Molecular characterization of a low-molecular-mass matrix metalloproteinase secreted by glomerular mesangial cells as PUMP-1

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A polymerase chain reaction (PCR)-based homology cloning strategy was used to define the spectrum of stromelysin-like matrix metalloproteinases (MMPs) synthesized by cultured glomerular mesangial cells (MC). Using this technique, cDNAs encoding an unusual, truncated member of the MMP family, punctuated (putative) metalloproteinase (PUMP-1), were exclusively isolated. Incubation with the cytokines interleukin 1 and tumour necrosis factor increased the abundance of PUMP-1 mRNA in mesangial cells. The mesangial PUMP-1 mRNA is processed in a tissue-specific manner, yielding a transcript containing repeated 3'-untranslated region ATTTA motifs commonly found in cytokines with limited mRNA stability. Polyclonal antibodies prepared against the C-terminal region of the PUMP-1 protein documented release of this enzyme by cultures of cytokine-stimulated MC and permitted identification of PUMP-1-expressing mesangial cells within clinical biopsy specimens of acute glomerulonephritis. These findings represent new molecular and clinical evidence that non-malignant cells process and secrete this unusual member of the MMP family in a cytokine-mediated, tissue-specific manner. Mesangial synthesis of PUMP-1 may contribute to the progression of injury during glomerular inflammatory states.

INTRODUCTION

The extracellular matrix (ECM) plays a critical role in normal glomerular structure and function. In addition, most forms of glomerular disease are typified by quantitative and qualitative changes in the composition of this specialized structure. Of particular significance may be the progressive accumulation of ECM which characterizes the sclerotic lesions of many forms of glomerulonephritis and diabetes mellitus. The glomerular ECM is composed primarily of type IV and V collagens, fibronectin, laminin, entactin and sulphated proteoglycans [1,2]. Many pathological conditions affecting the glomerulus are associated with altered rates or patterns of synthesis of these proteins, or with the appearance of additional components, such as interstitial collagen [3–6]. Glomerulosclerosis develops initially within the glomerular mesangium, a specialized interstitial space located between individual glomerular capillary loops. The mesangial cells contained within this structure possess a remarkably wide synthetic repertoire, including the ability to secrete a broad pattern of ECM proteins [2]. Given the complex compositional nature of the glomerular ECM, we have postulated that an extensive spectrum of matrix-degrading metalloproteinases (MMPs) must exist for the turnover of the ECM, particularly within the glomerular mesangium. Prior studies from our laboratories have demonstrated mesangial cell secretion of a specific type IV collagenase [7,8], which by amino-acid-sequencing analysis [8a] is structurally identical with the 72 kDa type IV collagenase recently cloned from H-ras-transformed tracheal bronchial epithelial cells [9]. In addition to the type IV collagenase, earlier studies suggested that mesangial cells secrete additional MMPs with stromelysin-like patterns of activity [10].

In the present report this stromelysin-like activity is characterized at the molecular level, using a PCR-based homology cloning strategy which exploits structural motifs common to members of the MMP family. These studies provided the unexpected finding that the stromelysin-like activity synthesized by mesangial cells is identical with that of punctuated (putative) metalloproteinase (PUMP-1), a recently described human MMP previously found in only a limited number of tumours or tumour cell lines [11,12].

MATERIALS AND METHODS

Mesangial cell cultures

The methods for procurement and maintenance of homogeneous cultures of rat and human glomerular mesangial cells (MC) have been reported in detail [7,8]. Conditioned medium suitable for enzymic studies was prepared by culturing washed mesangial cells for 3–4 days in RPMI 1640 medium supplemented with 0.2% lactalbumin hydrolysate, 10 µg of transferrin/ml, and 0.6 units of insulin/ml. Conditioned media were harvested, clarified by centrifugation (1000 g, 10 min) and stored at –80 °C prior to assay or purification procedures.

Enzyme assays

Casein-degrading activity was assayed with ³H-acetylated casein [13]. Gelatinase activity was determined as reported in detail [8], using [³H]gelatin as substrate. In both assays, the proteinases were fully activated by inclusion of 0.7 mM-*p*-aminophenyl mercury (II) acetate (PAMA) in the incubation mixture. One enzyme unit is defined as the amount which degrades 1 µg of substrate/h under the given assay conditions.

Abbreviations used: PUMP, punctuated (putative) metalloproteinase; MMP, matrix metalloproteinase; MC, mesangial cells; ECM, extracellular matrix; PAMA, *p*-aminophenyl mercury (II) acetate; CSPG-I, chondroitin sulphate proteoglycan; IL-1β, interleukin-1; TNF, tumour necrosis factor.

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The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under accession no. Z11887.

Assays utilizing rat serum fibronectin (kindly donated by Dr. R. Davies, Llandough Hospital, Cardiff, U.K.) as substrate were performed at 37 °C for 18 h, in a mixture containing 25 µg of fibronectin, 50 mM-Tris/HCl, pH 7.6, 5 mM-CaCl₂, 150 mM-NaCl, 0.01% Brij-35 and 0.7 mM-PAMA. Samples were boiled in SDS/PAGE sample buffer, electrophoresed on 5.5% acrylamide gels and stained with Coomassie Blue. ³⁵S-labelled human chondroitin sulphate proteoglycan (CSPG-I) was extracted and purified as previously described [14]. Proteoglycan-degrading activity was determined at 37 °C for 18 h in a mixture containing [³⁵S]CSPG-I (1.5 × 10⁵ c.p.m.), 50 mM-Tris/HCl, pH 7.6, 150 mM-NaCl, 5 mM-CaCl₂, 0.1% (v/v) Triton X-100 and 0.05% NaN₃ in a final volume of 300 µl. After incubation, 300 µl of 8 M-guanidine/HCl was added, the mixture was centrifuged at 10000 g for 2 min, and the supernatant was immediately placed on an analytical column (6 mm × 1.5 m) of Sepharose CL-4B equilibrated with 4 M-guanidine/HCl, 50 mM-sodium acetate, pH 6.0, 0.5% Triton X-100 and 0.05% NaN₃. Fractions of 0.6 ml were collected and analysed for radioactivity.

Electrophoretic procedures

Analytical SDS/PAGE was performed on vertical slabs according to Laemmli [15]. SDS/10% acrylamide substrate gels were prepared by including 1 mg of gelatin/ml (from rat tail type I collagen) or 1 mg of casein/ml in the acrylamide polymerization mixture. Enzyme samples were solubilized in non-reducing buffer and electrophoresed at 7 °C, using 25 mA/gel. Following electrophoresis, the gels were washed in cold 50 mM-Tris/HCl, pH 8.0, containing 50 mM-NaCl, 10 mM-CaCl₂, 1 µM-ZnCl₂ and 0.05% Brij-35. The gels were then incubated overnight in fresh buffer at 40 °C. Thereafter, the gels were stained with Coomassie Blue and zones of lysis were visualized. Standard proteins were utilized for assignment of molecular mass.

Enzyme purification

Human mesangial cell 68 kDa type IV collagenase was purified by sequential gel chromatographic and heparin-Sepharose affinity chromatography steps as reported in detail [8], to a specific activity of 759 units/mg. Human mesangial cell caseinolytic (PUMP-1) activity was partially purified as follows. Conditioned medium was concentrated by ultra/diafiltration against a YM-10 membrane (Amicon) and applied to a calibrated Sephacryl S200 SF column equilibrated with 150 mM-NaCl, 10 mM-Tris/HCl, pH 7.6, and 0.025% Brij-35. Caseinolytic activity of individual fractions was assessed by the assay procedures detailed above. Caseinolytic activity eluted as a single, poorly resolved peak with an apparent molecular mass of 40–20 kDa. The caseinolytic activity was dialysed against 20 mM-Tris/HCl, pH 7.8, and 0.01% Brij-35 (buffer A) and applied to a 2.1 mm × 220 mm Aquapore AX-300 h.p.l.c. anion-exchange column. The column was developed with a linear NaCl gradient of 0 to 250 mM in buffer A. Caseinolytic activity eluted as a single peak between 100 and 125 mM-NaCl, with a final specific activity of approximately 400 units/mg of protein. Rat MC caseinolytic activity was handled in an identical fashion through the gel permeation chromatography step (eluting as a broad peak between 40 and 20 kDa).

Immunological methods

A polyclonal rabbit anti-PUMP-1 IgG was prepared by immunizing rabbits with a synthetic peptide–keyhole limpet haemocyanin complex prepared with the heterodifunctional cross-linker *m*-maleimidobenzoyl *N*-hydroxysuccinimide ester [16]. Hyperimmune serum was precipitated with ammonium sulphate and the IgG fraction prepared by anion-exchange

chromatography on DEAE-dextran using standard methodology. The peptide consisted of the sequence CGGIQKL YGKRSNSRKK, which corresponds to the C-terminal residues (with the exception of the *N*-terminal cysteine used for coupling) 252–267 of the derived amino acid sequence of PUMP-1 [11]. Western blots were performed on electrophoresed h.p.l.c.-purified preparations of human mesangial cell caseinolytic activity by incubating membranes with primary anti-PUMP-1 IgG (1 µg/ml), secondary biotinylated goat anti-rabbit IgG and alkaline phosphatase-coupled biotin-avidin complex. Controls included blots incubated with secondary antibody alone or with primary antibodies which had been preabsorbed with 10 µg of synthetic PUMP-1 peptide/ml or with the partially purified caseinolytic fractions. Immunohistochemistry of renal biopsy tissue was performed on 5 µm frozen sections fixed for 20 min in buffered 4% paraformaldehyde. The sections were blocked with 5% goat serum/0.1% BSA, rinsed, and incubated overnight with primary antibodies at 1 µg of IgG/ml. The primary antibodies included the anti-PUMP-1 IgG and an affinity-purified anti-interleukin-1β (IL-1β) IgG prepared as reported in detail (F. T. Stevensen, F. Torrano, R. M. Locksley & D. H. Lovett, unpublished work). The sections were then rinsed, and incubated with 2.5 µg of affinity-purified biotinylated goat anti-rabbit IgG/ml in 0.1% BSA for 2 h. To reduce endogenous renal proximal tubule alkaline phosphatase activity, the sections were incubated in Bouin's fixative for 3 min at this point [17]. Rinsed sections were subsequently incubated with alkaline phosphatase ABC reagent (Vector, Burlingame, CA, U.S.A.), developed and counter-stained prior to dehydration and mounting. The staining was eliminated when the sections were incubated with anti-PUMP and anti-IL-1β IgGs which had been preabsorbed with 10-fold molar excess of the purified enzyme or cytokine for 2 h at 4 °C.

cDNA library construction and PCR homology cloning

A λgt11 cDNA library containing 1.5 × 10⁶ primary recombinants was prepared using standard methodology from doubly oligo(dT)-selected poly(A)⁺ RNA from human MC stimulated for 48 h with IL-1 (25 units/ml) and tumour necrosis factor, (TNF) (10 ng/ml). The library was amplified once before use. DNA templates from the gt11 cDNA library for PCR were prepared from phage lysates using LambdaSorb (Promega), followed by conventional extraction and ethanolic precipitation. Homology PCR was performed with 200 ng of gt11 cDNA template, dNTPs (200 µM each) and 2.5 units of Taq polymerase (Perkin-Elmer-Cetus). There were a total of 36 amplification cycles consisting of 30 s at 94 °C, 30 s at 53 °C, and 90 s at 72 °C. A final extension time of 10 min was used in all cases. The primer for the 5'-end encoding the activation locus was 5'-CCCAG(GA)TGTGAAGT(GT)CC(AT)GA(TC)GT(TG)G-3'. The primer (used as the inverse complement form) for the 3'-Zn locus was 5'-CATGAACT(TC)GGCCA(TC)TC(TC)TTGGG-3'. The primer (used as the inverse complement form) for a highly conserved sequence, QDDIKGIQ, which is found approx. 100 bp 3' to the Zn binding sequence, was 5'-CA(GA)GATGA(TC)AT(TA)(GA)A(AT)GGCAT(TC)CA(GA)-3'. Following amplification, the PCR reaction mixture was precipitated, dissolved in water, heat-denatured and applied to a Waters Gen-Pak FAX h.p.l.c. column (4.6 mm × 10 cm) equilibrated with buffer A (5 M-urea, 250 mM-NaCl, 30 mM-sodium phosphate, pH 7.5). The PCR products were eluted by using a linear gradient to 50% buffer B (5 M-urea, 1 M-NaCl, 30 mM-sodium phosphate, pH 7.5). Recovered products were freed of urea by repetitive diafiltration through Centricon-30 (Amicon) filters and quantified. For ligation into vectors, the PCR products were end-polished with T4 polymerase, ligated with *Eco*RI adaptors,

phosphorylated and subcloned into pGEM 7zf (Promega) using standard procedures. Sequencing of double-stranded plasmid DNA was performed according to Sanger *et al.* [18], using Sequenase II (USB). Screening of the mesangial λ gt11 cDNA library to obtain full-length metalloproteinase inserts was performed using the PRC-Zn PCR products as probes, which were labelled by the random hexamer method, with [α - 32 P]dCTP (NEN). Library screening, identification of positive clones and subcloning into plasmid vectors were performed using standard methodology [19].

Northern and Southern blot analyses

Poly(A)⁺ RNA (0.5 μ g/lane) from controls and from human MC incubated for 24 h with recombinant human IL-1 β (25 units/ml) and TNF (10 ng/ml) was electrophoresed on 1.2% denaturing agarose gels and transferred to nylon membranes. The blots were probed sequentially with a labelled full-length PUMP-1 cDNA probe and with a full-length β -actin cDNA probe. Hybridizations were performed under standard conditions [19], followed by washing at high stringency in 1% SDS/0.1 $\times$ SSC at 65 °C for 40 min. Southern genomic DNA analyses were performed by digesting 10 μ g of human placental genomic DNA with *Hind*III, *Pst*I, *Eco*RI or *Bgl*II. Digests were separated on 0.7% agarose gels, transferred to nylon membranes, and probed with the full-length PUMP-1 cDNA, followed by a high-stringency wash as above.

RESULTS

Re-evaluation of MMP secreted by glomerular mesangial cells

The first series of experiments was designed to determine whether cultured glomerular MC secrete, in addition to type IV collagenase, enzymes with patterns of activity characteristic of stromelysin. This appeared likely, as our original report concerning secretion of mesangial MMPs utilized a preferred stromelysin substrate, azocasein [10]. In the present paper we confirm that rat MC-conditioned medium degrades casein and in addition hydrolyses the core protein of 35 S-labelled CSPG (results not shown). Furthermore, we show that rat MC-conditioned medium contains activity capable of degrading fibronectin (Fig. 1). On S-200 gel permeation chromatography the latent caseinolytic activity eluted in fractions with a molecular mass of 40–20 kDa, which co-purified with activity against fibronectin (Fig. 1, lane e) as well as CSPG-I (results not shown). The activity of this low-molecular-mass fraction was completely inhibited by incubation with EDTA or 1,10-phenanthroline, but not phenylmethanesulphonyl fluoride (results not shown). This casein- fibronectin- and proteoglycan-degrading activity is distinct from the MC-derived type IV collagenase, which has no proteolytic effects upon either casein [8] or fibrinectin (Fig. 1, lane b). The substrate specificity of the 40–20 kDa proteinase(s), particularly its ability to degrade casein, indicate that cultured mesangial cells secrete MMPs other than type IV collagenase.

We next evaluated cultures of human MC for the presence of enzymes with similar stromelysin-like patterns of activity, as the availability of extensive human stromelysin-group nucleic-acid-sequence information would permit a more detailed structural characterization. Conditioned medium from human MC cultured without added growth factors or stimulants contained primarily the 68–66 kDa type IV collagenase activity (Fig. 2, lane a). Incubation of human MC with IL-1 β (25 units/ml) and TNF (10 ng/ml) for 48 h led to a significant change in the pattern of gelatinolytic activity detected with substrate gels, with new prominent bands at 45, 31 and 25 kDa (Fig. 2, lane b). As shown by the results obtained by incubating a sample of purified human

68 kDa type IV collagenase for 2 h at 37 °C prior to electrophoresis (Fig. 2, lane c) the smaller, catalytically active components (66 and 45 kDa) are probably derived from the high-molecular-mass form. The gelatinolytic proteins with apparent molecular mass between 31 and 26 kDa (Fig. 2, lane b) also revealed lytic bands in this same lower-molecular-mass range with casein substrate gels (Fig. 2, lane d).

PCR-based homology cloning strategy

We postulated that the lower-molecular-mass proteins with gelatinolytic and caseinolytic activity in the supernatants of cytokine-stimulated MC were derived from proteolytically active fragments of stromelysin-like enzymes [20]. The analysis of this problem was complicated by the fact that there are currently four recognized members of the stromelysin family (stromelysins 1–3 and PUMP-1) and that the casein substrate gels did not contain the 52 and 45 kDa bands characteristic of pro- and activated

Fig. 1. The degradation of soluble fibronectin

Rat serum fibronectin (FN, 25 μ g) was incubated at 37 °C for 18 h as follows: lane a, alone; lane b, with purified rat mesangial gelatinase; lane c, crude rat MC culture medium; lane d, crude rat MC culture medium without substrate; lane e, partially purified caseinolytic fraction; lane f, non-incubated fibronectin control. The reaction products were analysed on SDS/5.5%-polyacrylamide gels under reducing conditions, with Coomassie Blue staining.

Fig. 2. Identification of human MC neutral metalloproteinases by substrate gel electrophoresis

Aliquots of culture medium collected from confluent cultures of human MC were subjected to electrophoresis on 10% polyacrylamide gels containing either 1 mg of gelatin/ml (lanes a, b and c) or 1 mg casein/ml (lane d) as described in the Materials and methods section. Lane a, culture medium from unstimulated human MC; lanes b and d, culture medium from human mesangial cells stimulated with IL-1 β (25 units/ml) and TNF (10 ng/ml) for 48 h; lane c, purified human MC gelatinase (68 kDa) incubated for 2 h at 37 °C prior to electrophoresis. Solid arrow: 45 kDa type IV collagenase. Open arrows: 31 and 26 kDa proteins with caseinolytic activity.

Fig. 3. Analysis of MMP homology cloning PCR products

A sample (200 ng) of gt11 cDNA template for human MC was amplified by using primers for the activation locus and the Zn-binding locus (lane b, closed arrow), and primers for the activation locus and the conserved sequence QDDIKGIQ located 100 bp downstream from the Zn locus (lane c, open arrow). Samples were amplified for 36 cycles. One cycle comprised: 0.5 min of melting at 94 °C; 0.5 min of annealing at 53 °C; and 1.5 min extension time at 72 °C. A final extension time of 10 min was used in all cases. Following amplification the products are analysed on a 1.2% agarose gel with appropriate DNA markers (lane a). Bands were visualized after staining with ethidium bromide.

stromelysin [20,21]. Rather than attempt to purify and sequence each fragment, we performed PCR-based homology cloning, using as a template the cDNA prepared from IL-1/TNF-stimulated human mesangial cells. The PCR primers utilized the highly conserved activation and Zn-binding loci as the priming sites. Examination of the nucleotide sequences of these sites for stromelysins 1 and 2 and PUMP-1 permitted construction of primers with minimal degrees of nucleotide degeneracy. A minimally degenerate primer for a second, highly conserved sequence (Q²⁴⁸DDIKGIQ) located approximately 100 bp downstream from the Zn locus was also constructed. PCR amplification using the activation locus and Zn locus primers yielded a single product approx. 410 bp in size (Fig. 3, lane b), consistent with the distance between these priming sites in the stromelysin enzyme group. PCR amplification between the activation locus and the downstream QDDIK sequence yielded a single product approximately 100 bp larger than the above product (Fig. 3, lane c). The PCR products were subcloned and analysed by DNA sequencing. Of multiple clones subjected to sequence analysis from three individual PCR reactions, all were found to be identical with the sequence for PUMP-1. This finding was unexpected, as PUMP-1 mRNA has been detected exclusively within a limited number of human tumour extracts [11,22].

Cloning and sequencing of PUMP-1 cDNA clones

Given the absence of information on PUMP-1 expression by non-transformed cells, we elected to clone and sequence the full-length PUMP-1 cDNA from the mesangial cell cDNA library, using the 410 bp activation locus-Zn locus sequence as a probe. Library screening yielded multiple positive plaques, of which five were chosen for more complete analysis. Evaluation of the cloned phage cDNA inserts, using Agt11-specific PCR primers, revealed that all five clones were of equal size. Two of these clones were subsequently subjected to nucleotide sequence analysis. Each of these cDNA inserts was 1132 bp in size and encompassed the open reading frame of the PUMP-1 cDNA without variation from the sequence published by Muller and colleagues ([11]; Fig. 4). The mesangial PUMP-1 cDNA

Fig. 4. Nucleotide sequence of the cDNA and deduced amino acid sequences of the human MC-derived PUMP-1

The top line shows the nucleotide sequence of cDNA clones and the bottom the deduced amino-acid sequence. The sequence spanned 1132 bp and included the complete open reading frame of the PUMP-1 protein. The underlined sequences denote the sites used for the PCR-based homology cloning strategy described in the text. Two polyadenylation signal sequences are boxed; the repeated ATTTA motifs are boxed with solid lines placed above.

nucleotide sequence differs in the 3' untranslated region from the tumour-cell-derived PUMP-1 cDNA sequence. The mesangial transcript is extended at the 3' end by an additional 54 bp, which includes two ATTTA motifs. The ATTTA motif was originally described within the 3' untranslated regions of inflammatory cytokines and facilitates degradation of transiently expressed messages [23,24].

Southern genomic analysis of the PUMP-1 gene revealed a rather simple restriction enzyme digestion pattern (Fig. 5),

Fig. 5. Southern analysis of genomic DNA

Aliquots (10 μ g) of human placental genomic DNA were digested overnight with different restriction enzymes and the products were analysed on a 0.7% agarose gel, transblotted to nitrocellulose filters and hybridized to full-length 32 P-labelled PUMP-1 cDNA. Hybridization and washings were under high stringency as described in the Materials and methods section.

consistent with the existence of PUMP-1 as a single copy gene within the normal human genome. Digestions with four enzymes, *Bgl*II, *Hind*III, *Pst*I, and *Eco*RI revealed in each case one unique fragment hybridizing at high degrees of stringency with a full-length PUMP-1 cDNA probe. The size of this gene cannot exceed the 8.5 kb *Bgl*II fragment and is more likely the size of the 5.8–6.0 kb *Hind*III, *Pst*I fragments. Thus the PUMP-1 gene is considerably smaller than the genes for the other members of the stromelysin family, which are approx. 14 kb in size [25].

Western blot analysis of MC PUMP-1

The PUMP-1 cDNA open reading frame encodes for a protein 267 amino acid residues in size with an estimated molecular mass of approximately 29 kDa. This suggested that the low-molecular-mass caseinolytic bands present in cytokine-stimulated human MC supernates represented the PUMP-1 protein, rather than catalytically active fragments of stromelysin. To identify these bands, we prepared a rabbit polyclonal antibody to a synthetic peptide corresponding to residues 252–267 of the C-terminal region of the PUMP-1 protein. This region was chosen for its predicted function as an epitope [26,27], and because this particular region bears very little similarity to the corresponding sequences of stromelysin 1, 2 or 3. The caseinolytic activity from supernates of cytokine-stimulated human MC was partially purified to a specific activity of approx. 400 units/mg by using a sequence of gel permeation and h.p.l.c. anion-exchange chromatography as detailed in the Materials and methods section. Casein substrate gels of this material yielded two discrete bands of activity, 28 and 26 kDa in size (Fig. 6, lane b). Western blots of this same fraction identified these bands as PUMP-1 proteins (Fig. 6, lane a). The staining of these bands was specific and could be eliminated by pre-incubation of the anti-PUMP-1 antibody with either excess of synthetic peptide or with the h.p.l.c.-purified caseinolytic fraction.

Northern blot analysis of cytokine-stimulated MC

Northern blot analysis of poly(A)⁺ RNA from human MC revealed a 1400 bp, weakly positive transcript in non-stimulated cells (Fig. 7). Incubation with IL-1 β (25 units/ml) and TNF (10 ng/ml) for 48 h significantly enhanced (approx. 8-fold) the relative abundance of the PUMP-1 transcript. The mRNA for β -actin was not enhanced (results not shown).

Glomerular immunohistochemical localization of PUMP-1

Immunohistochemical evaluation of clinical renal biopsy material was performed, using the anti-PUMP-1 IgG in conjunction

Fig. 6. SDS/PAGE of partially purified human MC PUMP-1

Human MC low-molecular-mass caseinolytic protein was partially purified by h.p.l.c. as detailed in the Materials and methods section and subjected to SDS/PAGE. After electrophoresis the sample was transferred to nitrocellulose and probed with antibody to the C-terminal region of the PUMP-1 protein (lane a). A second sample was investigated by casein substrate 7% acrylamide gel electrophoresis (lane b).

Fig. 7. Northern blot analysis of human MC mRNA

Poly (A)⁺ RNA was prepared from control human MC (lane 1) and cells stimulated with cytokines [IL-1 β (25 units/ml) and TNF α (10 ng/ml; lane 2]. RNA (500 ng), after electrophoresis, was transferred to a nylon membrane and hybridized with a 32 P-labelled full-length PUMP-1 followed by autoradiography.

Fig. 8. Immunohistochemical localization of PUMP-1 and IL-1 β proteins in human glomeruli

Frozen sections (5 μ m) from renal biopsy material from a patient with rapidly progressive glomerulonephritis were probed with anti-serum to human PUMP-1 (a and b) and IL-1 β (c) as detailed in the Materials and methods section. (a) The reaction product is diffusely distributed within the glomerular tuft (gt) and shows foci of accumulation (arrows). (b) Examination at higher magnification reveals staining (arrow) of a cluster of mesangial cells (m). (c) Immunoreactive IL-1 β is localized to the glomerular endothelial cells (closed arrow) and to a monocyte (open arrow) within the capillary loop. Final magnifications: (a) $\times$ 150, (b) $\times$ 450, (c) $\times$ 600.

with staining for IL-1 β . Although normal human glomeruli do not contain detectable amounts of either PUMP-1 or IL-1 β , the expression of these proteins was greatly enhanced in several forms of acute proliferative glomerulonephritis. Shown in Fig. 8 are representative histochemical analyses of a case of pauci-immune rapidly progressive glomerulonephritis, in which mesangial hypercellularity was a prominent feature. Staining for the PUMP-1 protein was limited to the mesangial regions of the affected glomeruli, (Figs. 8a and 8b), while IL-1 β staining was readily evident within capillary endothelial cells and marginating mononuclear cells (Fig. 8c).

DISCUSSION

Using a combination of biochemical and immunological techniques, the present study provides evidence that glomerular mesangial cells synthesize PUMP-1, a unique member of the stromelysin family of MMPs. PUMP-1 was originally described as a PUTative MetalloProteinase obtained as a cDNA sequence from certain human malignancies [11]. Expression of this cDNA in transfected COS cells subsequently demonstrated that PUMP-1 is functionally active and exhibits a spectrum of substrate specificity with many similarities to that of stromelysin [28]. This includes the ability to degrade casein, gelatins, fibronectin and proteoglycans. In contrast to stromelysin 1, expressed PUMP-1 does not degrade native type III and IV collagens [28]. However, very recent evidence from Murphy *et al.* [29] indicates that PUMP-1 can degrade type IV collagen and elastin. The PUMP-1 cDNA hybridizes with a 1.2 kb RNA from involuting rat uteri, which suggests that PUMP-1 may encode for the human equivalent of a small matrix metalloproteinase extracted from the rat uterus, termed MMP-7 [30]. More recently, Miyazaki and colleagues have characterized a 29 kDa MMP secreted by a human rectal carcinoma cell line [12]. On the basis of partial amino-acid-sequencing data, these workers suggested that this enzyme is identical with PUMP-1 and proposed the name matrin. The purified enzyme exhibits the same matrix-substrate specificity as that found in the supernatants of PUMP-1-transfected COS cells [28].

Our studies provide the somewhat unexpected conclusion that PUMP-1 represents the primary stromelysin-like MMP produced by cultured human MC. It remains possible that a component of the caseinolytic activity in the rat MC supernatants represents stromelysin itself, or, alternatively, that stromelysin expression by MC varies among individual culture strains. The variability in MMP secretory profiles by cultured cells has been previously noted [31]. To date, studies utilizing a number of specific stromelysin nucleic acid probes and antibodies have failed to detect either the protein or the mRNA in unstimulated cultured mesangial cell line (D. H. Lovett, unpublished work).

There are several interesting features concerning the mesangial cell form of PUMP-1. First, the expression and synthesis of this enzyme *in vitro* is mediated by the inflammatory cytokines IL-1 and TNF. This stimulatory effect was synergistic, as the individual use of IL-1 and TNF led to only limited increases in PUMP-1 synthesis (results not shown). A similar pattern of synergistic action of IL-1 β and TNF α on stromelysin synthesis has been recently demonstrated with cultured rheumatoid synovial fibroblasts [32]. Second, the mesangial PUMP-1 transcript differs in the 3' untranslated region from the published tumour-cell-derived transcript [11] by the addition of a 54 bp sequence containing two ATTTA motifs. These motifs significantly limit mRNA stability, suggesting that alternative splicing or tissue-specific transcript trimming exists for this particular MMP gene product, a phenomenon not evident with other members of the collagenase supergene family. The presence

of these instability motifs in the mesangial PUMP-1 transcript provides for an additional level of mesangial cellular control of PUMP-1 synthesis. Deletion of these elements in tumour cell transcripts may permit enhanced enzymic synthesis facilitating metastatic potential.

Finally, one of the more important features of this study may be the demonstration that the PUMP-1 protein is expressed *in vivo* during an acute glomerular inflammatory process associated with IL-1 synthesis. Monocytic infiltration has been noted in several forms of experimental and human glomerulonephritis, including pauci-immune rapidly progressive glomerulonephritis, and post-streptococcal disease [33]. Both mesangial cells and monocytes from animals with murine lupus nephritis express high levels of IL-1 mRNA [34], and significant quantities of IL-1 have been detected in the supernatants of cultured glomeruli derived from patients with pauci-immune rapidly progressive glomerulonephritis [35]. Thus, it may be that the intraglomerular release of IL-1 (and presumably TNF) by activated monocytes or intrinsic glomerular cells induces mesangial synthesis of PUMP-1. Cytokine-induced synthesis of this enzyme, which exhibits such a broad range of substrate activity, could conceivably contribute to the further evolution of injury recently postulated to result from glomerular protease release [36,37].

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