Expression of Platelet-derived Growth Factor Receptors in Normal and Diseased Human Kidney

An Immunohistochemistry and In Situ Hybridization Study

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Abstract

We studied the expression of PDGF-α and -β receptors in 10 normal and 40 pathologic human kidneys (five minimal change disease, five membranous nephropathy, 25 IgA nephropathy, five lupus nephritis), by both immunohistochemistry and in situ hybridization techniques. In normal-appearing kidneys, both PDGF-α and -β receptors were expressed at the glomerular and interstitial level, the latter receptor more intensely than the former. The distribution and degree of expression of both receptors in nonproliferative glomerulonephritides were comparable with those found in normal-appearing kidneys. PDGF-β receptor gene and protein expression were upregulated in proliferative nephritides both at the glomerular and the interstitial level and strictly correlated with the grade of histologic lesions. Finally, PDGF β receptor expression was observed at a low level in normal-appearing renal vessels, and strikingly increased in injured arteries. Diseased kidneys displayed only a slight increase of PDGF-α receptor expression, chiefly at the interstitial level. Noteworthy, a few cases of lupus nephritis showed a moderate increase of PDGF-α receptor also at the glomerular level.

These data establish PDGF-β receptor activation as a candidate for driving glomerular and interstitial proliferation and, probably, expansion of extracellular matrix in proliferative glomerulonephritis, while the role of PDGF-α receptor activation at the renal level remains to be elucidated. (*J. Clin. Invest.* 1994. 94:50–58.) Key words: growth factors • PDGF receptors • glomerulonephritis • interstitial fibrosis • mesangial cells

Introduction

Platelet-derived growth factor (PDGF) is a multifunctional polypeptide which is expressed by several cell types within the kidney, including resident endothelial, mesangial, vascular smooth muscle, and interstitial cells, as well as immigrant mononuclear cells. Its biologic effects range from mitogenic activity to regulation of extracellular matrix metabolism, chemotactic and vasoactive properties, and regulation of immune–inflammatory responses (1–8).

PDGF is a disulfide-bonded dimeric glycoprotein composed of two nonidentical but highly homologous chains, known as A and B chains, which give rise to the formation of three different isoforms: AA, AB, and BB (1, 7). The activity of PDGF is mediated through its interaction with a high affinity cell surface receptor, a transmembrane glycoprotein of 180 kD with intrinsic tyrosine kinase activity. Two types of PDGF receptors (α and β) have been cloned and sequenced (9–11). Although sharing identical structural features, α and β receptors (R)[1] present only 43% overall sequence homology at the amino acid level (10). The ligand binding specificity of these two receptors also differs considerably: PDGF-αR binds both chains, whereas PDGF-βR binds only the B-chain (12). Moreover, preliminary studies lead to hypothesize that PDGF-α and -βRs may transduce common as well as unique intracellular signals (13). Both in vitro and in vivo experiments have clearly shown that PDGF can modulate cell proliferation and the production of extracellular matrix in physiologic as well as in pathologic processes (14–19). Mesangial cells have been reported to express both PDGF protein and its receptor in vitro, which makes this growth factor a potential autocrine regulator of mesangial cell activity (15). Furthermore, Muller and co-workers (20–21) have shown that cultured renal interstitial fibroblasts respond to the mitogenic effect of PDGF. This indirectly suggests that also interstitial cells would express specific PDGF receptors on their surface. Indeed, Alpers et al. recently showed a widespread constitutive expression of PDGF-βR protein by cortical and medullary peritubular interstitial cells in normal human and primate kidneys (22). Finally, several observations indicate that the responsiveness of cells to the different isoforms of PDGF in vivo depends not only on the availability of the ligand, but also on the regulated expression of PDGF receptors on the cell surface (23).

We report herein the first successful detection of both α and β receptors of PDGF within normal human kidney, by using in situ hybridization and immunohistochemical techniques. Moreover, we demonstrate that proliferative glomerulonephritides (GN)[1] exhibit a marked upregulation of PDGF-βR, associated with minor modifications of αR expression.

Methods

Tissue. 10 normal-appearing kidney portions, obtained from patients undergoing nephrectomy for renal carcinoma, and 40 percutaneous renal biopsies from patients affected by nonproliferative (five minimal change disease and five membranous nephropathy) and proliferative (25 IgA

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1. *Abbreviations used in this paper:* GN, glomerulonephritides; HMC, human mesangial cells; R, receptor.

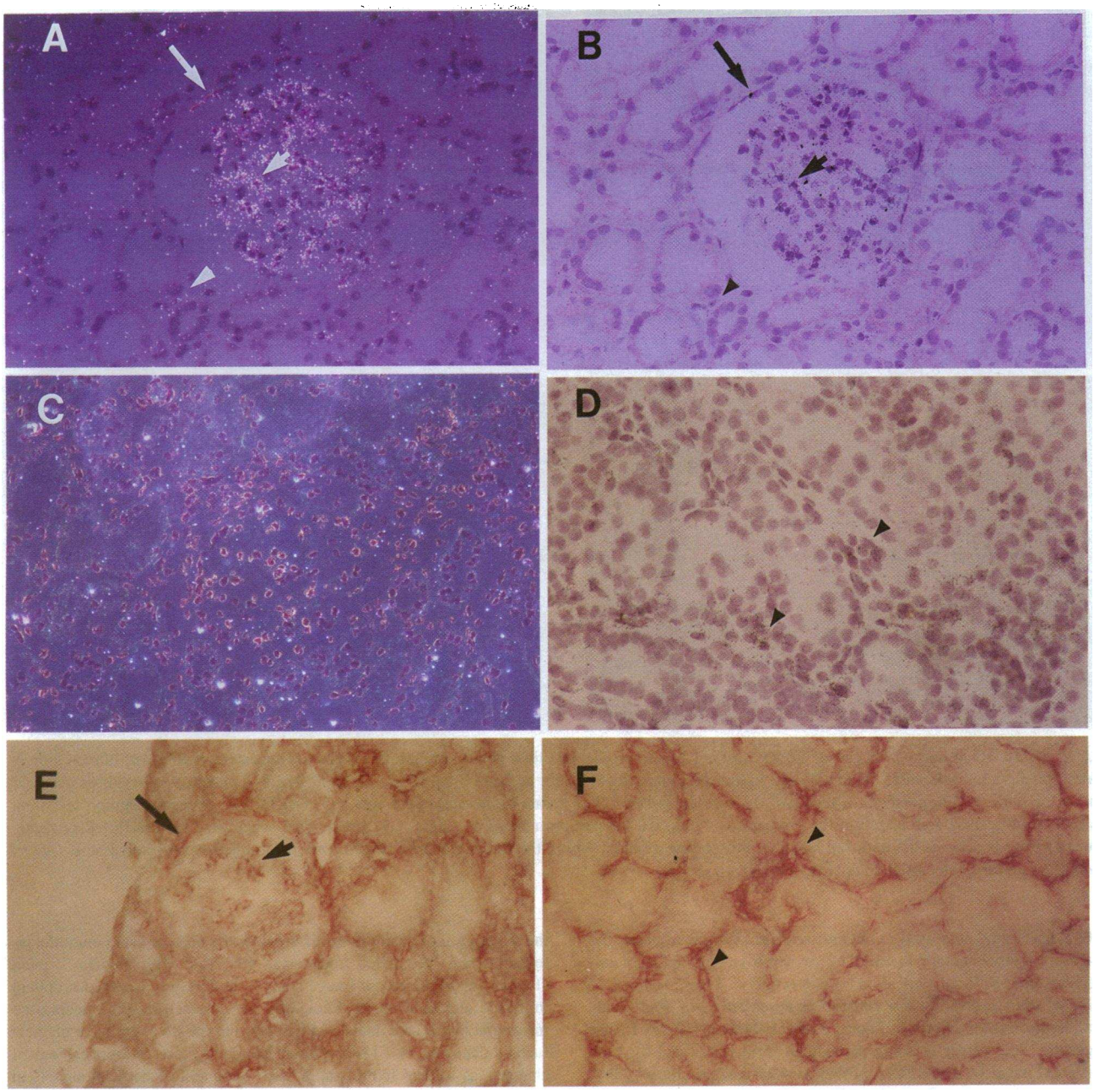

Figure 1. PDGF-βR in normal-appearing kidney. Dark- (*A*) and bright- (*B*) field photomicrographs of normal-appearing portions of human kidney revealing positive hybridization for PDGF-βR within the glomerulus (×200; positive hybridization is indicated by collection of white or black grains in the dark- and bright-field, respectively). Note the striking collection of grains in the mesangial area. (*C*) In situ hybridization of a section from the same tissue specimen shown in *A* and *B*, which demonstrates the lack of cell-associated collection of silver grains when control sense derived from PDGF-βR cDNA was tested (×200, dark-field photomicrograph). (*D*) Bright-field photomicrograph revealing the presence of positive hybridization for β receptors within the interstitium (*arrowheads;* ×200). (*E* and *F*) Immunohistochemistry for PDGF-βR protein. Immunostaining is seen as the dark red alkaline phosphatase product within the glomerulus (*E*) and the interstitium (*F*). Note the positivity of the signal within the mesangial area (*short arrows*), the parietal epithelial cells (*long arrows*) and the interstitial cells (*arrowheads*).

nephropathy and five lupus nephritis) GN were studied. The tissue was immediately included in OCT compound, snap-frozen in liquid nitrogen, and stored in the same liquid until used. Frozen sections (4 μm thick) were collected onto polylisine-coated slides, dried briefly on a hot plate at 80°C and fixed in 4% paraformaldehyde for 20 min. After two washes in PBS, dehydration in graded ethanols, and short air drying, sections were immediately used for in situ hybridization. For immunohistochemical studies, frozen sections (4 μm thick) were air dried overnight and stored at −80°C until used.

Probes. For the preparation of RNA probes, the cDNA fragments listed in Table I were subcloned into the plasmid pGEM1 (Promega Biotec, Florence, Italy) at the appropriate restriction sites. After linearization of the plasmids with either HindIII or EcoRI restriction endonuclease, T7 or SP6 RNA-polymerase (Bethesda Research Laboratories,

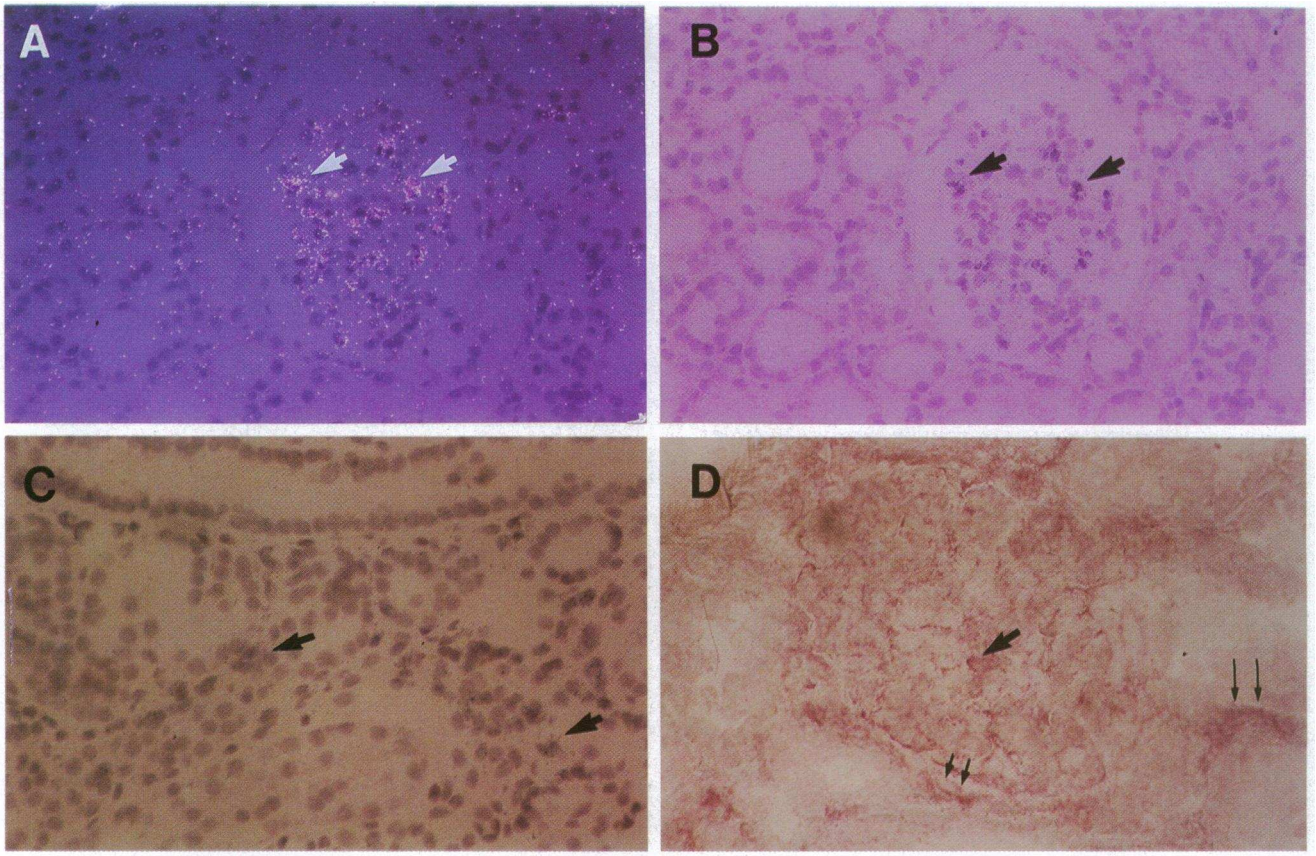

Figure 2. PDGF-αR in normal-appearing kidney. Dark- (*A*) and bright- (*B*) field photomicrographs of normal-appearing portions of human kidney revealing positive hybridization for PDGF-αR within the glomerulus (×200; positive hybridization is indicated by collection of white or black grains in the dark- and bright-field, respectively). Note the collection of grains in the mesangial area. (*C*) Bright-field photomicrograph revealing the presence of positive hybridization for α receptors within the interstitium (*arrows;* ×200). (*D*) Immunohistochemistry for PDGF-αR protein. Immunostaining is seen as the dark red alkaline phosphatase product within the mesangial area (*thick arrows*), the parietal epithelial cells (*short thin arrows*) and the interstitial cells (*long thin arrows*).

Gibco, Milan, Italy) was used to obtain run off transcripts of either the anti-sense (complementary to mRNA) or sense (anticomplementary, negative control) ^{35}S-labeled strands, respectively. Transcription and labeling of RNA probes were performed as described (24). Prehybridization, hybridization, removal of nonspecifically bound probe by RNase A digestion, and further washing procedures were performed for both positive and negative strand RNA probes as described previously (24). Autoradiography was performed by dipping the dehydrated slides into Ilford G5 nuclear emulsion (Ilford, Mobberley Cheshire, United Kingdom). The exposed slides were developed using Kodak D19 developer (Kodak, Hemel Hampstead, United Kingdom), counterstained in hematoxylin-eosin and finally mounted. Sections from normal and diseased kidneys were processed in parallel, using the same batches of probes and reagents.

Immunohistochemistry. The immunohistochemical detection of PDGF-α and -βRs was performed on frozen sections using chromatographically purified mouse antibodies, specific for human PDGF-α and -βRs (Genzyme, Cambridge, MA) (25, 26). Immobilized mouse antibodies were detected by the immunoalkaline phosphatase (APAAP) method with an affinity-purified rabbit anti-mouse immunoglobulin serum (Dako, Milan, Italy) and APAAP complex (1:50 dilution, Dako). Alkaline phosphatase was developed with New Fuchsin (Sigma, Milan, Italy) (25).

Microscopy studies. Computer morphometry (Quantimet 500, Image Analysis System, Leica S.pA., Milan, Italy) was used to quantify the histologic lesions and the optical density of the signal generated by the silver grains (in situ hybridization) or the new fuchsin (immunohistochemistry). The video image was generated with a video camera (Leica) and digitized for image analysis at 256 grey levels. An optical threshold and filter combination was set to select only the nuclei, the mesangial matrix, the interstitial fibrosis, and the silver grains or the new fuchsin deposits. Two observers independently assessed morphological changes, as well as hybridization and immunohistochemical signals in a double-blind protocol, as previously described (17, 27). Total glomerular area, glomerular matrix area, glomerular cellularity, number of interstitial cells infiltrates, areas of interstitial fibrosis, and number of pixels generated by hybridization and immunohistochemical signals were quantified in eight randomly selected glomeruli or interstitial areas from each IgA nephropathy biopsy. The structures of interest were interactively discriminated by the operators using the cursor and then automatically measured for total area. The resulting counts divided by the total area gave the measures desired. For the quantitation of in situ hybridization and immunohistochemistry signals, four groups were established: (*a*) sections hybridized with PDGF-βR antisense RNA; (*b*) sections hybridized with PDGF-βR sense RNA; (*c*) sections immunostained with mouse anti-PDGF-βR serum; and (*d*) sections immunostained with non immune mouse serum.

Using the Statview II software (BrainPower Inc., Calabasas, CA), the single data for each variable were tabulated and Pearson's correlation coefficients were computed by linear-least squares regression.

Cell culture and Northern blot analysis. Human mesangial cells (HMC) were cultured as previously reported (28). Total cellular RNA

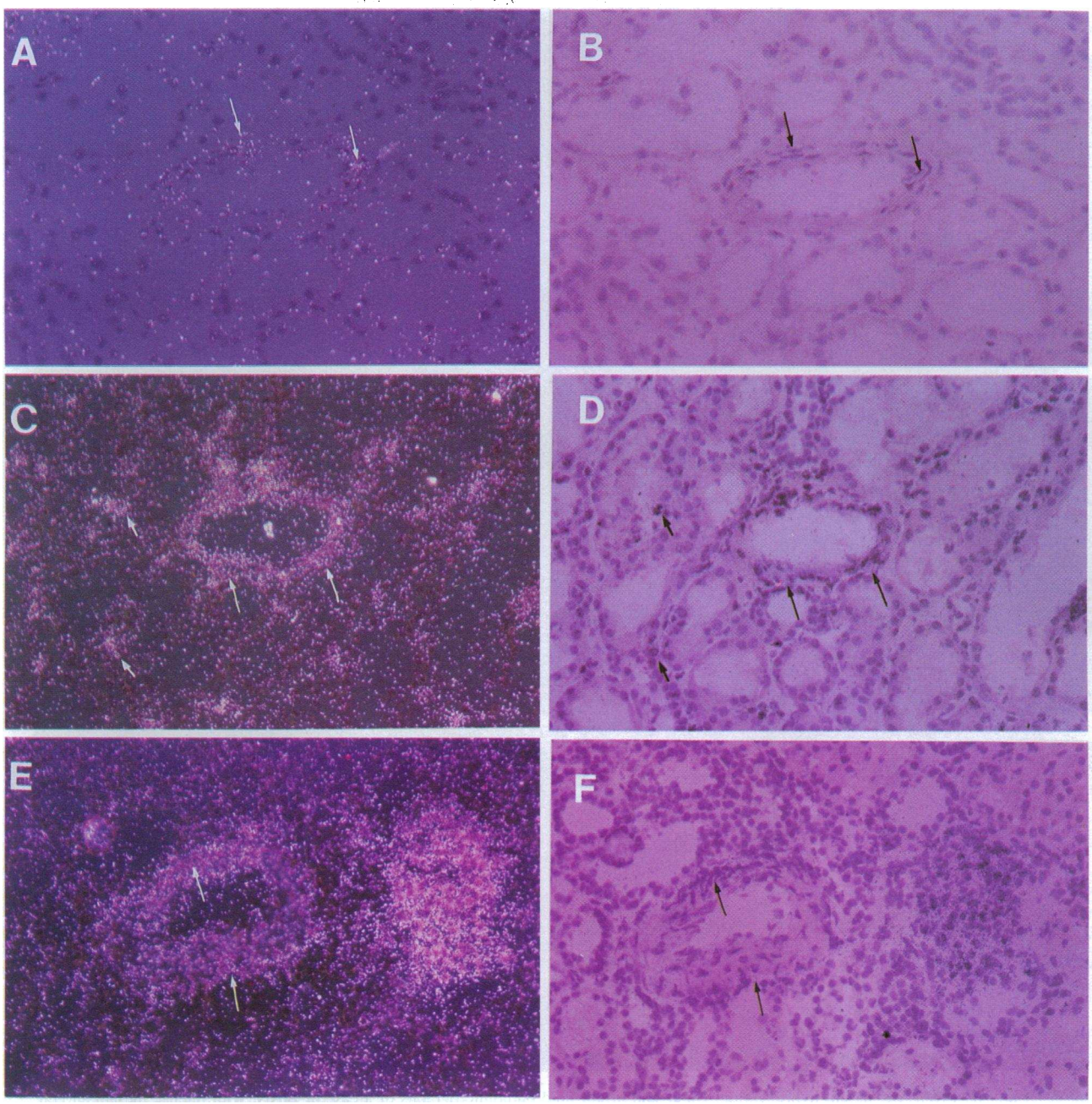

Figure 3. PDGF-βR expression in normal and pathologic renal vessels. Dark- (*A, C,* and *E*) and bright- (*B, D,* and *F*) field microphotographs (×200). Normal appearing vessels (*A* and *B*) show a slight, but consistent positivity of PDGF-βR signal, mainly within smooth muscle cells of the media and adventitial connective tissue cells (*long arrows*). Kidneys from class IV lupus nephritis (*C–F*) exhibit an up-regulation of PDGF-βR both within the media and the adventitia of small arteries (*long arrows*). An increased cellularity can be appreciated within the pathologic vessels. The same pattern is present into the interstitium (*short arrows*). Note also the strong positivity of a glomerulus in the right part of *E* and *F*.

was extracted by the method of Chomczynski and Sacchi (29). HMC RNA was fractionated on 1.2% agarose gel with 6% formaldheyde and analyzed for human PDGF-βR mRNA by Northern Blot (30).

Statistical analysis. Quantitative data were compared by analysis of variance (p). Pearson's correlation coefficients were computed by linear-least squares regression.

Results

In normal-appearing human kidneys, in situ hybridization revealed PDGF-βR mRNA expression within the glomerulus (Fig. 1, *A* and *B*). At the glomerular level, the hybridization signal was prominent in the mesangial area. Parietal epithelial cells displayed a constitutive expression of PDGF-βR, although to a lesser extent. The lack of hybridization with control "sense" probe (Fig. 1 *C*), along with the lack of detection of silver grains at the renal edge (not shown), apparently demonstrated that the hybridization signal detected was highly specific. Finally, a widespread and intense hybridization signal was found within cells of cortical as well as medullary interstitium (Fig. 1, *A, B,* and *D*). Tubular profiles were uniformly negative.

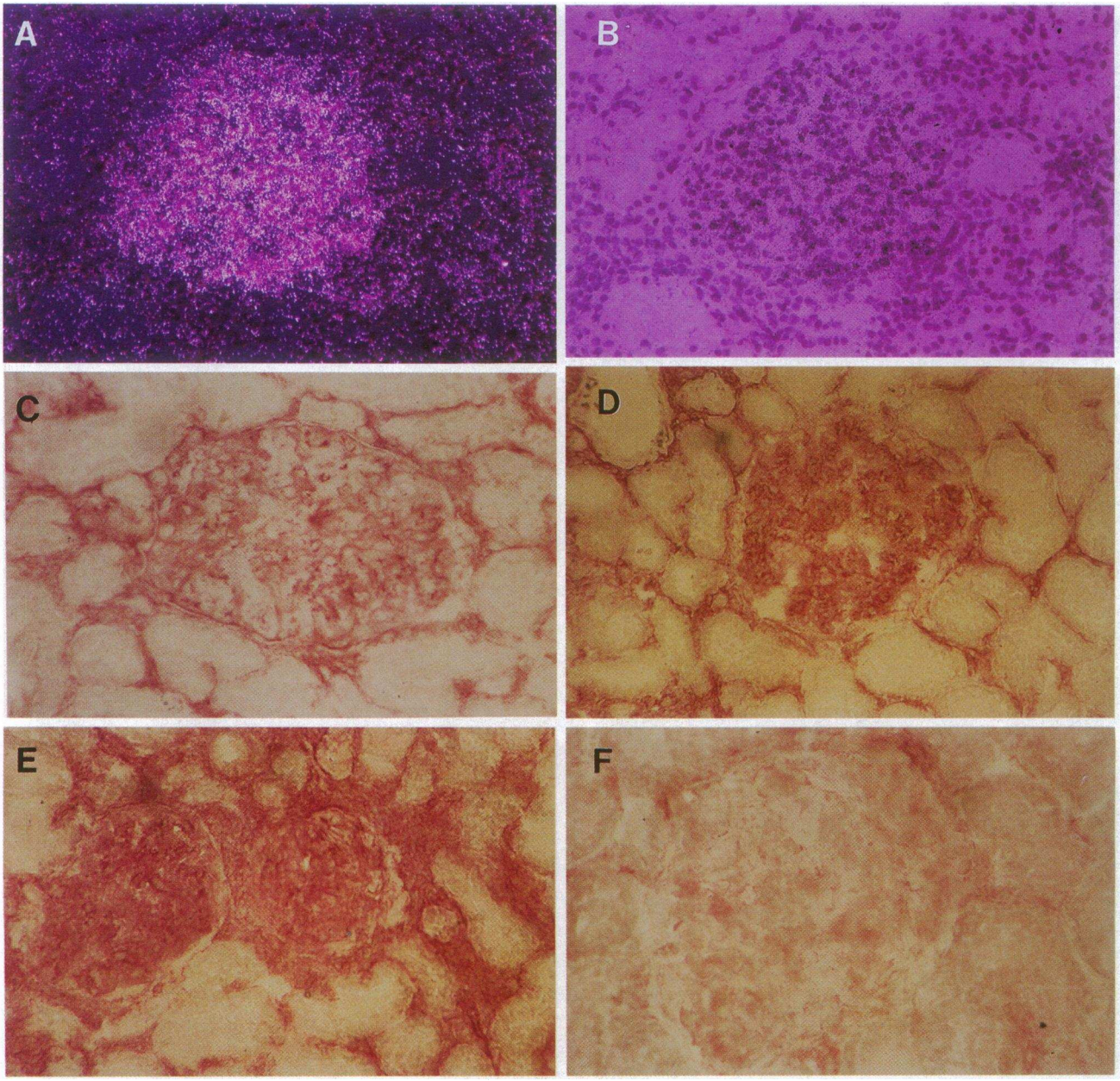

Figure 4. (*A* and *B*) PDGF-βR in situ hybridization. Dark- and bright-field photomicrographs of a case of IgAN, with intense mesangial cell proliferation (×200). Note the striking collection of bright (*A*) or dark (*B*) grains within the mesangial area. (*C–E*) An increased expression of PDGF-βR immunoreactivity is observed both in the glomerulus and the interstitium of IgAN (×200), compared to normal appearing kidney (see Fig. 1). Signal intensity is positively correlated with the grade of the histologic lesions: mild lesions (*C*) show a less intense signal than moderate (*D*) and severe (*E*) lesions (see results). (*F*) Nonproliferative GN, namely membranous GN, does not show any significant difference with normal-appearing kidney (see Fig. 1).

Table I. Plasmids Used for the Preparation of Probes

Plasmid	Species/gene	Fragment/size	Predominant region encoded	Reference
pUC 19	Human/PDGF-αR	EcoRI–Acc I/750 bp	Tyrosine kinase segment	9
pUC 19	Human/PDGF-βR	PstI–PstI/751 bp	Transmembrane, juxtamembrane and 1st tyrosine kinase segments	43

Table II. Correlation between PDGF-βR Expression and Grade of Glomerular Lesions in IgAN Patients

	PDGF-βR mRNA*	PDGF-βR protein*	Glomerular cellularity‡	Mesangial matrix area§
PDGF-βR mRNA*	1			
PDGF-βR protein*	0.868	1		
Glomerular cellularity‡	0.722	0.871	1	
Mesangial matrix area§	0.672	0.746	0.621	1

Tabular entries are Pearson's correlation coefficients (r); $t \geq 2.631$, $P < 0.02$. * Number of pixels per glomerulus. ‡ Measured as number of nuclei/1,000 μm^2 glomerular area in eight glomeruli/biopsy. § Measured as percent of total glomerular area occupied by mesangial matrix.

Similar patterns of distribution were observed by APAAP staining. Protein expression was present diffusely within mesangial area and focally at the apical surface of parietal epithelial cells (Fig. 1 *E*). PDGF-βR expression was scattered in the interstitium, and especially in mesenchimal peritubular cells, whereas tubular cells were constantly negative (Fig. 1 *F*).

PDGF-αR expression, as evaluated by in situ hybridization and immunohystochemical techniques, strictly mirrored the distribution of PDGF-βR reported above (Fig. 2, *A–D*). Noteworthy, both gene transcription and protein synthesis of PDGF-αR in normal human kidney exhibited a rather low level of expression, thus suggesting that PDGF-βR is the major target for PDGF within resident renal cells.

Finally, smooth muscle cells of the media and adventitial connective tissue cells of intrarenal arteries showed a fairly detectable, but constant, hybridization with PDGF-βR probe (Fig. 3, *A* and *B*). An increased expression of PDGF-βR mRNA and its protein was observed in the kidney specimens of patients suffering from proliferative GN, namely IgA nephropathy and class IV lupus nephritis. In IgA nephropathy (Fig. 4, *A–E*), PDGF-βR upregulation was detected chiefly within the mesangium, the glomerular parietal epithelial cells, and the interstitium, while tubular profiles remained consistently negative. Moreover, a strict correlation was observed between PDGF-βR expression and the severity of histologic lesions (Tables II and III; Fig. 4, *C–E*). In lupus nephritis (Fig. 5, *A–C*), PDGF-βR expression mirrored the pattern observed in IgA nephropathy. Interestingly, an increased expression of PDGF-βR was observed also in glomerular crescents of lupus nephritis (Fig. 6, *A–C*) being strictly limited to its cellular component. In contrast, specific staining in non proliferative GN, i.e., membranous GN (Fig. 4 *F*) and minimal change disease (not shown), was similar to that observed in normal tissues. Finally, kidneys with proliferative glomerular lesions exhibited an upregulation of PDGF-βR within the vessel media and the adventitia both at transcriptional (Fig. 3, *C–F*) and posttranscriptional levels (not shown). The increased PDGF-βR expression was strictly correlated with the severity and extension of the vascular lesions (hypercellularity and sclerosis, both $P < 0.01$).

As regards PDGF-αR expression, diseased kidneys exhibited only a slight increase, predominantly at the interstitial level. Noteworthy, a few cases of lupus nephritis showed a moderate increase of PDGF-αR both at the glomerular and the interstitial level (not shown).

Table III. Correlation between PDGF-βR Expression and Grade of Interstitial Lesions in IgAN Patients

	PDGF-βR mRNA*	PDGF-βR protein*	Intersitial cellularity‡	Interstitial fibrosis§
PDGF-βR mRNA*	1			
PDGF-βR protein*	0.858	1		
Interstitial cellularity‡	0.842	0.851	1	
Interstitial fibrosis§	0.627	0.671	0.745	1

Tabular entries are Pearson's correlation coefficients (r); $t \geq 2.672$, $P < 0.02$. * Number of pixels per single 1,000 μm^2 tubulo–interstitial area. ‡ Measured as number of nuclei/1,000 μm^2 tubulo-interstitial area in eight randomly selected microscopy fields/biopsy. § Percent of single 1,000 μm^2 tubulo–interstitial area occupied by fibrosis in eight randomly selected microscopy fields/biopsy.

Discussion

It has been previously reported that human mesangial cells in vitro predominantly express PDGF-βR (8). In the present study, we confirm, by Northern blot analysis, that cultured mesangial cells exhibit a strong constitutive expression of a 5.7-kb transcript for PDGF-βR (Fig. 7). This expression apparently accounts for the relevant mitogenic response elicited by PDGF-BB and -AB and, conversely, the very weak effect evoked by PDGF-AA isoform in cultured cells (8). Recently, it has been elegantly shown that rat mesangial cells in vivo proliferate also in response to exogenous infusion of PDGF-BB (31). Finally, Silver et al. (32) demonstrated that mesangial cells, when stimulated by PDGF, can express the growth factor. Thus, PDGF can act on mesangial cells in an autocrine fashion. An upregulation of PDGF and its β receptor in mesangial region has been described in experimental mesangial proliferative GN (16–19), along with a reduction of mesangial cell proliferation after anti-PDGF antibody treatment of rats with proliferative glomerular lesions (anti-Thy.1 model) (33). Furthermore, an increased expression of PDGF-βR protein was reported also in diseased human kidneys (34). This study provides further support to the hypothesis that PDGF-βR upregulation in mesangium may contribute to the amplification of mesangial cell response to PDGF during glomerular injury in humans.

Alpers et al. (22) demonstrated that normal human and primate kidneys frequently express PDGF-βR protein on the apical and lateral surface of glomerular parietal epithelial cells. The increase of PDGF-βR gene expression and protein at the surface of parietal epithelial cells described here seems particularly interesting. It suggests that parietal epithelial cells may also participate in the inflammatory response to glomerular damage, possibly through the binding of PDGF-B chain released by infiltrating or adjacent glomerular cells, in a classical paracrine fashion (35).

Renal interstitial cells from rabbits and humans have been shown to proliferate in response to PDGF in vitro (20–21). Immunohistochemistry studies clearly localized PDGF-βR protein on the surface of peritubular cortical and medullary interstitial cells of human kidney (22). Here we confirm the constitutive expression of PDGF-βR gene and protein by interstitial

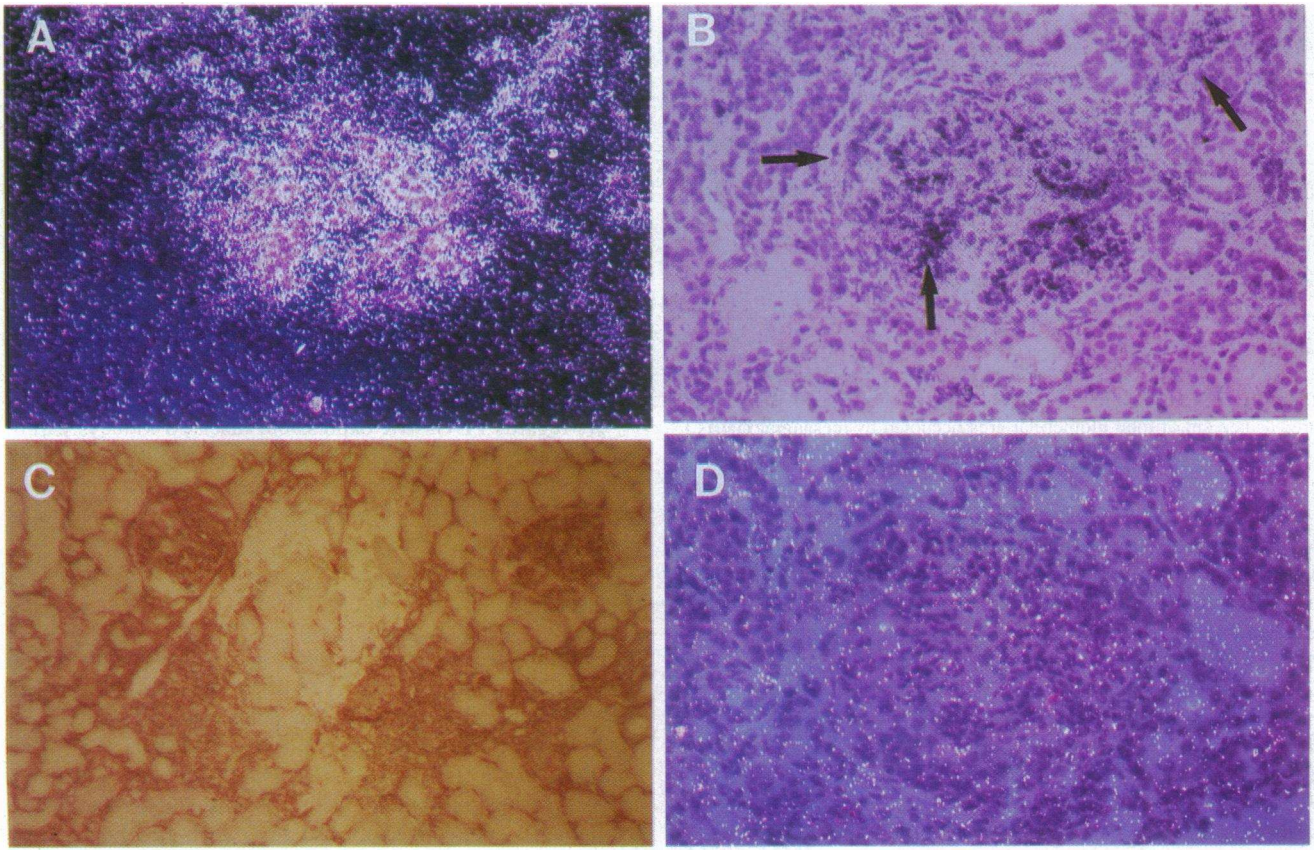

Figure 5. PDGF-βR expression in class IV lupus nephritis. (*A* and *B*) Dark- and bright-field photomicrographs (×200), showing an intense signal of hybridization within the mesangium, parietal epithelial cells and interstitium (*arrows*). (*C*) Immunohistochemistry shows the upregulation of PDGF-βR at the protein level. An increased expression of β receptor protein is evident both within the glomerulus and the interstitial area, while tubular profiles are consistently negative (×100). (*D*) Sense probe control. Dark-field microphotograph (×200) of a section serial to that shown in *A* and *B*. This section was hybridized with sense probe for PDGF-βR and developed in parallel with that shown in *A* and *B*. Note the absence of hybridizing cells, compared with *A* and *B*.

cells at both the cortical and medullary level. The increase of PDGF-βR expression in the interstitium of diseased kidneys, on the other hand, supports the view that the extent of interstitial involvement in human nephropathies, regardless of the primary site of injury, may be crucial for the fate of renal lesions. Again, the release of PDGF by infiltrating cells (36, 37) or by adjacent tubular epithelial cells (20, 21) may serve as a stimulus for the recruitment and activation of interstitial fibroblasts expressing an upregulated PDGF-βR.

Two findings need to be further addressed. First, PDGF-αR expression was uniformly low in normal specimens and showed only minor modifications in almost all diseased kidneys. In fact, PDGF-R is subject to regulation by PDGF itself as well as by other peptides (38–42). It has been argued that very few PDGF-αRs, in the presence of very high amounts of PDGF A-chain mRNA, may represent a mechanism aimed to prevent autocrine growth of mesangial cells in normal glomeruli (42). Were this view true, it may well be that overexpression of PDGF or other growth factors in human GN could modulate PDGF-αR expression in target cells, while increasing PDGF-βR mRNA transcription.

Second, we found a positive and constant signal for PDGF-βR in smooth muscle cells of the media of arterial vessels. This finding seemingly contradicts previous reports (22, 33) which depicted, by immunohystochemical techniques, a renal distribution of PDGF-βR closely resembling that described here, except for the above finding. Indeed, PDGF exerts a well recognized proliferative action on vascular smooth muscle cells in vitro and plays a pathogenetic role in the process of atherogenesis, largely due to its mitogenic effect on smooth muscle cells. Furthermore, injured arteries of diseased kidneys usually display a striking expression of PDGF-βR (Fig. 3, *C–F*) (34). Therefore, the presence of a scattered positive signal for PDGF-βR in the media of undamaged kidney arterial vessels is largely expected, and may become apparent according to the different methodology of investigation adopted.

To our knowledge, this is the first report demonstrating PDGF-αR and -βR mRNA expression in vivo in normal and diseased kidney by in situ hybridization techniques. Moreover, we showed for the first time an upregulation of PDGF-βR expression at the interstitial level in human proliferative GN. On the basis of PDGF properties, the demonstration of an upregulation of PDGF-βR expression within the glomerulus and the interstitium of diseased kidneys strongly supports the role of PDGF receptor activation in the modulation of inflammatory and proliferative events seen in proliferative GN. Furthermore,

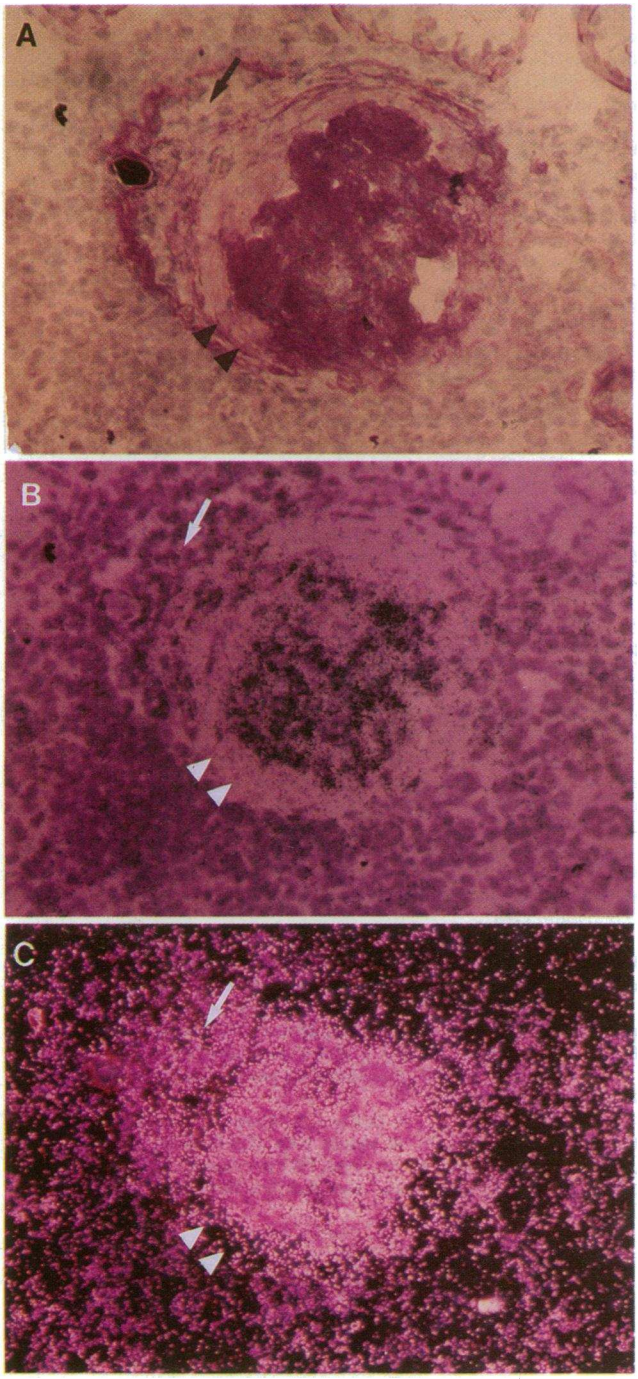

Figure 6. (*A*) Light microscopy photomicrograph showing a fibrocellular crescent of a glomerulus stained with periodic acid Schiff's solution (PAS). (*B*) Bright- and (*C*) dark-photomicrographs of the same glomerulus shown in A demonstrating a strong positivity for PDGF-βR transcript within the cellular portion of crescents (*arrows*) and, conversely, the lack of a hybridization signal in the sclerosed portions (*arrowheads*). An intense signal is present within the glomerulus and the interstitium (×250).

the constitutive expression of PDGF-βR transcript by vascular smooth muscle cells prompts the hypothesis that the growth factor may also contribute to the regulation of vascular response in physiologic as well as pathologic states.

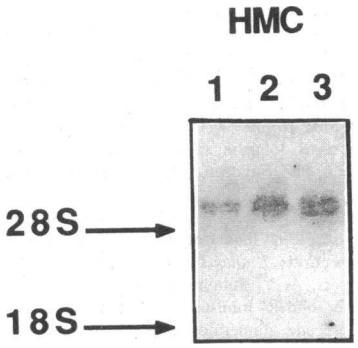

Figure 7. Northern blot analysis of PDGF-βR mRNA expression by HMC. Total cellular RNA was extracted from cultured human mesangial cells and analyzed by Northern blot for PDGF-βR. 20 μg of total cellular RNA from three different HMC lines are represented. RNA size standards are indicated on the left.

Acknowledgments

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