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Up-regulation of extracellular matrix proteoglycans and collagen type I in human crescentic glomerulonephritis

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Background. The pathogenesis of crescentic glomerulonephritis (CGN) involves cellular migration and proliferation in the urinary space, frequently followed by fibrous organization. Extracellular matrix proteoglycans (PGs) may regulate these events via effects on cellular migration, interactions with growth factors, including transforming growth factor-β (TGF-β), and control of collagen fibrillogenesis. The expression of PG in human CGN is unknown.

Methods. Renal tissues from 18 patients with CGN were examined immunohistochemically for versican, decorin, biglycan and collagen type I, and were compared with morphologically normal tissues from six tumor nephrectomies. Synthesis of decorin, biglycan, and procollagen type I mRNAs was evaluated by in situ hybridization.

Results. Versican was strongly expressed in cellular crescents and periglomerular areas, whereas decorin and biglycan accumulated in collagen type I-enriched regions, including fibrocellular and fibrous crescents, and interstitial fibrosis. PG and collagen type I accumulation colocalized with myofibroblasts in crescents, periglomerular areas, and interstitium.

Conclusions. The temporal and spatial patterns of expression demonstrated in this study provide evidence to support pathogenic roles for PG in the evolution of CGN. Based on known biological properties of this molecule, versican may facilitate migration of cells in developing crescents. Decorin and biglycan may contribute to progression of CGN, perhaps via interactions with collagen type I in the remodeled extracellular matrix.

The pathogenesis of crescentic glomerulonephritis (CGN) and the factors that determine progression to end-stage renal failure in some cases of CGN are incompletely understood [1]. A pathologic hallmark of progressive renal disease in general is the accumulation of interstitial collagens (that is, types I and III) in the lesions of glomerulosclerosis and interstitial fibrosis, implying a central pathogenic role for expansion and remodeling of the extracellular matrix in these processes. Another class of extracellular matrix molecules, the proteoglycans (PG), has multiple biologic properties that may be relevant to the development and progression of CGN, including effects on cell adhesion and migration, modulation of growth factor bioavailability, and regulation of collagen fibrillogenesis [2]. The expression of PG in CGN is largely unknown.

Versican is a high molecular weight chondroitin sulfate PG synthesized by smooth muscle cells, mesangial cells, and fibroblasts, which is widely distributed in extracellular matrices [2]. Versican is selectively expressed in embryonic tissues that act as barriers to neural crest cell migration and axonal outgrowth, suggesting a role in guiding cell migration [3]. Synthesis of versican may be required for smooth muscle cell proliferation in vitro [4], and versican accumulates in the neointima of atherosclerotic arteries, consistent with a role in the migration and/or proliferation of smooth muscle cells during neointima formation [5]. Recently, versican has been shown to function as a ligand for the leukocyte adhesion molecule L-selectin in kidney tissue, suggesting a possible role for versican in leukocyte trafficking in inflammatory diseases of the kidney [6]. In normal adult human kidney, versican has been localized to the tubulointerstitium [5], but its expression in glomerular disease is unknown.

Decorin and biglycan are members of the family of small, leucine-rich chondroitin/dermatan sulfate PG that may be particularly relevant to the pathogenesis of progressive renal disease [7]. A putative role for these molecules in fibrosing disease has been inferred from their ability to bind and inactivate the fibrogenic cytokine transforming growth factor-β (TGF-β) in some models of fibrosing disease [7]. In the Thy 1 rat model of mesan-

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gioproliferative glomerulonephritis, synthesis of decorin and biglycan is dramatically up-regulated in glomeruli, possibly in response to increased TGF-β activity [8]. Administration of exogenous decorin [9] or transfection of decorin cDNA into skeletal muscle to increase circulating levels of decorin [10] has been reported to reduce proteinuria, ameliorate extracellular matrix accumulation, and decrease glomerular TGF-β levels [10], consistent with a role for decorin as a negative regulator of TGF-β activity. Decorin may also have a role in fibrosing disease via regulation of collagen fibrillogenesis, as indicated by the demonstration of specific interactions between decorin core protein and collagen proteins in vitro [11], and the finding of abnormal collagen fibrillogenesis in decorin-deficient mice [12]. Accumulation of decorin in areas of interstitial fibrosis has been shown to correlate with renal outcome in human glomerular diseases [13]. The precise functions of biglycan are unknown. Like decorin, its synthesis may be regulated by TGF-β and platelet-derived growth factor (PDGF) [14], both of which have been implicated in human renal disease [15]. Biglycan also may have roles in regulating TGF-β activity [16] and collagen fibrillogenesis [17]. Decorin has been localized to collagen-enriched areas in developing and mature human kidney [13, 18], whereas biglycan was localized to a variety of cell types in human fetal kidney, including tubular epithelium and glomerular endothelial cells, suggesting distinct roles for these molecules during development [18]. We recently identified colocalization of decorin and biglycan with collagen type I in sclerosing glomerular diseases of diverse etiology, and specific codeposition of decorin and collagen type I in amyloid deposits [19]. The expression of decorin and biglycan in inflammatory human glomerular diseases, including CGN, is unknown.

In this study, we sought evidence to support the hypothesis that the temporal expression of versican, decorin, and biglycan helps mediate the pathologic events in crescent formation by examining their expression in human renal biopsies with features of CGN at different stages of disease (represented by predominantly cellular crescents and predominantly fibrous crescents, respectively). The distribution of these PGs was compared with the synthesis and deposition of collagen type I and with the localization of myofibroblasts and monocyte/macrophages in these tissues and in morphologically normal kidney.

METHODS

Tissue selection and pathologic examination

Archived paraffin-embedded diagnostic renal biopsy tissues from cases of CGN in which there was remaining tissue in excess of that needed for patient diagnosis were selected for study. Clinical data were obtained from the physician referral forms submitted with the biopsy. Renal biopsy tissues utilized for this study were fixed in 10% buffered formalin. The pathologic diagnosis in each case was established by light microscopy, immunofluorescence microscopy, and electron microscopy, using standard techniques. The types of crescent identified in Jones' silver methenamine stained glomeruli were classified as follows. Cellular crescents consisted of two or more flattened layers of cells in the urinary space, without appreciable accumulations of extracellular matrix. Fibrous crescents comprised abundant extracellular matrix, with few if any identifiable nuclear profiles, and were usually associated with obliteration of the adjacent glomerular capillary tufts and/or global glomerulosclerosis. Most cases also showed a variable number of fibrocellular crescents that contained, in addition to cells, a variable amount of silver-positive extracellular matrix. The percentage of glomeruli exhibiting glomerulosclerosis (segmental or global) was evaluated in each case; in many instances, sclerotic glomeruli also showed features of fibrous crescent formation. Cases were subsequently divided into two groups based on whether the glomeruli showed predominantly cellular crescents (group A, $N = 10$) or predominantly fibrous crescents ± sclerosis (group B, $N = 8$; Table 1 and Fig. 1). The extent of chronic tubulointerstitial injury, defined as interstitial fibrosis, tubular dilation and/or atrophy, and associated mononuclear interstitial inflammatory cell infiltration was graded semiquantitatively as follows: 0 = no injury; 1 = involving <25% of cortical area; 2 = involving 25 to 75% of cortical area; and 3 = involving >75% of cortical area. For controls, macroscopically normal renal tissues were obtained from nephrectomies for localized renal carcinoma.

Antibodies

Rabbit polyclonal antibody specific for human versican (VC-E) was provided by Dr. Richard Le Baron (University of Texas, San Antonio, TX, USA) [20]. Polyclonal rabbit antisera LF-51, LF-30/136, and LF-67 (to human decorin, human biglycan, and human collagen type I, respectively) were kind gifts of Dr. Larry Fisher (National Institute of Dental Research, Bethesda, MD, USA). LF-30/136 was generated against a synthetic peptide corresponding to amino acids 5 to 17 of the core protein of human PG-II/decorin, conjugated to keyhole limpet hemocyanin [21]. LF-51 was generated against a synthetic peptide corresponding to amino acids 11 to 25 of the core protein of the secreted form of human bone proteoglycan I (PG-I)/biglycan, conjugated to bovine serum albumin (BSA) [22]. LF-67 was generated against a synthetic C telopeptide antigen of the human collagen αI(I) chain, consisting of 26 amino acids, unconjugated [23]. The specificities of these antisera have been previously confirmed in Western blot and immunoprecipitation experiments [20–24]. Additional staining was per-

Table 1. Clinical and pathologic characteristics of cases of crescentic glomerulonephritis (CGN) examined

Case[a]	Diagnosis	Glomeruli *% global sclerosis*	T.I.[b]	C	FC	F	CD68 +/ Cresc.[c]
				%			
Group A							
1	CGN	19(35)	2	15	6	6	5.7
2	CGN/ANCA	10(20)	2	10	60	0	9.3
3	CGN	8(0)	1	35	0	0	6.7
4	CGN	24(5)	2	60	0	0	10.1
5	CGN/IgAN	21(4)	0	10	10	5	15
6	CGN/IgAN	13(0)	0	14	0	0	4.0
7	CGN	6(0)	1	16	66	0	16
8	CGN	18(14)	1	38	36	6	10
9	CGN	42(5)	1	33	7	0	14.5
10	CGN/Lupus	14(7)	1	14	3	0	10.3
Mean		(9%)	1.1	24.5	18.8	1.7	10.2
Group B							
11	CGN/ANCA	20(50)	1	0	5	5	ND
12	CGN/ANCA	11(18)	2	0	46	0	ND
13	CGN	26(58)	2	12	8	16	5.5
14	CGN	48(12)	2	0	6	12	4.8
15	CGN	6(83)	2	0	0	66	ND
16	CGN/WG	17(25)	2	6	12	18	1.5
17	CGN	19(24)	1	0	50	16	4.5
18	CGN/Lupus	37(5)	1	0	13	3	16
Mean		(34%)	1.62	2.2	17.5	17.0	6.5

[a] Group A, predominantly cellular CGN; Group B, predominantly fibrous CGN (CGN, crescentic glomerulonephritis; ANCA, antineutrophil cytoplasmic autoantibody present; WG, Wegener's granulomatosis; IgAN, IgA nephropathy)

[b] T.I., chronic tubulointerstitial injury score (see text for scoring parameters); C, FC, F, percentage of glomeruli involved by cellular, fibrocellular and fibrous crescents, respectively

[c] Average number of CD68+ cells per crescent; ND, not done

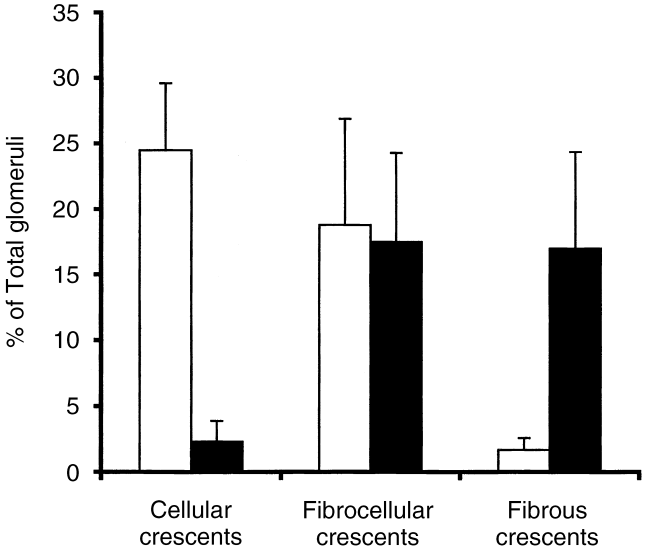

Fig. 1. Distribution of crescents by light microscopy in patients with predominantly cellular crescents (□) and those with predominantly fibrous crescents (■).

formed using commercial antibodies to decorin (6-B-6, monoclonal mouse antihuman dermatan sulfate PG; Seigaku, Tokyo, Japan) and collagen type I (polyclonal rabbit anti-human; Chemicon, Temecula, CA, USA). For α-smooth muscle actin (α-SMA) and CD68 immunostaining, commercial murine antihuman antibodies were used (both from Dako, Carpinteria, CA, USA), as previously described [25].

Immunohistochemistry

Sections were deparaffinized in xylene and rehydrated in a descending ethanol series. Endogenous peroxidase was quenched with 3% H_2O_2 for 10 minutes. For versican, decorin, and biglycan staining, sections were digested with chondroitinase ABC lyase (ICN Biomedicals, Costa Mesa, CA, USA) at 250 mU/mL in 0.1 mol/L Tris, 0.01% BSA, for 60 minutes at 37°C. For collagen type I staining, sections were digested in proteinase K (5 μg/mL; Sigma, St. Louis, MO, USA) at 37°C for 15 minutes. For CD68 staining, sections were heated with Antigen Unmasking Solution (Vector, Burlingame, CA, USA) in a household vegetable steamer for 20 minutes. For polyclonal rabbit antibody staining, sections were blocked with normal goat serum for 10 minutes. Sections were incubated with primary antibody, diluted in 1% BSA/phosphate-buffered saline (PBS) for one hour at room temperature (dilutions 6-B-6, 1/100; LF-36/130, 1/250; VC-E, LF-51, LF-67, anticollagen I, 1/500), followed by a biotinylated goat anti-rabbit antibody (Vector) or biotinylated horse anti-mouse (Vector), and ABC-Elite reagent (Vector). The reaction product was visualized with 3,3′ diaminobenzidine (Sigma) and nickel chloride enhancement. The slides were counterstained with methyl green. The immunohistochemical staining

in each case was graded by an observer who was blinded to the histopathologic diagnosis. A score was assigned to each individual glomerulus as follows: 0 = no staining product visualized; 1 = weak and/or segmental staining; and 2 = strong and/or global staining. The final score (S) per section was calculated as the weighted mean,

$$S = (N_1 \times 0 + N_2 \times 1 + N_3 \times 2)/(N_1 + N_2 + N_3)$$

where N_i (i = 1 to 3) is the number of glomeruli in each category. Negative controls for immunohistochemistry included substitution of the primary antibody with equal amounts of an irrelevant rabbit or mouse IgG (Dako).

Molecular probes

Two pBluescript SK plasmids, containing either human bone decorin cDNA (plasmid P2) or human bone biglycan cDNA (plasmid P16), were generous gifts of Dr. Larry Fisher (National Institute of Dental Research). Plasmid p16 contains a 1658 bp insert with the complete protein-encoding sequence of the human biglycan gene [21]. Plasmid p2 contains a 1.6 kb insert with the protein-encoding sequence of the human decorin gene [21]. A 1.8 kb cDNA coding for human pro-αI(I) chain of type 1 procollagen (Hf677) [26, 27] was subcloned in pcDNA3 (Invitrogen, Carlsbad, CA, USA), and the sequence was confirmed by dideoxy sequencing. The plasmids were linearized with Xba I and Kpn I (p16), BamH I and Kpn I (P2), and *Hind* III and Xba I (Hf677) and were transcribed into both antisense and sense (negative control) riboprobes in T3-, T7-, or SP6-primed reactions [21] using reagents from Promega (Madison, WI, USA; except ^{35}S-UTP; New England Nuclear, Boston, MA, USA).

In situ hybridization

Formalin-fixed, paraffin-embedded tissues were deparaffinized following standard protocol. The sections were washed with 0.5 × standard sodium citrate (SCC; GIBCO, Grand Island, NY, USA) and digested with 5 μg/mL proteinase K (Sigma) in Tris buffer (500 mmol/L NaCl, 10 mmol/L Tris, pH 8.0) for 30 minutes at 37°C. Several 0.5 × SCC washes were followed by prehybridization for two hours in 50 μL of prehybridization buffer [50% formamide, 0.3 mol/L NaCl, 20 mmol/L Tris, pH 8.0, 5 mmol/L ethylenediaminetetraacetic acid (EDTA), 1 × Denhardt's solution, 10% dextran sulfate, 10 mmol/L dithiothreitol (DTT), 500 μg/mL yeast tRNA] at 50°C. The hybridization was started by adding 500,000 cpm of ^{35}S-labeled riboprobe in 50 μL of prehybridization buffer and was allowed to proceed overnight at 50°C. The hybridization was started by adding 500,000 cpm of ^{35}S-labeled riboprobe in 50 mL of prehybridization buffer and allowed to proceed overnight at 50°C. Sections were then washed with 0.5 × SSC followed by RNase A (20 μg/mL, 30 minutes at room temperature), 2 × SSC washes (2 × 2 minutes), three high-stringency washes with 0.1 × SSC/0.1% Tween 20 (Sigma) at 50°C, and several 2 × SSC washes. The slides were dipped in NTB2 nuclear emulsion (Kodak, Rochester, NY, USA) and exposed in the dark at 4°C for one to four weeks. After developing, sections were counterstained with hematoxylin and eosin, dehydrated, and coverslipped. Positive cellular labeling was defined as five or more silver grains in a single cell. For negative controls, simultaneous hybridization with the sense riboprobe was performed on replicate tissue sections.

Statistical analysis

The values of the groups are expressed as means ± SEM. Data were analyzed for statistical significance by Student *t* test, assuming unequal variances, using SPSS program 9.0. A P value <0.05 was considered significant.

RESULTS

General observations: Clinicopathologic diagnoses

Specific clinicopathologic diagnoses in four cases included diffuse proliferative lupus nephritis (WHO Class IV, $N = 2$), Henoch-Schönlein purpura ($N = 1$), and recurrent active Wegener's disease ($N = 1$). One biopsy, obtained from a 14-year-old girl with features of a pulmonary–renal syndrome and a positive perinuclear-ANCA (pANCA) test, showed limited mesangial IgA deposits that were interpreted as suggestive of subclinical IgA nephropathy, with a superimposed antineutrophil cytoplasmic antibody (ANCA)-related CGN. Three additional cases were diagnosed as ANCA-related CGN. In the remaining 10 individuals, the results of serologic testing for ANCA and for antiglomerular basement membrane (GBM) antibodies were not available at the time of biopsy, and these cases were diagnosed as CGN of nonimmune complex or pauci-immune complex type. These data are summarized in Table 1.

Pathologic examination: General observations

Pathologic findings are summarized in Table 1 and Figures 1 and 2. All biopsies showed segmentally necrotizing and CGN (mean 45% of glomeruli involved by crescents, range 16 to 83%), with varying degrees of glomerulosclerosis (mean 20.7%, range 0 to 83%). The distribution of different crescent types in groups A and B is illustrated in Figure 1. Predictably, the percentage of glomerulosclerosis was greater in group B (34.4 ± 9.4 vs. 9 ± 3.5, $P < 0.05$), as was the chronic tubulointerstitial injury score (1.6 ± 0.2 vs. 1.2 ± 0.2, $P = 0.09$). The degree of interstitial fibrosis, tubular atrophy, and arteriosclerosis in individual cases was generally commensurate with the severity of glomerulosclerosis. Rupture of Bowman's capsule basement membrane was identified in 15 cases. Features of arterial vasculitis were not identified in any case.

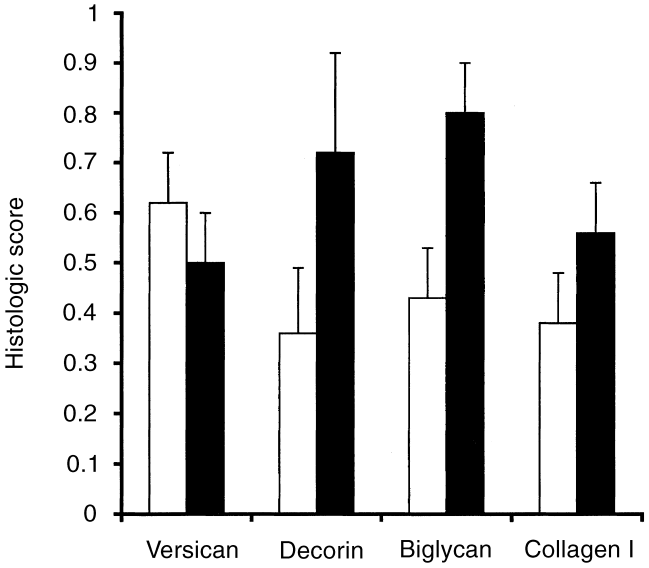

Fig. 2. Immunostaining scores for versican, decorin, biglycan, and collagen type I in glomeruli of patients with crescentic glomerulonephritis (CGN). Symbols are: (□) patients with predominantly cellular crescents; (■) those with predominantly fibrous crescents.

Immunolocalization of versican

In normal kidney (obtained from tumor nephrectomy specimens), versican showed a patchy distribution in the interstitium, with accentuation of staining around glomeruli and in perivascular adventitia, but was not identified in normal glomeruli or in tubular epithelium. Versican was identified in cellular crescents, in some cases accompanied by focal staining in the glomerular tuft adjacent to crescents (Fig. 3). Versican was also present in fibrous crescents. The overall glomerular versican staining score was slightly increased in cases with predominantly cellular crescents (group A) compared with cases with predominantly fibrous crescents (group B, 0.62 ± 0.1 vs. 0.50 ± 0.1, $P = 0.5$). Mesangial areas in most glomeruli were negative. Globally sclerotic glomeruli were typically negative for versican, except where these also showed fibrous crescent formation. There was patchy interstitial staining for versican, particularly in perivascular adventitia, in a pattern similar to that seen in normal kidney. There was no apparent difference in the intensity or distribution of versican accumulation in the interstitium in cases with predominantly cellular crescents and those with predominantly fibrous crescents. Diffuse accumulations of versican were noted in the neointima of arteriosclerotic vessels (data not shown).

Immunolocalization and in situ hybridization for decorin and biglycan

Decorin protein and mRNA were not identified in morphologically normal-appearing glomeruli. The glomerular decorin staining score was increased in group B (0.36 ± 0.13 vs. 0.72 ± 0.2, $P = 0.2$). These scores reflect the inclusion of decorin staining in fibrocellular crescents (Fig. 4A) and in globally sclerotic glomeruli in both groups. Decorin was not clearly identified in cellular crescents. Decorin also accumulated in areas of interstitial fibrosis. Decorin mRNA expression was localized to cells at sites of decorin accumulation, in fibrocellular and fibrous crescents (Fig. 4B), and in sclerotic glomeruli. In the interstitium decorin mRNA was localized to peritubular interstitial cells (Fig. 4C) with morphologic features of fibroblasts or myofibroblasts [28], but was not identified in tubular epithelial cells or in infiltrating mononuclear inflammatory cells.

Biglycan was not identified by immunohistochemistry in morphologically normal glomeruli or in tubular epithelial cells. Biglycan accumulated in fibrous crescents and sclerotic glomeruli (Fig. 5A) and in the interstitium, in a pattern similar to that of decorin, with accentuation in perivascular adventitia and in areas of fibrosis. Similar to decorin, the glomerular staining score for biglycan was also increased in group B (0.43 ± 0.07 vs. 0.8 ± 0.14, $P = 0.05$). Biglycan, but not decorin, also accumulated in the neointima of arteriosclerotic vessels in a pattern similar to that of versican. Biglycan mRNA was strongly expressed by parietal epithelial cells (Fig. 5B) and by occasional intracapillary glomerular cells (either mesangial and/or endothelial cells) in morphologically normal glomeruli. An intense signal for biglycan mRNA was identified in the majority of both cellular and fibrocellular crescents (Fig. 5C). Biglycan mRNA was also widely expressed by interstitial fibroblasts/myofibroblasts (Fig. 5D) and by neointimal cells and occasional endothelial cells (data not shown). Biglycan mRNA was not identified in tubular epithelial cells or in infiltrating leukocytes.

In situ hybridization and immunolocalization of collagen type I

Collagen type I accumulated in fibrous crescents, in sclerotic glomeruli, and in areas of interstitial fibrosis in a distribution similar to that of decorin and biglycan (Fig. 6A). Glomerular staining was increased in group B (0.38 ± 0.1 vs. 0.56 ± 0.1, $P = 0.2$). Procollagen type I mRNA was not identified in normal-appearing glomeruli. In CGN, an intense signal for procollagen type I mRNA was identified in a subpopulation of crescent cells, typically spindle shaped cells in fibrocellular or fibrous crescents (Fig. 6B), but also focally in some cellular crescents. Because of the complexity of the crescentic lesions, it was unclear whether some mesangial cells in these cases also expressed procollagen type I. In general, the number of crescent cells expressing procollagen type I mRNA was fewer than those expressing biglycan mRNA (compare Fig. 6B with Fig. 5C). Procollagen type I mRNA also localized to spindle-shaped cells in the interstitium and periglomerular areas.

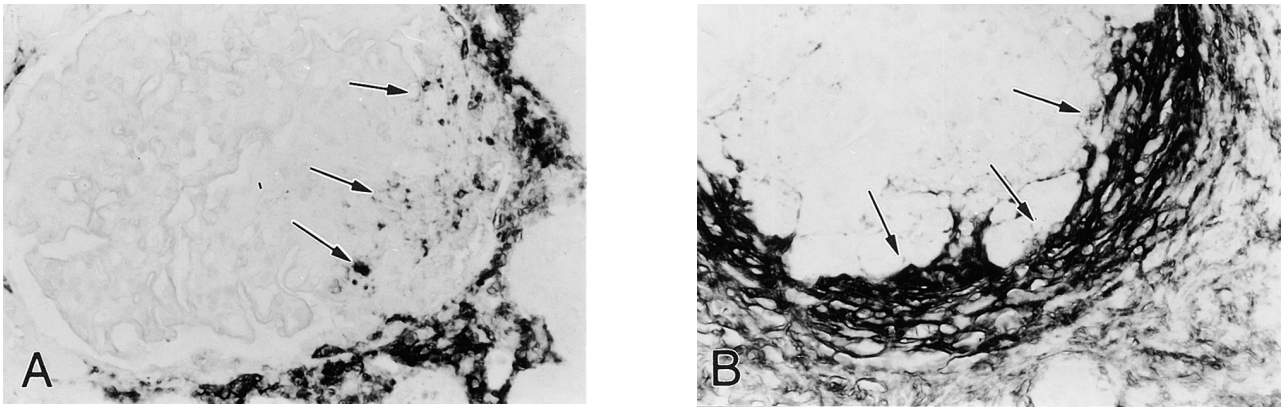

Fig. 3. (*A*) Versican accumulation in cellular crescent. Note also staining in adjacent glomerular tuft. (*B*) Versican accumulation in fibrous crescent.

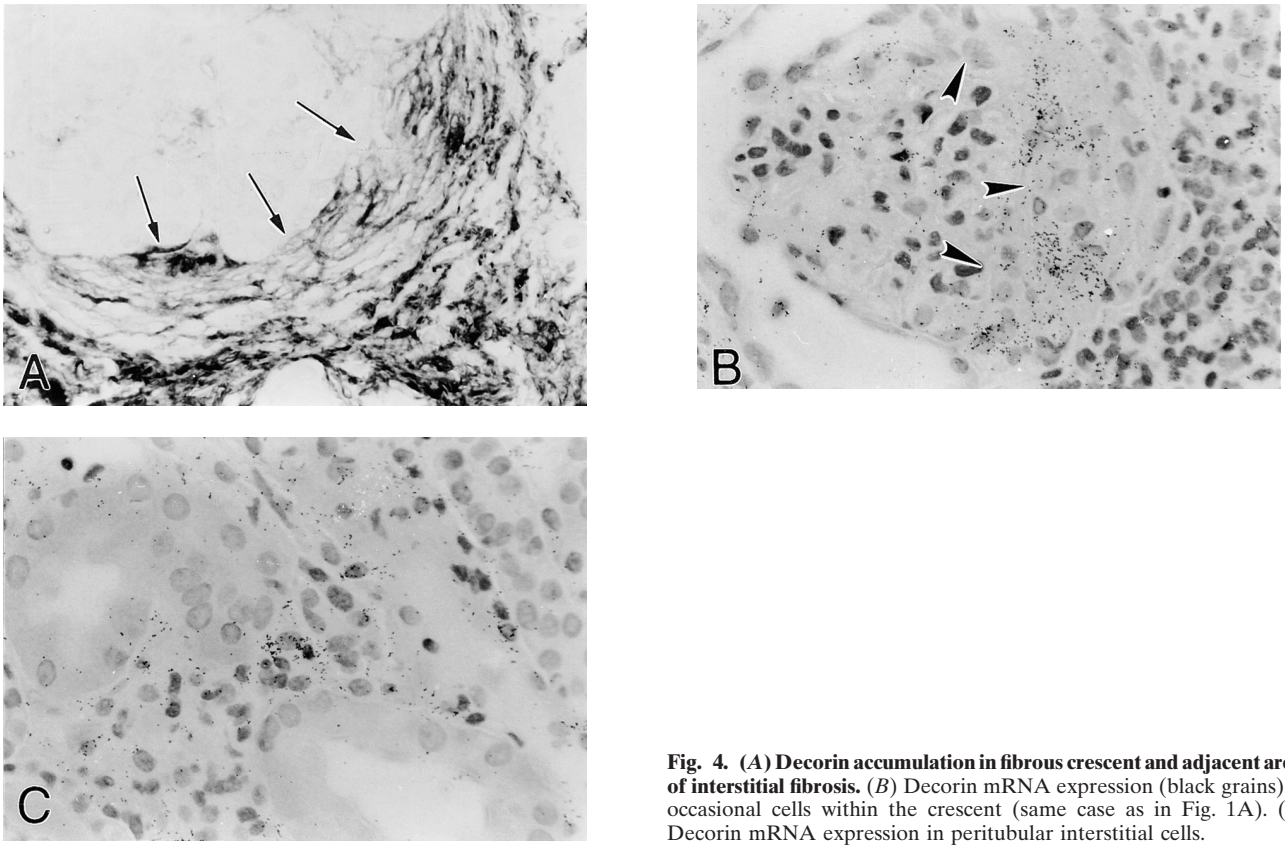

Fig. 4. (*A*) Decorin accumulation in fibrous crescent and adjacent areas of interstitial fibrosis. (*B*) Decorin mRNA expression (black grains) by occasional cells within the crescent (same case as in Fig. 1A). (*C*) Decorin mRNA expression in peritubular interstitial cells.

Localization of α-SMA–positive cells and CD68 positive cells (macrophage/monocytes)

In both normal and disease tissues, α-SMA was widely expressed by vascular smooth muscle cells and by occasional interstitial cells with the morphologic appearance of fibroblasts/myofibroblasts. In addition, rare intraglomerular cells in morphologically normal glomeruli stained for α-SMA. Most crescentic glomeruli showed enhanced immunostaining for α-SMA in mesangial areas (Fig. 7A), with variable, frequently numerous α-SMA+ cells in fibrocellular and fibrous crescents (Fig. 7A, B). α-SMA+ cells were mostly absent from cellular crescents. Some glomeruli were encircled by α-SMA+ cells that focally extended into the underlying crescent through breaks in Bowman's capsule (Fig. 7B). Occasional CD68-positive cells were present both within capillary loops and in the urinary space of morphologically normal glomeruli and within interstitial inflammatory

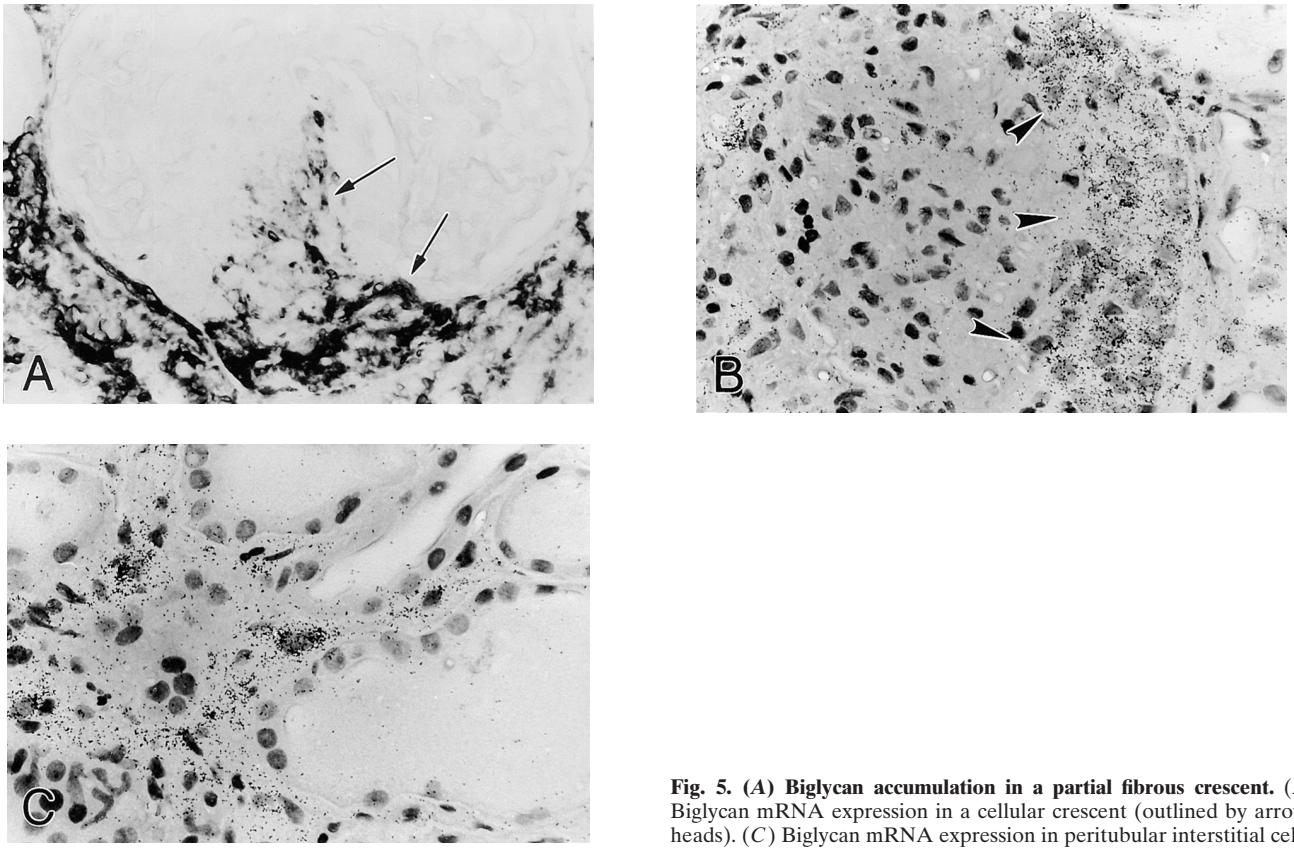

Fig. 5. (*A*) Biglycan accumulation in a partial fibrous crescent. (*B*) Biglycan mRNA expression in a cellular crescent (outlined by arrowheads). (*C*) Biglycan mRNA expression in peritubular interstitial cells.

cell infiltrates. Significant numbers of CD68-positive cells were identified in most crescents (mean 8.9, range 1.5 to 16), but in no case were they the predominant cell type (Fig. 7C). Glomerular CD68+ cells were more numerous in group A (10.2 ± 1.3 vs. 6.5 ± 2.5, $P = 0.2$).

Serial immunohistochemical staining

Replicate tissue sections were stained for versican, decorin, biglycan, CD68, and α-SMA (Fig. 8). Versican, decorin, and biglycan codistributed with α-SMA–positive cells in fibrocellular and fibrous crescents (data not shown), in the interstitium, and in neointimal thickenings of arteries (versican and biglycan only). However, versican was also identified in cellular crescents that did not contain appreciable numbers of α-SMA+ cells (Fig. 8A), and versican was not detected in mesangial areas, many of which contained numerous α-SMA+ cells (Fig. 8D). No apparent association was observed between the presence of CD68+ cells and versican, decorin, and biglycan accumulations in crescents, interstitium, or neointimal thickenings.

Attempts to characterize the phenotype of decorin, biglycan, and procollagen mRNA+ cells using combined immunohistochemical staining/in situ hybridization techniques were unsuccessful. Examination of replicate tissue sections demonstrated that decorin and biglycan mRNA expression overlapped with the distribution of α-SMA+ cells in the peritubular interstitium and in crescents. Biglycan mRNA was also strongly expressed by parietal epithelial cells in normal glomeruli that lacked α-SMA+ cells. In replicate tissue sections, procollagen type I mRNA expression overlapped with the presence of α-SMA+ cells, in crescents and in interstitium, and also possibly in mesangial areas adjacent to crescents.

DISCUSSION

The molecular and cellular events mediating crescent formation have recently been reviewed [1]. These include synthesis of chemotactic molecules {for example, monocyte chemoattractant protein-1 (MCP-1) [29] and ostopontin [30, 31]}, up-regulation of leukocyte adhesion molecules [32], macrophage proliferation [33], and expression of prosclerotic growth factors, including PDGF [34] and TGF-β [35, 36]. Rupture of Bowman's capsule [37, 38], which is associated with, and may be related to macrophage accumulation in Bowman's space [1], is followed by the influx of fibroblasts or myofibroblasts from the interstitium, and these cells may synthesize the interstitial collagens (types I and III) that accumulate in fibrocellular and fibrous crescents [37, 39]. In experimental CGN, procollagen I mRNA synthesis is up-regulated

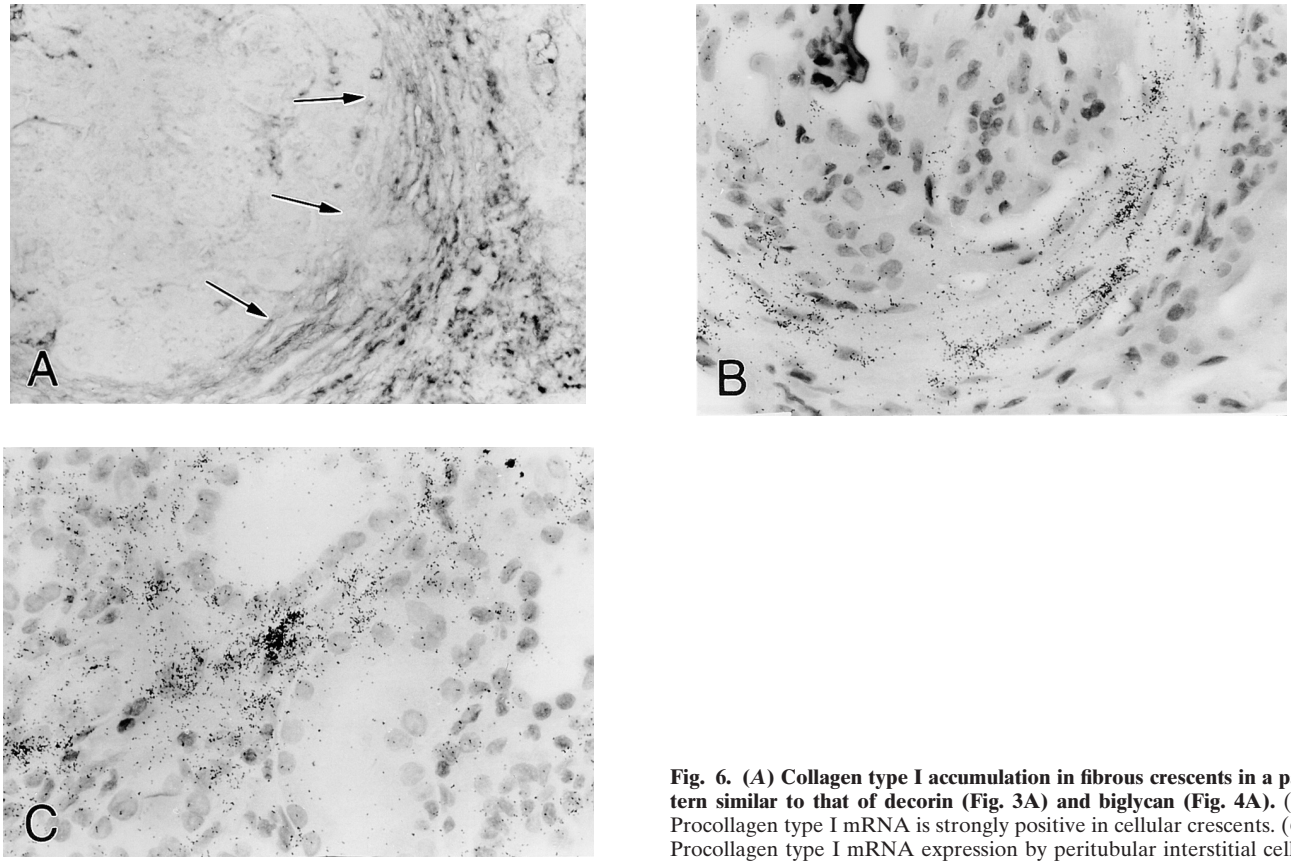

Fig. 6. (*A*) Collagen type I accumulation in fibrous crescents in a pattern similar to that of decorin (Fig. 3A) and biglycan (Fig. 4A). (*B*) Procollagen type I mRNA is strongly positive in cellular crescents. (*C*) Procollagen type I mRNA expression by peritubular interstitial cells.

in extraglomerular compartments early in the course of disease [40], followed by its expression in periglomerular areas and in Bowman's space [41], suggesting that collagen-synthesizing cells migrate across Bowman's capsule from the interstitium as crescents undergo fibrous organization. The present study demonstrates that PG synthesis and deposition occur in human CGN, and these molecules may contribute to the fate of crescents and renal outcome. α-SMA+ myofibroblasts may contribute to the accumulations of PG and collagen type I, as evidenced by their overlapping patterns of distribution in fibrous/fibrocellular crescents and in interstitial fibrosis. However, we did not positively identify the crescent cell type(s) responsible for matrix molecule synthesis and specifically cannot exclude a contribution from native glomerular mesangial and/or epithelial cells in many cases.

We present evidence that versican may have a role in cellular migration during the evolution of CGN. The localization of versican to cellular crescents provides strong evidence for a pathogenic role for versican in CGN. Versican has previously been localized to the interstitium and periglomerular regions of adult human kidney, but was not identified in normal glomeruli or tubules [5]. Versican forms a loose, highly hydrated extracellular matrix with hyaluronic acid and may facilitate cell migration by interfering with cell attachment to various substrata, including fibronectin, collagen, and laminin [2]. Others have localized hyaluronate in experimental CGN [42, 43], and it is possible that versican/hyaluronate may have a role in the migration of leukocytes during crescent formation, perhaps via interactions with CD44 and/or L-selectin expressed on the surface of infiltrating leukocytes [6]. Although we did not identify a spatial relationship between versican deposition and CD68+ cells within individual crescents, the finding that glomerular staining for versican was increased in cases with predominantly cellular crescents that also showed increased numbers of glomerular CD68+ cells suggests that these events are related. In all likelihood, the high frequency of chronic disease in the human renal tissues studied (as evidenced by the frequent finding of fibrocellular crescents and chronic tubulointerstitial injury in all cases) will tend to obscure whatever associations may exist between PG accumulation and the early stages of CGN. These results are also consistent with a pathogenic role for versican in fibrous crescent formation, perhaps related to effects on migration of α-SMA+ myofibroblasts in human CGN. This interpretation is consistent with in vitro observations that migration and mitosis of smooth muscle cells is dependent on the formation

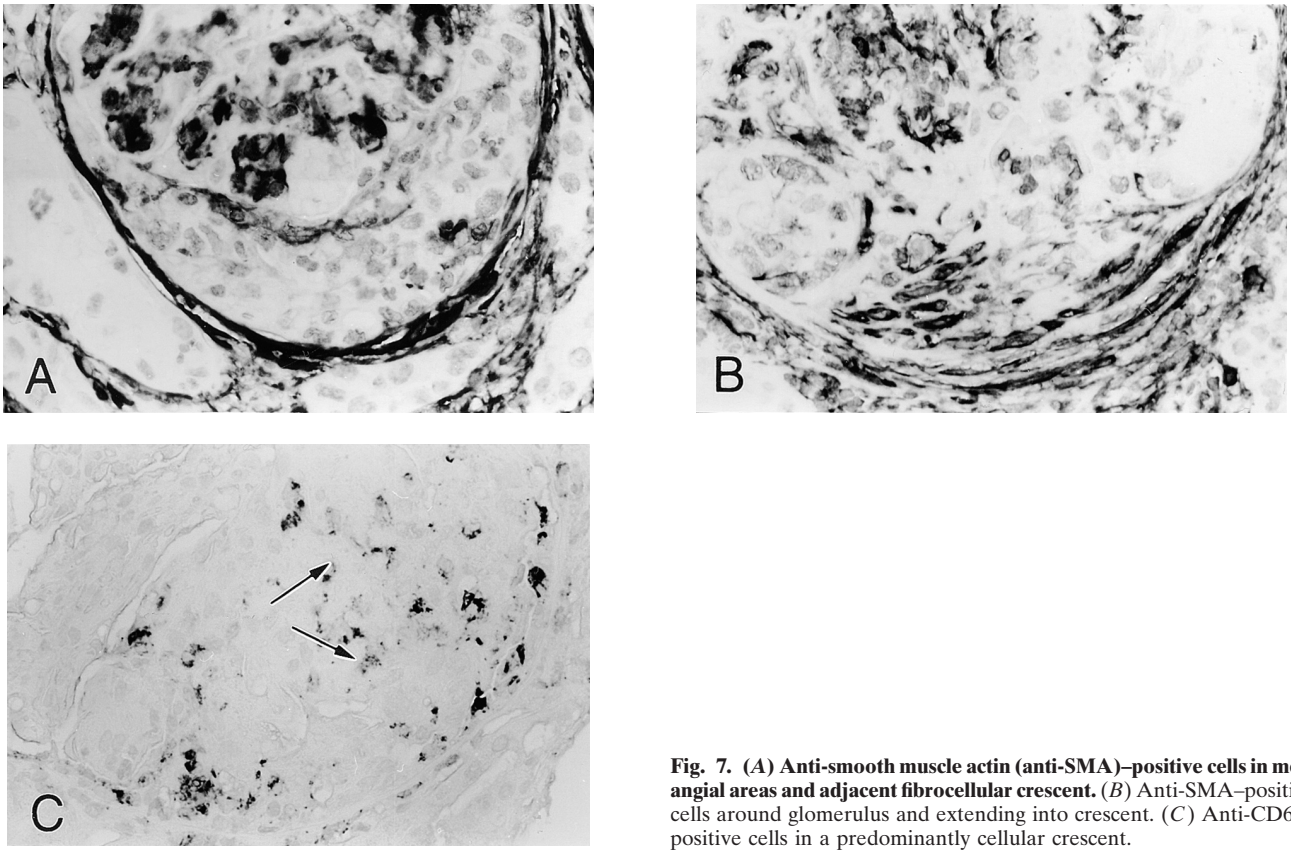

Fig. 7. (*A*) Anti-smooth muscle actin (anti-SMA)–positive cells in mesangial areas and adjacent fibrocellular crescent. (*B*) Anti-SMA–positive cells around glomerulus and extending into crescent. (*C*) Anti-CD68–positive cells in a predominantly cellular crescent.

of a versican-enriched pericellular matrix [4]. Up-regulation of versican in CGN may be linked to the activity of TGF-β [35, 36, 44] and PDGF [34, 45] in CGN, consistent with in vitro evidence demonstrating regulation of versican synthesis by smooth muscle cells in response to these growth factors [46].

The overlapping distribution patterns of decorin and biglycan with collagen type I in fibrous crescents and interstitial fibrosis strongly suggest a role for these PG related to collagen fibrillogenesis at these sites. Although collagen fibrillogenesis is largely a self-assembly process determined by the primary amino acid sequence of the collagen molecules themselves, this process may be modulated by other extracellular molecules, including decorin and biglycan. Ultrastructural studies confirm a close relationship between a small interstitial PG, putatively identified as decorin, and collagen fibrils [47], and decorin knockout mice manifest bizarre collagen morphology [12], supporting a role for decorin in maintaining interfibrillar spacing [2]. Although the function of biglycan in collagen fibrillogenesis is less well understood, the existence of biologically relevant biglycan/collagen interactions is supported by the observation that biglycan codeposits with collagen type I in some models of fibrosing disease [24, 48]. In addition to a role in collagen fibrillogenesis, decorin and biglycan may modulate TGF-β activity at sites of fibrosing injury. Binding of TGF-β by decorin/biglycan may serve a reservoir function, contributing to a locally high concentration of this growth factor in the extracellular matrix [9], or alternately sequestering the growth factor and thereby limiting its local effects. PG binding may have activating as well as suppressant effects on TGF-β activity [2], and the biologic significance of PG accumulations at sites of fibrosing injury remains undetermined. Nonetheless, this study is consistent with a pathogenic role(s) for PG in progressive renal disease related to CGN. Of note, we observed distinct patterns of decorin and biglycan mRNA expression. Glomerular expression of decorin mRNA was limited to occasional cells in fibrous crescents and in sclerotic glomeruli, whereas constitutive synthesis of biglycan mRNA was identified in nondiseased glomeruli, most consistently in parietal epithelial cells, but also in a limited number of other glomerular cells. Biglycan mRNA expression was strikingly up-regulated in crescents, including cellular crescents. While we have speculated on the importance of these molecules for growth factor activity that might mediate evolution of the injury process, the actual significance of these observed differences in PG expression remains unclear.

In summary, we report PG expression in human CGN and progressive renal fibrosis. Versican synthesis is up-

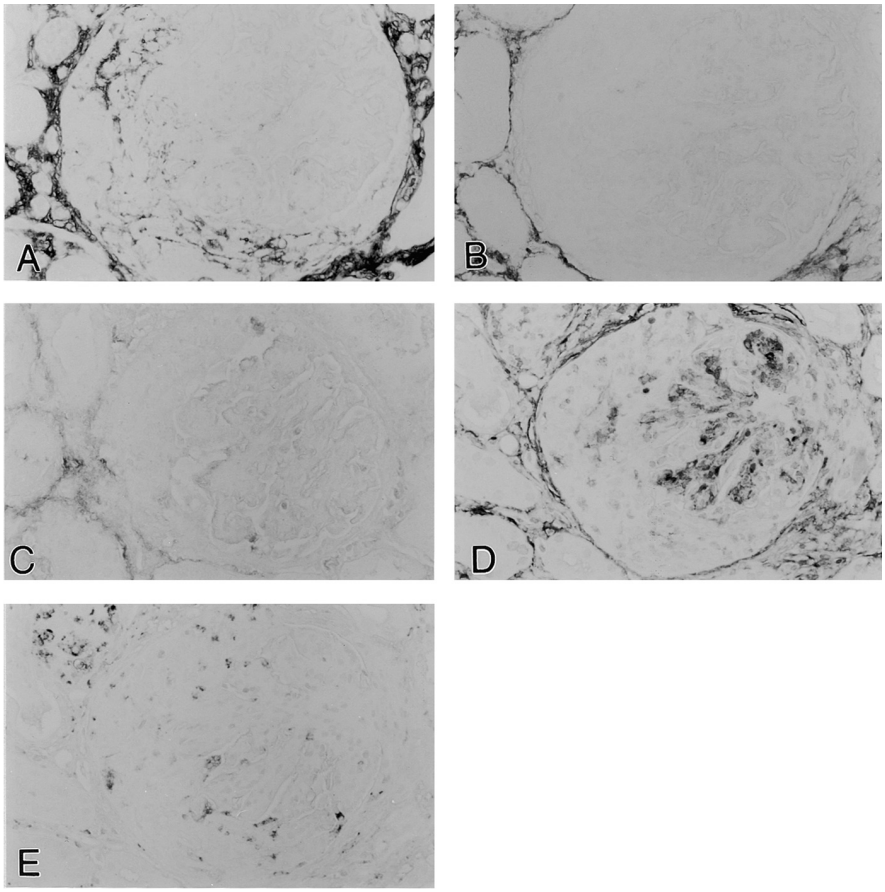

Fig. 8. (*A*) Versican accumulation is seen in a predominantly cellular crescent. (*B*) In a replicate tissue section, no decorin is localized within the crescentic glomerulus. (*C*) Biglycan-positive cells can be seen in the interstitium, but not in the crescent in a replicate tissue section. (*D*) Anti-SMA–positive cells can be seen in the mesangial area of the glomerular tuft, and a few SMA-positive cells can be seen in the crescent. (*E*) Anti-CD68–positive cells can also be seen in both the crescent and the tuft.

regulated early in CGN, as evidenced by its appearance in cellular crescents, consistent with a role in cellular migration during crescent formation. Versican most likely also contributes to the activity and/or migration of α-SMA+ myofibroblasts in the development of fibrous crescents. Decorin and biglycan may stabilize collagen type I-enriched matrices, thus contributing to glomerulosclerosis, interstitial fibrosis, and progression of CGN. Determining the precise roles of these molecules at different stages of disease could lead to novel and more therapeutic strategies aimed at critical pathogenic steps in CGN and progressive renal fibrosis.

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