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Constitutive Expression and Modulation of the Functional Thrombin Receptor in the Human Kidney

Yichun Xu,* Ute Zacharias,* Marie-Noëlle Peraldi,* Ci-Jiang He,* Changyong Lu,† Jean-Daniel Sraer,* Lawrence F. Brass,‡ and Eric Rondeau*

From the INSERM U 64 and Association Claude Bernard, Tenon Hospital, Paris, France; INSERM U 120,† Robert Debré Hospital, Paris, France; and the Hematology-Oncology Section,‡ University of Pennsylvania, Philadelphia, Pennsylvania*

Thrombin exerts procoagulant effects and has also many cellular effects mediated by cell surface receptors. A functional thrombin receptor from human platelets has been cloned and sequenced. In the present study, by reverse transcription and polymerase chain reaction, using specific primers designed from the thrombin receptor cDNA sequence, we show that the mRNA encoding for this receptor can be amplified from freshly isolated human glomeruli obtained by microdissection of normal kidney cortex. By immunohistochemistry using a specific monoclonal antibody, ATAP2, directed against the extracellular N-terminus of this receptor, we find that this functional thrombin receptor is constitutively expressed in the normal human kidney. The three glomerular cell types, endothelial, mesangial, and epithelial cells, were positively stained, as were the endothelial cells of renal arteries, arterioles, venules, and peritubular capillaries. Occasionally, interstitial cells and smooth muscle cells in the media of renal arteries were also stained. Proximal and distal tubular cells were not stained. By* in situ *hybridization, using a digoxigenin-labeled cDNA probe specific for thrombin receptor, the thrombin receptor mRNA was found to have the same distribution as the thrombin receptor protein detected by immunohistochemistry. A lighter staining of glomerular endocapillary cells was observed in cases of thrombotic microangiopathy and extracapillary glomerulonephritis, two renal diseases associated with* in situ *thrombin generation and fibrin formation. In one case of thrombotic microangiopathy, we observed an increase in thrombin receptor mRNA. This suggests that thrombin receptor protein is not always correlated with thrombin receptor mRNA level. Internalization and degradation of thrombin receptor protein have been demonstrated* in vitro *and could also occur after activation* in vivo. *This is the first demonstration of the constitutive expression of the functional thrombin receptor in the human kidney. These results suggest that thrombin may exert glomerular and vascular effects within the kidney in normal and in pathological conditions. (Am J Pathol 1995, 146:101–110)

Apart from its procoagulant activity, thrombin possesses a variety of cellular effects[1] upon inflammatory cells,[2] platelets,[3] vascular smooth muscle cells,[4,5] and endothelial cells.[6–8] These effects seem to be mediated through a single class of thrombin receptor that belongs to the G protein-coupled receptor family. A cDNA encoding for this functional thrombin receptor has been isolated.[9] Interestingly, receptor activation occurs when thrombin cleaves the extracellular N-terminus of the receptor, exposing a tethered ligand.[10] Synthetic peptides containing the initial residues of the neo-N-terminus are able to activate the thrombin receptor.[11] Monoclonal antibodies raised against these synthetic peptides have been shown to inhibit platelet activation by thrombin.[12]

Many cases of experimental and human glomerulonephritis are associated with fibrin deposition, in-

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Address reprint requests to Dr. Eric Rondeau, INSERM U 64, Hôpital Tenon, 4 rue de la Chine, 75020 Paris, France.

filtration by inflammatory cells, and proliferation of intrinsic glomerular cells, ie, endothelial, mesangial, and epithelial cells.[13] We and others have previously shown that human glomerular mesangial and epithelial cells in culture possess thrombin receptors and proliferate in response to thrombin.[14–18] However, to date, direct evidence of thrombin receptor expression *in vivo* in the human kidney has not been provided. In the present report, using *in situ* reverse transcription-polymerase chain reaction (RT-PCR), immunohistochemical methods, and *in situ* hybridization, we demonstrate that the thrombin receptor is expressed *in vivo* in the normal human kidney, in the glomerulus, in endothelial cells of intrarenal vessels, and in some interstitial cells. Furthermore, we show that the pattern of expression of thrombin receptor antigen is modified in fibrin-associated glomerular diseases such as thrombotic microangiopathy and extracapillary glomerulonephritis.

Materials and Methods

Normal human kidney specimens were obtained by nephrectomy performed for adenocarcinoma (n = 7). Percutaneous renal biopsies were also studied. They were obtained from patients with hemolytic and uremic syndrome and biopsy-proven thrombotic microangiopathy (n = 7) and from patients with crescentic glomerulonephritis (n = 7). The histological diagnosis was performed by conventional light microscopy and immunofluorescence studies. Kidney samples were frozen in liquid nitrogen immediately after nephrectomy or biopsies for immunohistochemical and *in situ* hybridization studies or fixed in Dubosc Brazil solution for conventional light microscopy analysis. It was not ensured that the control tissues used for the study had normal histology.

Preparation of cDNA Probe for Thrombin Receptor

The human thrombin receptor cDNA probe was prepared by a RT-PCR technique. Specific thrombin receptor primers, 20 mer, were designed according to the previously published cDNA sequence[9]: sense, 5′-268-TGTGCGGCC CGCTGTTGTCT-288–3′; anti-sense, 5′-1120-TGCGGTTGGCAACTGCG-GAA-1140–3′). They were synthesized on an Applied Biosystem DNA synthesizer model 380 with phosphoramidate chemistry. After detritylation and alkaline deprotection, crude material was used for all applications without further purification. Total RNA from cultured human glomerular epithelial cells, which we have shown to express a functional thrombin receptor,[17,19] was reverse transcribed with a cDNA synthesis kit (Boehringer Mannheim, Indianapolis, IN). PCR amplification of the cDNA was performed with 50 pmoles of each primer, 10 μl of 10X buffer (100 mmol/L Tris-NaCl pH 8.3, 50 mmol/L KCl, and 35 mmol/L $MgCl_2$), 2 μl of 25 mmol/L dNTP, and 2.5 U of Taq polymerase in 100 μl of sterile distilled water overlaid by mineral oil. The size of the amplified product used as a probe was 872 bp as predicted from the cDNA sequence of thrombin receptor.[9] Its identity with thrombin receptor cDNA was confirmed by restriction enzyme analysis and DNA sequencing of a 200-bp fragment by the dideoxynucleotide chain termination method.

Amplification of Thrombin Receptor mRNA from Isolated Glomeruli by In Situ *RT-PCR*

Glomeruli were microdissected from cortical fragments taken at a distance from the tumor as described by Peten et al.[20] In brief, cortical fragments were placed at 4 C in an RNAse inhibitor solution (vanadyl ribonucleoside complex; Life Technologies, Gaithesburg, MD) in a microdissecting dish cooled at 4 C and glomeruli were separated from tubules. At least 10 to 30 glomeruli were isolated from each kidney. Five washed isolated glomeruli, free of tissue debris and vanadyl ribonucleoside complex, were transferred to a PCR tube containing a human placental RNAse inhibitor (Boehringer Mannheim) and permeabilized immediately before RT with a mixture containing 0.9% Triton X-100, 1.2 U/μl of placental RNAse inhibitor, and 5 mmol/L dithiothreitol. RT was performed with a cDNA synthesis kit (Boehringer Mannheim) as described.[20] PCR amplification of glomerular cDNA was performed as for preparation of the cDNA probe. The entire reaction mix was separated in a 4% agarose gel by electrophoresis and DNA bands were detected with ethidium bromide staining and UV trans-illumination. DNA molecular weight markers (DNA-MWM III; Boehringer Mannheim) were run simultaneously in the same gel. Controls consisted of tubes containing rat glomerular cDNA also obtained by microdissection and *in situ* RT and cDNA obtained from total RNA of HepG2 cells, which are derived from a human hepatoma and which have been shown to be a target for thrombin.[21] With these primers, no amplification of thrombin receptor cDNA could be obtained from purified genomic human DNA. With another set of primers specific for the housekeeping gene GAPDH, a single band could be

amplified from both rat and human glomeruli ensuring the quality of glomerular RT products (not shown).

Immunohistochemical Studies

Thrombin receptor was detected by using ATAP2, a monoclonal antibody that recognizes an epitope within the initial eight residues (SFLLRNPN) of the neo-N-terminus.[22] The antibodies were detected by the alkaline phosphatase-anti-alkaline-phosphatase complex (DAKO PATTS) technique.[23] In brief, 5-μ cryostat sections were first incubated with the specific monoclonal anti-thrombin receptor antibody, then with polyclonal rabbit anti-mouse immunoglobulins and alkaline phosphatase-anti-alkaline phosphatase mouse complex. The enzyme was revealed with Naphtol-AS-phosphate and Fast red TR (Sigma Chemical Co., St. Louis, MO). Sections were then counterstained with hematoxylin. In control experiments, the primary antibody was omitted or preincubated with an excess of 14-mer (SFLLRNPNDKYEPF) synthetic peptide. The intensity of staining detected in kidney glomeruli, vessel tubules, and interstitium was semiquantitatively graded as follows: –, no staining; 1+, light; 2+, moderate; and 3+, strong staining. Scores of patient biopsies were determined by comparison with normal kidney tissues, which were studied at the same time and which exhibit a very reproducible staining.

In Situ *Hybridization*

In situ hybridization was performed by using a digoxigenin-labeled thrombin receptor cDNA probe as previously described,[24,25] with slight modifications. The cDNA probe was labeled by digoxigenin-11 UTP incorporation with a DIG DNA labeling kit (Boehringer Mannheim). Slides were first rinsed in alcohol, dried at room temperature, baked at 180 C for 2 hours and stored dust-free at room temperature. Cryostat tissue sections (8 μ) were cut, placed on slides, and fixed in 4% formaldehyde in phosphate-buffered saline for 10 minutes, rinsed in phosphate-buffered saline, and then dehydrated in graded alcohols and air dried. Slides were stored at –20 C until analyzed.

For hybridization, the sections were first prehybridized with a mixture containing 4X SSC (3 mol/L NaCl, 0.3 mol/L trisodium citrate for 20X), 1X Denhart (2% bovine serum albumin, 2% Ficoll, 2% polyvinylpyrrolidone for 100X), 50% deionized formamide, 250 μg/ml yeast RNA, 500 μg/ml salmon sperm DNA, and 5% dextran in a wet chamber at 37 C for 1 hour. Hybridization was then carried out in the same mixture as prehybridization plus 0.26 ng/μl digoxigenin-labeled thrombin receptor probe. After denaturation of cDNA probe by heating at 100 C for 5 minutes 10 μl of hybridization solution was applied to each section and then the sections were covered by siliconed coverslips. Hybridization was performed in a wet chamber at 40 C overnight. After the hybridization, the sections were successively washed in 40% formamide 4X SSC and then 2X SSC at room temperature for 2 hours.

For the reaction of anti-digoxigenin antibodies to the digoxigenin, slides were preincubated in buffer A (100 mmol/L Tris-HCl, 150 mmol/L NaCl, pH 7.5) containing 2% sheep serum at 37 C for 1 hour and then incubated with 3 U/ml anti-digoxigenin antibodies coupled with alkaline phosphatase in buffer A at 4 C overnight. The slides were then washed in buffer B (100 mmol/L Tris-HCl, 100 mmol/L NaCl, and 50 mmol/L $MgCl_2$, pH 9.5). Alkaline phosphatase was then revealed by an alkaline phosphatase substrate buffer containing 0.15 mg/ml 5-bromo-4-chloro-3-indolyl phosphate 0.30 mg/ml nitroblue tetrazolium, 100 mmol/L Tris buffer, and 1 mmol/L $MgCl_2$. The enzymatic reaction was stopped by buffer C (10 mmol/L Tris-HCl, 1 mmol/L EDTA). Negative controls were performed by hybridization of the sections with digoxigenin-labeled pBR 328 DNA or incubation without the digoxigenin-labeled thrombin receptor probe.

Results

RT-PCR detection of thrombin receptor mRNA in microdissected glomeruli

As shown in Figure 1, a single band of cDNA was amplified by PCR from cDNA obtained from human isolated glomeruli after RT. It had the expected 872-bp molecular weight as determined by comparison with molecular standards. A similar band was obtained by the same method from HepG2 cells, a hepatoma cell line also expressing thrombin receptors.[21] In contrast, no cDNA could be amplified from rat isolated glomeruli, due to species differences in the thrombin receptor sequence at the sites of primer hybridization.[26] This negative result also assured lack of PCR contamination. No band could be amplified from human genomic DNA (not shown). To determine whether the expression of thrombin receptor mRNA *in vivo* was associated with thrombin receptor protein synthesis and to identify which cells exhibit this receptor, immunohistochemical and *in situ* hybridization studies were performed on normal kidney

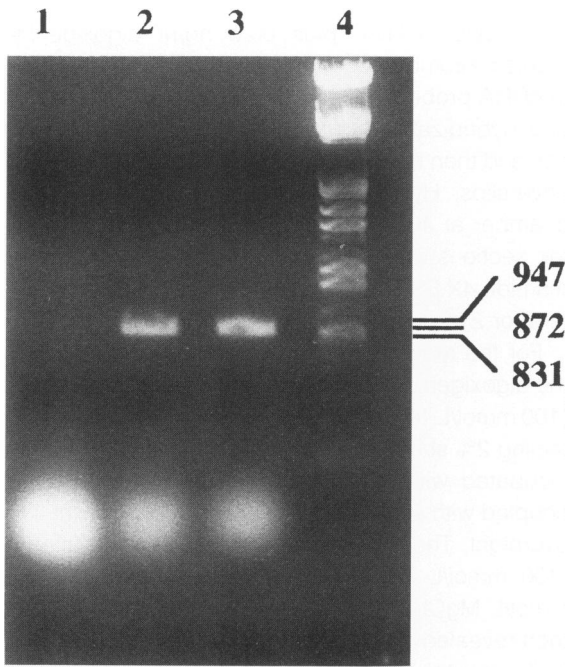

Figure 1. *Expression detection of the human thrombin receptor mRNA by RT-PCR. Five microdissected glomeruli obtained from normal rat* (lane 1) *and human* (lane 3) *kidney were used for* in situ *RT and PCR amplification. Total RNA (5 μg) extracted from HepG2 cells* (lane 2) *were also reverse transcribed and used for PCR amplification of the human thrombin receptor cDNA fragment. Products of amplification were separated in a 4% agarose gel electrophoresis and their molecular weights were determined by comparison with DNA molecular weight markers. The amplified product (expected mw, 872 bp) is found between the bands corresponding to 947 and 831 bp of the DNA mw markers.*

sections and on renal biopsies of patients with thrombotic microangiopathy and with proliferative glomerulonephritis.

Immunohistochemical Studies

As shown in Figure 2, thrombin receptor was detected in the normal human kidney. It was mainly located at the glomerulus, in endothelial, mesangial, and visceral epithelial cells as well as in parietal glomerular epithelial cells (Figure 2A, B). Proximal and distal tubular cells were negative. The endothelial cells lining renal arteries and arterioles were also positively stained (Figure 2B, C). In some arteries and arterioles, a small staining could also be detected in the myocytes of the media (Figure 2C). Occasionally, interstitial peritubular cells were weakly stained. As shown in Table 1, the scores of staining of the glomeruli, vessels, and interstitium from the seven normal kidneys were similar.

Preincubation of the ATAP2 MAb with saturating concentrations of the 14-mer synthetic peptide, SFLLRNPNDKYEPF, completely suppressed immunostaining of the thrombin receptor on kidney sections (Figure 2D).

In Situ *Hybridization Studies*

As shown in Figure 3, by *in situ* hybridization with digoxigenin-labeled thrombin receptor cDNA probe, the thrombin receptor mRNA was detected in the normal human kidney. In accordance with immunohistochemical results, thrombin receptor mRNA was detected in glomerular cells and in endothelial cells of renal vessels (Figure 3A–C). In glomeruli, the staining was stronger in endocapillary cells than in epithelial cells. Proximal and distal tubular cells were negative as found by immunohistochemical studies. In the absence of the digoxigenin-labeled thrombin receptor probe or after hybridization with digoxigenin-labeled pBR 328 DNA, no signal was detected (Figure 3D).

Thrombin Receptor Expression in Renal Diseases

The kidney sections of two groups of patients (seven with thrombotic microangiopathy and seven with proliferative extracapillary glomerulonephritis) were examined by immunohistochemistry with the anti-thrombin receptor antibody and semiquantitatively scored as described in Materials and Methods (Table 1). In the seven cases of thrombotic microangiopathy, a decreased glomerular staining of endocapillary cells (mesangial and/or endothelial cells) but not of visceral epithelial cells (Figure 4A) was found when compared with normal glomeruli (Figure 2B and Table 1). Peritubular interstitial cells (fibroblasts, capillary endothelial cells, and mononuclear cells) were also stained (Figure 4A). By *in situ* hybridization, in the case with the more severe glomerular lesions, an increased expression of the thrombin receptor mRNA was observed in endocapillary cells and podocytes (Figure 4B) as well as in the peritubular cells (Figure 4C).

In the seven cases of proliferative extracapillary glomerulonephritis, a lighter staining of the endocapillary cells was also observed, compared with normal kidneys (Table 1). In addition, crescents, which consist of proliferating parietal epithelial cells and macrophages, were faintly stained (Figure 4D). An increased staining of periglomerular and peritubular cells was also observed (Figure 4E).

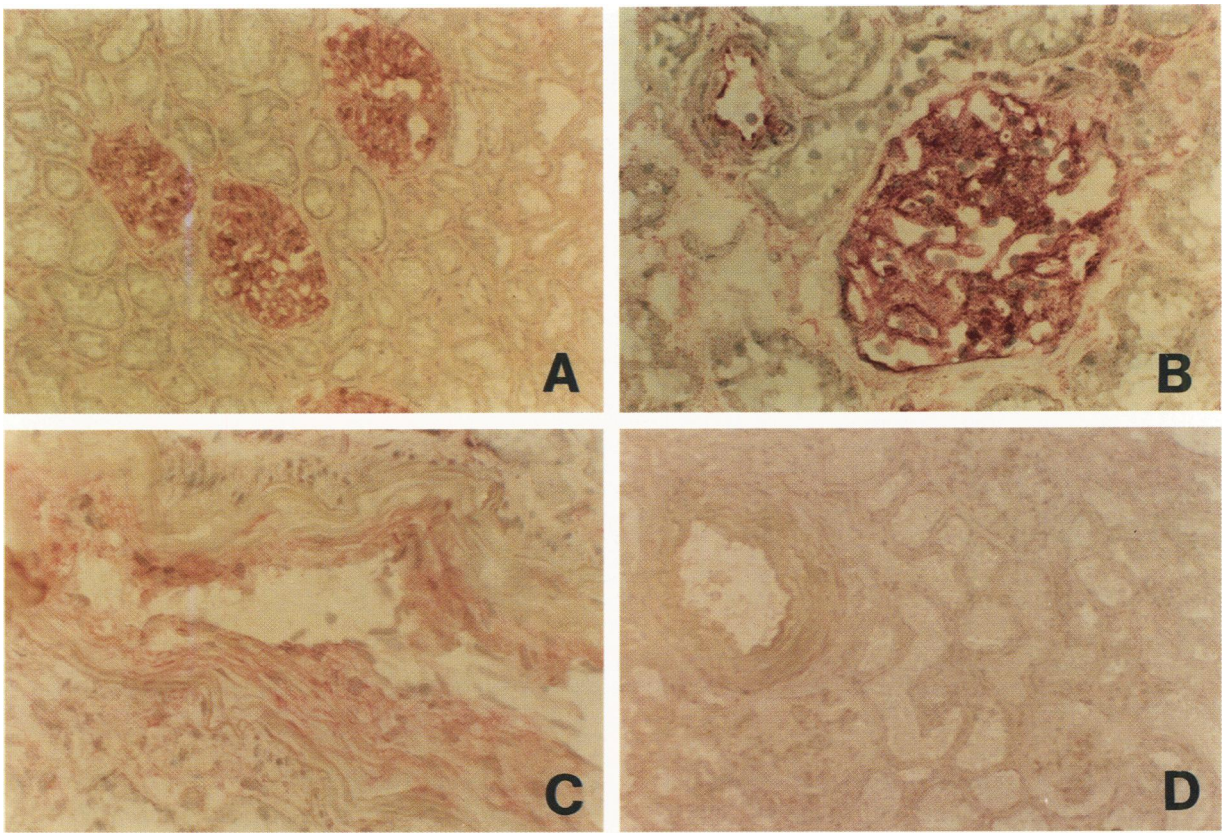

Figure 2. *Immunohistochemical detection of thrombin receptor antigen in the normal human kidney (alkaline phosphatase-anti-alkaline phosphatase complex technique). A strong staining (3+) was observed in the glomeruli (A; original magnification, × 100). All of the glomerular cell types (ie, endothelial, mesangial, visceral, and parietal cells) were positively stained (B; original magnification, × 250). Endothelial cells of arterioles and some peritubular interstitial cells were also positively stained (B). Tubular epithelial cells were always negative (B). Endothelial cells and smooth muscle cells of an artery with moderate intimal proliferation exhibited a positive staining with the thrombin receptor antibody (C; original magnification, × 250). Glomerular and vascular staining were completely suppressed by preincubation of the thrombin receptor antibody with an excess of 14-mer (SFLLRNPNDKYEPF) synthetic peptide identical to the N-terminus epitope of the thrombin receptor, which is recognized by ATAP2 antibody (D).*

Discussion

In this study, we report for the first time the expression of thrombin receptor in the human kidney by PCR amplification of the specific cDNA from isolated glomeruli, immunohistochemical localization of the protein, and localization of thrombin receptor mRNA by *in situ* hybridization. Immunohistochemical and *in situ* hybridization studies gave concordant results, except for glomerular visceral epithelial cells in which thrombin receptor mRNA signal was low or undetectable whereas thrombin receptor protein was clearly demonstrated. This may be related to a low expression of thrombin receptor mRNA in these cells and/or to a low sensitivity of the *in situ* hybridization method. Although unlikely, we cannot exclude that the visceral epithelial staining represents expression or trapping of released receptor peptides.

Until recently, almost all studies on thrombin receptor expression have been performed on platelets, monocytes, or lymphocytes or on cultured cells, mainly endothelial and smooth muscle cells.[5] This receptor was detected by functional assays and its exact localization *in vivo* was not known. Using *in situ* hybridization, Soifer et al[27] showed that thrombin receptor mRNA was expressed widely in mesenchymal cell populations during early mouse organogenesis, particularly in developing heart blood vessels and the germinal epithelium of the hindbrain. With time, thrombin receptor expression became more restricted and was found in some neurons and endocardial and endothelial cells and within lung and liver. In this study, however, the thrombin receptor protein was not analyzed nor was the expression of thrombin receptor mRNA within the kidney. Nelken et al[28] were the first to report thrombin receptor expression *in vivo* on tissue sections of normal and atherosclerotic human arteries using both *in situ* hybridization and immunohistochemistry. According to these authors, in normal appearing arteries, thrombin receptor was expressed exclusively in the endothelial layer. In human

Table 1. *Semiquantification of thrombin receptor antigen expression by immunochemical studies, in the human kidney, of normal and pathological conditions*

Group	Case	Subtype	Glomeruli			Arteries, arterioles		Interstitial cells
			Endocapillary cells	Podocytes	Crescents	Endothelial cells	Myocytes	
Normal	1		3+*	3+	0	3+	2+	1+
	2		3+	3+	0	3+	1+	1+
	3		3+	3+	0	2+	1+	1+
	4		3+	3+	0	2+	1+	1+
	5		3+	3+	0	2+	–	1+
	6		3+	3+	0	1+	–	1+
	7		2+	2+	0	2+	1+	1+
Thrombotic microangiopathy	1	G	1+	3+	0	0	0	2+
	2	G	1+	3+	0	3+	2+	2+
	3	G+A	1+	3+	0	3+	–	3+
	4	G	1+	2+	0	0	0	1+
	5	G	1+	3+	0	2+	–	2+
	6	G+A	1+	3+	0	0	0	2+
	7	A	1+	2+	0	2+	1+	1+
Proliferative extracapillary glomerulonephritis	1		1+	3+	3+	0	0	3+
	2		1+	3+	1+	2+	–	2+
	3		1+	3+	1+	2+	–	3+
	4		1+	2+	0	0	0	2+
	5		2+	2+	0	1+	–	2+
	6		2+	3+	1+	0	0	2+
	7		1+	2+	3+	0	0	2+

G and A, glomerular or arteriolar form of thrombotic microangiopathy, respectively.
* Sections were scored as follows: 0, structure not observed on the section used for immunohistochemical studies; –, no staining; 1+, light; 2+, moderate; and 3+, strong staining.

atheroma, the receptor was widely expressed in regions rich in macrophages and in vascular smooth muscle cells. Their results suggest that thrombin could play an important role in sclerotic and inflammatory processes in the human vasculature. Similarly, in the vessels of the human kidney, thrombin receptor is expressed by endothelial cells, and occasionally by smooth muscle cells in the renal arteries, suggesting that thrombin could play a role in renal vascular injury, such as chronic nephroangiosclerosis or acute thrombotic microangiopathy.

Numerous cases of glomerulonephritis are associated with fibrin deposition, infiltration of inflammatory cells, and proliferation of intrinsic glomerular cells.[13] A pathogenic role for thrombin in these glomerular lesions is likely as thrombin is required for fibrin formation and has chemotactic and mitogenic effects on monocytes and lymphocytes.[2,4] Furthermore, we and others have reported numerous effects of thrombin on cultured glomerular mesangial and epithelial cells.[14,17,18,29] Thrombin was shown to stimulate mesangial and epithelial cell proliferation and to induce the synthesis of plasminogen activators and plasminogen activator inhibitor type 1, resulting in a decreased fibrinolytic activity,[14,17] an effect that could inhibit local fibrinolytic activity and promote fibrin accumulation. Thrombin may also affect glomerular hemodynamics as it has been shown to increase endothelin gene expression and endothelin release by cultured human mesangial cells *in vitro*.[29] Furthermore, in bovine glomerular endothelial cells, thrombin as well as bradykinin, ATP, and platelet activating factor were shown to increase intracellular calcium concentrations and the release of endothelial-derived relaxing factor activity.[30] Finally, in rat mesangial cells, thrombin has been shown to induce cell proliferation and contraction and release of prostaglandins *in vitro*.[31] All of these *in vitro* results suggest the presence of functional thrombin receptors on glomerular cells. However, cell phenotypes in culture conditions may not always correlate with the more differentiated phenotype *in vivo*. Our present study clearly demonstrates that the functional thrombin receptor is expressed by glomerular cells in the normal human kidney. This result emphasizes the relevance of previous studies on the effect of thrombin on cultured glomerular cells and also establishes thrombin receptor activation as a candidate for contributing to sclerotic and inflammatory processes in the human kidney.

Thrombotic microangiopathy and extracapillary glomerulonephritis are the two main human kidney diseases associated with renal fibrin deposits.[13] Local thrombin generation induces fibrin formation and may also activate the cellular thrombin receptor. Interestingly, we found that the glomerular expression of thrombin receptor antigen at the cell surface of endocapillary cells was decreased in these cases. This decrease could correspond to a decreased synthesis of the receptor and/or to an increased degradation. *In*

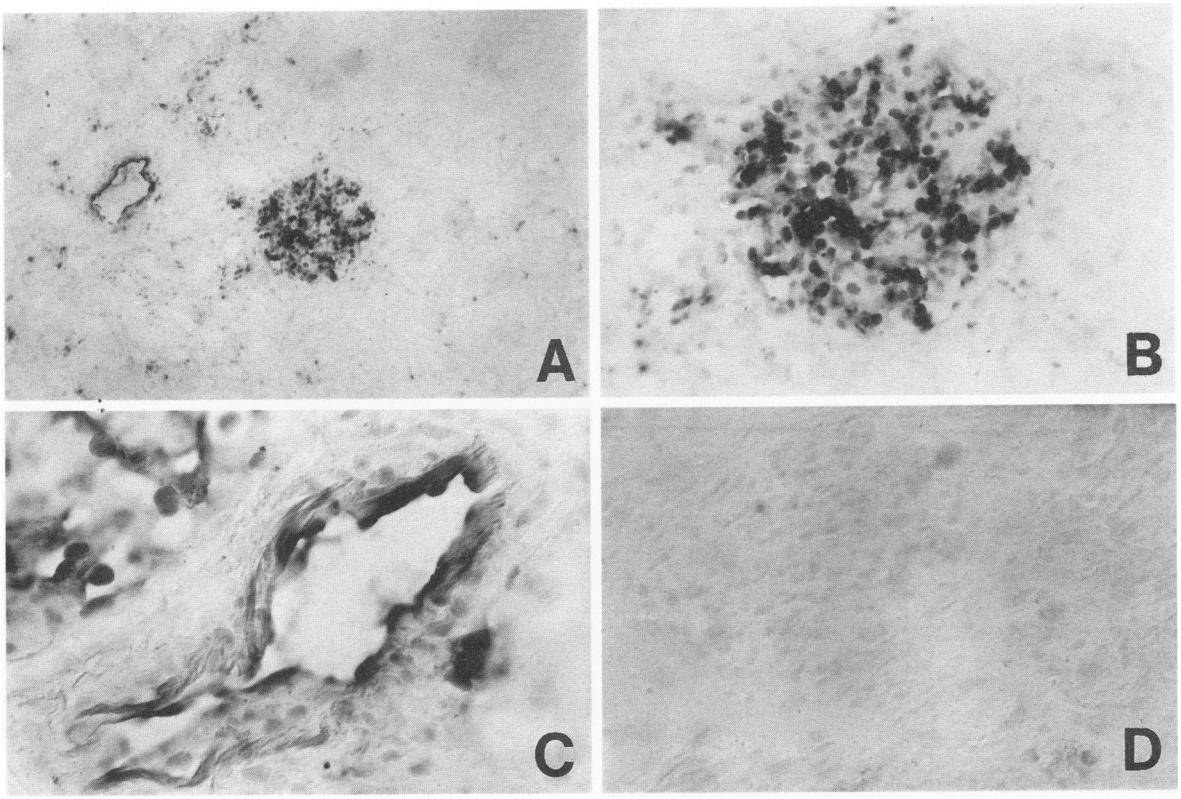

Figure 3. *Detection of the thrombin receptor mRNA by* in situ *hybridization in the normal human kidney. By* in situ *hybridization with a specific digoxigenin-labeled cDNA probe, the thrombin receptor mRNA was detected in the glomeruli* (**A** *and* **B**; *original magnification,* × *100 and* × *250, respectively). It was also detected in the endothelial cells of arterioles* (**A** *and* **C**) *whereas it was not found in the media. No signal was detected when* in situ *hybridization studies were performed with a digoxigenin-labeled pBR 328 DNA* (**D**) *or in the absence of the thrombin receptor probe* (*not shown*).

vitro, a rapid internalization and degradation of thrombin receptors have been shown to occur after addition of thrombin to HEL cells, a megakaryoblastic human erythroleukemia cell line that expresses the functional thrombin receptor.[22] After withdrawal of thrombin from the culture medium, thrombin receptor is re-expressed at the cell surface within hours. Internalization and degradation of thrombin receptor is likely to occur also *in vivo*. Importantly, the lower thrombin receptor protein expression was associated with an increased level of thrombin receptor mRNA in thrombotic microangiopathy, suggesting separate regulation of the receptor protein and the receptor mRNA at least in some pathological conditions. Zhong et al[26] reported an increased thrombin receptor mRNA after addition of basic fibroblast growth factor to rat vascular smooth muscle cells. Whether thrombin itself or cytokines or growth factors could increase the thrombin receptor gene transcription *in vivo* remains to be elucidated.

Crescent formation is a complex phenomenon including glomerular capillary necrosis, coagulation, parietal epithelial cell proliferation, and inflammatory cell infiltration.[32] The low, but detectable, level of thrombin receptor antigen in crescents could also indicate local thrombin generation and thrombin receptor activation, followed by internalization and degradation. Although thrombin generation may be transient during renal diseases, it has been shown that active thrombin can be trapped within fibrin where it is protected from its main inhibitor, antithrombin III, and from which it can be released locally for days or weeks in an experimental model of balloon-induced de-endothelialization *in vivo*.[33] This may explain prolonged cellular effects of thrombin *in vivo* and the decreased thrombin receptor antigen expression in fibrin-associated human nephropathies.

An interstitial inflammatory reaction is frequently observed in extracapillary glomerulonephritis.[34] We found an increased expression of thrombin receptor antigen in interstitial peritubular cells (Figure 4E). These results suggest that these cells are potential targets for thrombin but that they are not stimulated enough by thrombin *in vivo* to exhibit thrombin re-

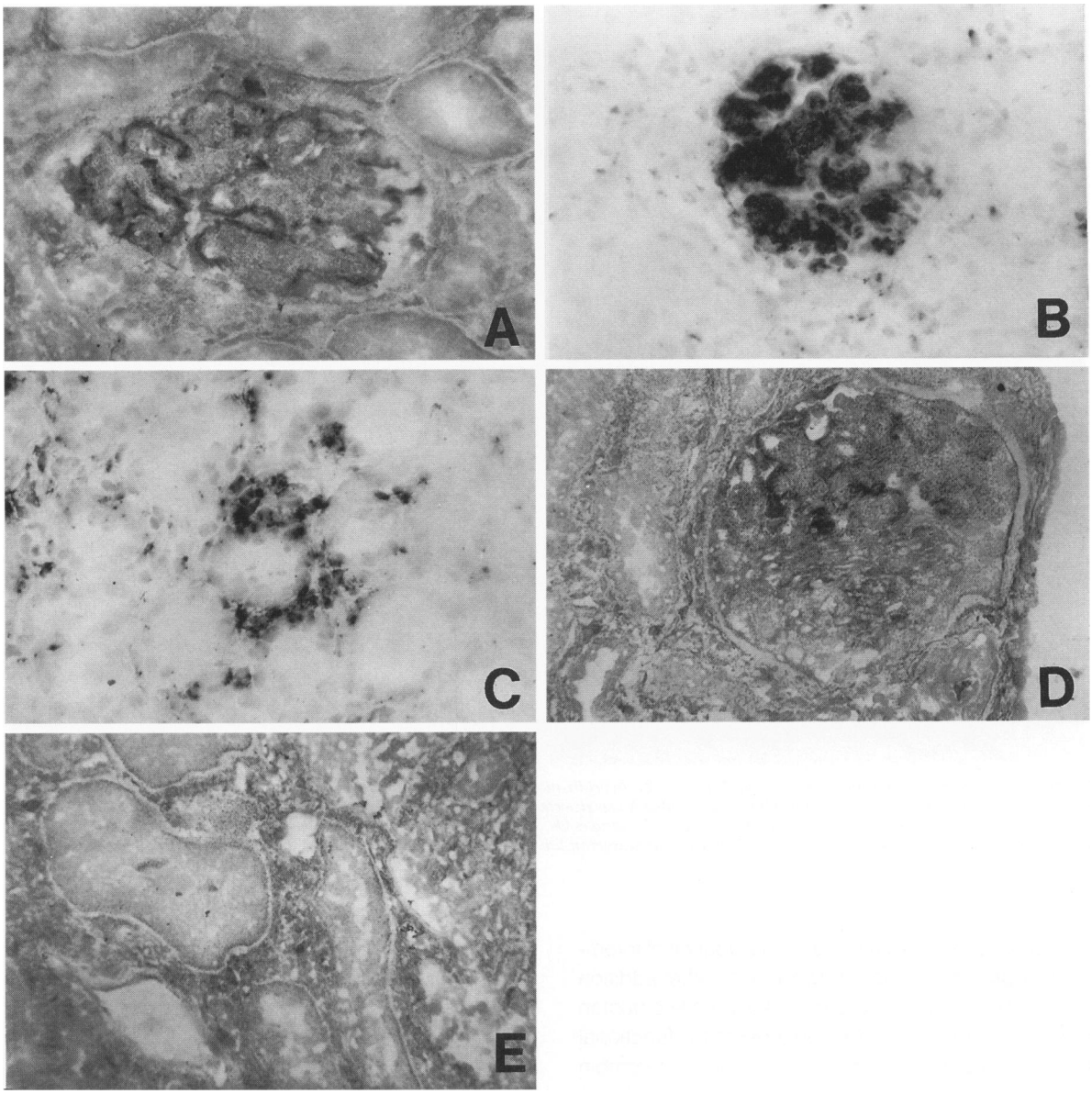

Figure 4. *Analysis of thrombin receptor expression by immunohistochemistry* (**A, D,** *and* **E**) *and* in situ *hybridization* (**B** *and* **C**) *in human kidney diseases. In thrombotic microangiopathy* (**A, B,** *and* **C**; *original magnification,* × *250), a decreased staining of endocapillary cells was observed compared with normal glomeruli, whereas podocyte staining appeared unchanged* (**A**). *By* in situ *hybridization, thrombin receptor mRNA was strongly detectable in glomeruli* (**B**) *as well as in peritubular capillary endothelial cells* (**C**). *By immunohistochemistry, in crescentic glomerulonephritis* (**D** *and* **E**; *original magnification,* × *250), a decreased staining of endocapillary cells was observed, and a low but detectable staining of proliferating parietal cells and/or infiltrating macrophages was found in crescents* (**D**). *An increased staining was found in peritubular interstitial cells* (**E**).

ceptor down-regulation. Accordingly, fibrin deposits are rarely observed in the interstitium during glomerulonephritis.

In conclusion, we demonstrated by RT-PCR, immunohistochemistry, and *in situ* hybridization that the functional thrombin receptor is expressed in the normal human kidney, mainly by glomerular cells and by endothelial cells of intrarenal vessels and to a low extent in peritubular interstitial cells. Tubular epithelial cells do not express this receptor. In thrombotic microangiopathy and in extracapillary glomerulonephritis, two renal diseases associated with fibrin deposition, a lower expression of thrombin receptor antigen was demonstrated in glomeruli whereas it was increased in the interstitium. It is suggested that prolonged stimulation of thrombin receptor *in vivo* by thrombin could induce internalization and degradation of the thrombin receptor protein.

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