34). Wistar rats were thus considered in this model as appropriate, and RAHu/FV decreased proteinuria in this study. This may be the result of decreased ECM accumulation. In conclusion, the findings of

Fig. 12. Analysis of mRNA expression in isolated glomeruli. A: Northern blot analysis of type IV collagen, fibronectin, TGF-β, and CTGF mRNA levels in the pool of isolated glomeruli from the normal group, the disease control group, and 0.5 mg IgG/kg of the RAHu/FV-injected group. The levels of GAPDH mRNA served as the housekeeping gene. Glomerular mRNA levels of type IV collagen (B), fibronectin (C), TGF-β (D), and CTGF (E) were decreased in the RAHu/FV-injected group compared with the upregulated expression in disease controls for data on all blots. We repeated this experiment two times and essentially obtained the same results. Representative data are shown.
the present study demonstrated that anti-coagulant treatment attenuates the glomerular mesangial deposition of not only fibrin but also ECM components. The present findings suggest that ECM component accumulation may progress partly in accordance with coagulation in the mesangial area through mesangial factor V expression in MsPGN.

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