Kidney International, Vol. 68 (2005), pp. 2621–2628

Immunolocalization of fibroblast growth factor-1 (FGF-1), its receptor (FGFR-1), and fibroblast-specific protein-1 (FSP-1) in inflammatory renal disease

MICHELE ROSSINI, BOONYARIT CHEUNSUCHON, ELLEN DONNERT, LI-JUN MA, JAMES W. THOMAS, ERIC G. NEILSON, and AGNES B. FOGO

Department of Pathology, Vanderbilt University Medical Center, Nashville, Tennessee; and Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee

Immunolocalization of fibroblast growth factor-1 (FGF-1), its receptor (FGFR-1), and fibroblast-specific protein-1 (FSP-1) in inflammatory renal disease.

Background. The fibroblast growth factor (FGF) family has functions in development, cell proliferation, migration, and differentiation. While FGF-2 induces fibrosis, the role of FGF-1 in inflammation and fibrosis is less defined. We examined the expression of FGF-1 and FGF receptor (FGFR-1) to determine if renal diseases with varying etiologies of inflammation, including lupus nephritis (LN), acute interstitial nephritis (AIN) and acute rejection superimposed on chronic allograft nephropathy (CAN), showed varying patterns of expression. We also examined the expression of fibroblast-specific protein-1 (FSP-1), which has been linked to epithelial-mesenchymal transition (EMT) and fibrosis, to determine whether it was linked to potential profibrotic and inflammatory FGF-1 mechanisms.

Methods. Proliferative LN (PLN) ($N = 12$), nonproliferative lupus nephritis (NPLN) ($N = 5$), AIN ($N = 6$), CAN ($N = 4$), and normal kidneys ($N = 3$) were studied. FGF, FGFR-1, and FSP-1 were localized by immunohistochemistry, and intensity scored on a 0 to 3+ scale. Double staining with CD68 and separate immunohistochemical staining for CD4 and CD8 with serial sections analysis were done to identify if T lymphocytes or macrophages showed staining for FGF-1 and FGFR-1 or FSP-1.

Results. In normal kidneys, FGF-1 was expressed in mesangial cells (0.67 ± 0.58), glomerular endothelial (0.67 ± 0.58), visceral, and parietal epithelial cells (1.67 ± 0.58). FGFR-1 showed a similar pattern of staining but also was expressed in tubular epithelium, and arterial endothelium and smooth muscle. Expression of FGF-1 was increased over normal in glomerular parenchymal cells only in CAN in podocytes (2.30 ± 0.58 vs. 3.00 ± 0.00) ($P < 0.05$) and parietal epithelial cells (1.67 ± 0.58 vs. 2.25 ± 0.50) ($P < 0.05$). Infiltrating glomerular and interstitial inflammatory cells in diseased glomeruli also expressed FGF-1 and FGFR-1. Tubular cells expressed slightly increased FGFR-1 in renal diseases vs. normal, whereas tubules remained negative for FGF-1 in diseased kidneys. FSP-1 expression was prominent in the interstitium in all kidneys with interstitial inflammation, and most prominent in CAN. Interstitial FSP-1+ cells were consistent with a myofibroblast-type morphology, and did not stain with CD-68. FSP-1 expression was closely associated with inflammatory cells expressing FGF-1 and FGFR-1. FSP-1 also showed positivity within crescents and occasional podocytes in PLN.

Conclusion. The expression of FGF-1 and FGFR-1 in infiltrating lymphocytes and macrophages, and of FGFR-1 in tubules, is supportive, but does not prove causality, of the possibility that FGF-1 might have both autocrine and paracrine functions in renal inflammation. However, the initial stimulus for renal inflammation, whether immune complex, hypersensitivity or rejection, did not alter expression patterns of FGF-1 or its receptor. The colocalization of inflammatory infiltrates with interstitial fibrosis supports the possibility of a contribution of FGF-1 for chemotaxis and associated fibrosis, further supported by interstitial FSP-1 expression closely associated with these inflammatory cells expressing FGF-1 and FGFR-1.

Key words: fibroblast growth factor, FGFR-1, kidney disease, inflammation.

Received for publication October 26, 2004
and in revised form May 16, 2005, and June 28, 2005
Accepted for publication July 14, 2005

Progressive renal disease, regardless of the initiating insult, is characterized by glomerulosclerosis, tubulointerstitial fibrosis, and vascular sclerosis. Numerous factors, including growth factors, chemokines, cytokines, and reactive oxygen species, have been implicated in the process of progressive renal scarring. Tubular epithelial cells contribute to this process by changing their phenotype with expression of a fibroblast-specific protein-1 (FSP-1), and migrating into the interstitium, so-called epithelial-mesenchymal transition (EMT) [1, 2].

The fibroblast growth factor (FGF) family includes at least 23 heparin-binding proteins with different biologic functions in developmental and repair processes of tissues [3]. FGFs act through binding to high-affinity tyrosine kinase receptors (FGFR) encoded by four genes that by alternative mRNA splicing can generate seven functional FGFR variants [4]. During embryonic development, FGF/FGFR signals play a major role in mesoderm organization, body axis and neural axis formation, and tissue/organ induction [5–7].

While some FGF members such as FGF-2 [basic FGF (bFGF)] have been proven to play a role in the pathogenesis of renal disease by inducing fibrosis and contributing to renal damage in immune-mediated injury [8–10], the role of FGF-1 [acidic FGF (aFGF)] in renal disease is less defined. FGF-1 may play a role in inflammatory disease such as rheumatoid arthritis. FGF-1 expression was increased in synovial cells of patients with rheumatoid arthritis and enhanced expression of its receptor (FGFR-1) was found in perivascular CD4+ T lymphocytes at sites of injury. Furthermore, T cells activated by FGF-1 were also increased in the peripheral blood of these patients [11]. Transforming growth factor-β (TGF-β) increased FGF-1 production by cultured synovial cells [12].

FGF-1 may also play a role in the fibrotic response to tissue injury. FGF-1 and FGFR are actively synthesized during the development of pulmonary fibrosis induced in rats with paraquat plus hyperoxia [13]. The entry of fibroblasts into the interstitium and the process of EMT require initial degradation of matrix. FSP-1 is a marker of EMT. FGF-1 promotes collagenase and urokinase-like plasminogen activator receptor (u-PAR) production, both of which promote these processes. Expression of FSP-1 and FGF-1 therefore could be linked, reflecting a potential interrelationship in inflammation, EMT, and fibrosis.

Several studies have evaluated the localization of FGF-1 and its receptor FGFR-1 in the normal kidney, both in experimental models and in humans. Ichimura et al [14] observed expression of FGF-1 in distal tubule elements, as well as in blood vessel and glomeruli of adult rats. Also in rats, Cancilla et al [15] observed expression of FGFR-1 in glomeruli, thin limbs, distal straight tubules, distal convoluted tubules, medullary collecting ducts, and smooth muscle of renal arteries. In humans, Floege et al [16] observed expression of FGFR-1 protein and mRNA in tubular cells of the distal nephron and in vascular smooth muscle cells.

However, few studies have explored the role expression of FGF-1 in renal disease, or even more specifically in inflammatory/fibrotic renal diseases. Ichimura et al [14] showed that FGF-1 may play an autocrine and paracrine role in the regenerative changes observed in the rat proximal tubular epithelium following administration of S-(1,1,2,2,-tetrafluoroethyl)-L-cysteine, In vascular lesions of kidney allografts with chronic allograft nephropathy (CAN), there was increased expression of both FGF-1 protein and mRNA in resident inflammatory and tubular epithelial cells in tubulointerstitial lesions [17], and increased expression of FGF-1 and its receptors in vascular lesions of kidney allografts with CAN [18], suggesting that FGF-1 may act as an important mediator of growth and repair responses associated with development of vascular and tubulointerstitial lesions occurring in CAN.

Table 1. Clinical findings at time of biopsy/nephrectomy

Diagnosis	Age *years*	Systolic blood pressure *mm Hg*	Serum creatinine *mg/dL*	Proteinuria *g/24 hours*
Proliferative lupus nephritis	27.6 ± 4.4	132.4 ± 5.7	1.6 ± 0.4	4.5 ± 1.7
Nonproliferative lupus nephritis	20.6 ± 3.0	111.3 ± 4.9	0.9 ± 0.2	2.3 ± 0.7
Acute interstitial nephritis	43.8 ± 6.5	124.0 ± 13.0	5.1 ± 1.6	0.3 ± 0.2
Chronic allograft nephropathy	31.3 ± 4.0	N/A	10.1 ± 1.3	N/A
Normal	24.7 ± 8.7	N/A	N/A	N/A

Data are shown as mean ± SE. N/A is data not available.

We therefore sought to examine the expression of FGF-1 and its receptor specifically in various inflammatory renal diseases with interstitial inflammation, including lupus nephritis (LN), acute interstitial nephritis (AIN), and acute rejection superimposed on CAN, and examine any linkage to type of inflammatory infiltrate and fibrosis. We also further examined the colocalization of FSP-1, which has been linked to EMT and interstitial fibrosis.

METHODS

Tissue specimens

Formalin-fixed, paraffin-embedded renal tissues from core needle biopsies in normal control kidneys ($N = 4$) proliferative LN (PLN) ($N = 12$), nonproliferative LN (NPLN) ($N = 5$), AIN ($N = 6$), and from transplant nephrectomies with acute rejection and CAN ($N = 4$) were included in this study after the diagnostic workup was completed. Clinical data from these patients at renal biopsy are shown in Table 1. Of note, normal tissue was from patients in whom further clinical records were unavailable to us due to HIPA regulations. There were nine women and three men with PLN, four women and one man with NPLN, three women and two men with AIN, and two women and two men with CAN. Gender was known for three normal controls, and included two women and one man. As expected by clinical indications for biopsy, patients with AIN and CAN had higher serum creatinine levels than PLN or NPLN, whereas the latter had higher levels of proteinuria. Corticosteroid treatment had been given to seven PLN and four each NPLN and AIN patients. In addition, one NPLN patient had received cytotoxic therapy. Most LN patients had received nonsteroidal anti-inflammatory drugs, nine PLN and three NPLN. All transplant patients were on various immunosuppressive drugs, including corticosteroids in all and calcineurin inhibitors in most.

Normal control kidneys showed no evidence of abnormalities by light, immunofluorescence, and electron microscopy. The PLN group was composed of seven patients

with World Health Organization (WHO) class IV [International Society of Nephrology/Renal Pathology Society (ISN/RPS) IV S (A), one; S (A/C), three; and G (A/C), three] and five with WHO class III (ISN/RPS III A, two; and A/C, three) LN [19]. Three of class IV and two of class III cases had crescents. All PLN cases had increased intraglomerular leukocytes and interstitial infiltrates. The NPLN group was composed of two patient with WHO class II (ISN/RPS II) and three with WHO class V (ISN/RPS V) LN. All these latter biopsies had less interstitial inflammatory infiltrates and few intraglomerular leukocytes compared to the PLN group. All biopsies in the AIN group showed significant interstitial eosinophils. Therefore, drug-induced hypersensitivity was thought to represent the most likely underlying etiology. Only a few intraglomerular leukocytes were observed in this group. All cases in the CAN group were transplant nephrectomy specimens removed due to pain. All these CAN cases also had superimposed acute vascular and cellular rejection, type 1 and type 2, according to Cooperative Clinical Trials in Transplantation (CCTT) criteria [20].

Immunohistochemistry

FGF-1, FGFR-1, and FSP-1. Thin sections (2 μm) of the renal biopsies were deparaffinized and dehydrated by a series of xylene and alcohol washes. Following quenching of endogenous peroxidase activity with 3% (vol/vol) H_2O_2 in methanol × 10 minutes, sections were microwaved (20 minutes) in citrate buffer for antigen retrieval. The tissues were blocked with 1% (wt/vol) bovine serum albumin (BSA) and incubated with polyclonal antibodies directed against FGF-1 (1:100) (Sigma-Aldrich, St. Louis, MO, USA) or FGFR-1 (1:200) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), directed at the high-affinity signaling isoform with active kinase domain, thus detecting functional receptors, for 90 minutes at room temperature, or against FSP-1 (polyclonal rabbit anti-FSP-1) (1:200), at 37°C for 1 hour in a humidified chamber [1]. This specificity was further verified by using an FGF-Ig fusion protein to first immunoprecipitate FGFR-1 and then blot with the anti-FGFR-1 reagent [21]. In addition, monoclonal antibodies directed at the first Ig loop of FGFR-1 and inhibition with a FGFR-1 specific peptide also confirmed the specificity of the anti-FGFR-1 reagent (unpublished observations). Using recombinant FGF-1, the anti-FGF-1 reagent is highly specific for FGF-1 on Western blot, and, importantly, does not cross-react with FGF-2 (bFGF), FGF-5, or FGF-7 [11, 12]. Positive control included synovial tissue with known FGF-1 and FGFR-1 expression by Western blot, and fibrotic kidneys with FSP-1 expression. Negative controls for all antibodies included incubation of sections with preimmune serum and deletion of the primary antibody, a process that completely prevented immunostaining. Secondary antibody was added (goat antirabbit (1:50) (Southern Biotechnology Associates, Birmingham, AL, USA) and incubated for 30 minutes. Peroxidase-stained sections were then developed with 3,3′-diaminobenzidine (DAB) and counterstained with hematoxylin (Sigma-Aldrich).

Double staining with CD68 and separate stainings for CD4 and CD8 with serial sections analysis were done to identify if any macrophages or T lymphocytes showed staining for FGF-1 and FGFR-1 as follows.

CD68. Sections stained with FGF-1, FGFR-1, and FSP-1 as above were double stained with antibodies against macrophages. Powerblock (BioGenex, San Ramon, CA, USA) diluted 1:10 with PBS Triton (Sigma-Aldrich) was used to block remaining endogenous peroxidase activity. The slides were then incubated with antibodies against CD68 (1:100) (Sigma-Aldrich) at room temperature for 90 minutes. Antibody binding was detected by incubating with prediluted secondary antimouse (BioGenex) for 30 minutes followed by streptavidin-conjugated alkaline phosphatase (BioGenex) for 30 minutes. Peroxidase-stained sections were developed with Fast Red chromogen (Sigma-Aldrich). Following chromagen development, slides were coverslipped using aqueous-based mounting media. Tissue from tonsil was used a positive control.

CD4 and CD8. Additional serial sections were stained separately with antibodies against CD4 and CD8 (1:20) (Novocastra, Newcastle upon Tyne, UK). Endogenous peroxidase activity was exhausted with 0.5% (vol/vol) H_2O_2. For CD4 antigen retrieval, slides were immersed in buffer solution, placed in pressure cooker, and microwaved for 15 minutes. For CD8, slides were immersed in buffer solution and microwaved for 20 minutes. Primary antibodies were then added for 60 minutes, antimouse antibody (Ready to Use) (BioGenex) applied for 30 minutes followed by horseradish peroxidase–conjugated streptavidin for 15 minutes. The chromogen reaction was developed with DAB and the slides were counterstained with hematoxylin.

Staining scoring. Immunostaining for FGF-1, FGFR-1, CD4, CD8, and CD68 was evaluated by light microscopy in a blinded fashion. The intensity of each glomerular cell type staining was scored on a 0 to 3+ scale: 0, no staining identified; 1+, less than 25% of glomerular cells positive; 2+, 25% to 75% of glomerular cells positive; and 3+, more than 75% of glomerular cells positive. Resident glomerular cells were identified by morphology and anatomic location, as double staining could technically not be performed due to different pH requirements for each of the antibodies. Macrophages were identified by positivity for CD68. Expression of FGF-1, FGFR-1, and FSP-1 was evaluated by double immunostaining technique for CD68. FSP-1 staining was qualitatively assessed, and identification of staining in CD68-negative cells done by morphologic assessment. CD4 and CD8+ T cells were identified by staining and their expression

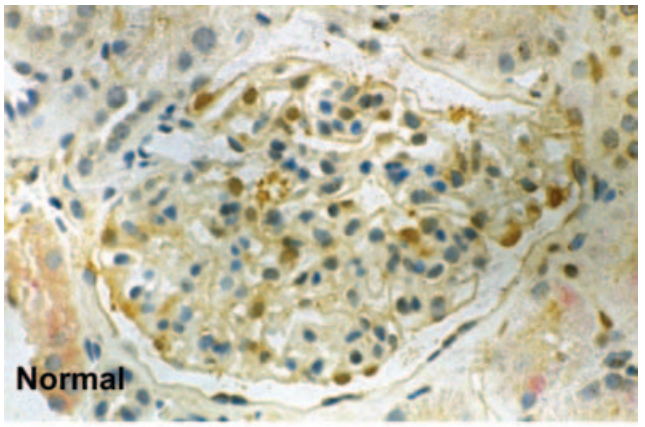

Fig. 1. Fibroblast growth factor-1 (FGF-1) expression was moderately increased in podocytes and parietal epithelial cells in occasional diseased kidneys, as seen here in chronic allograft nephropathy (CAN) (anti-FGF-1 antibody, 400×).

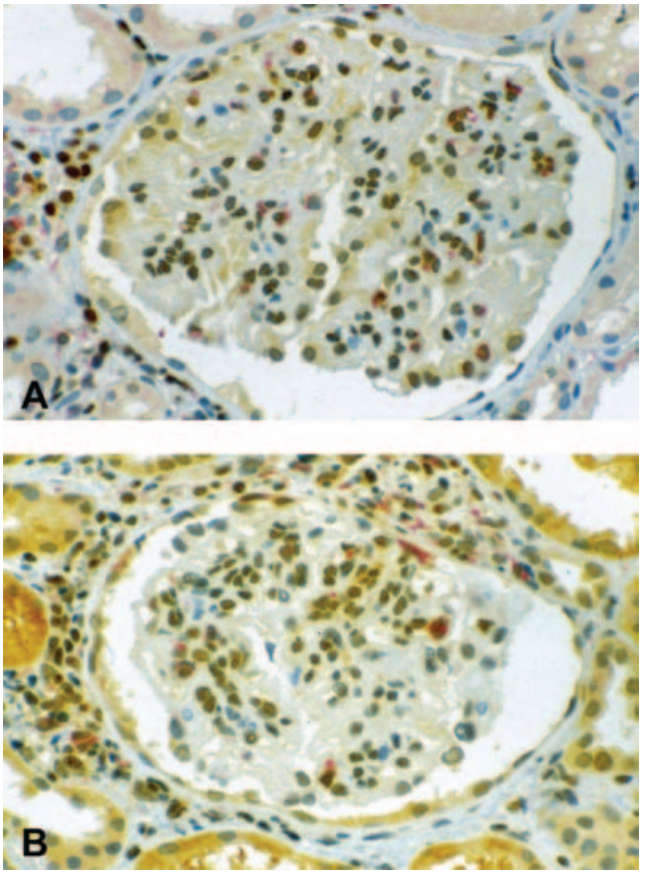

Fig. 2. Infiltrating inflammatory cells, identified as macrophages by double staining (red) in glomerulus of proliferative lupus nephritis, also showed expression of fibroblast growth factor-1 (FGF-1) (brown) (*A*) and FGF receptor-1 (FGFR-1) (brown) (*B*) [anti-FGF-1 + CD68 antibodies (A), anti-FGFR-1+ CD68 antibodies (B), both 400×].

of FGF-1 and FGFR-1 was evaluated by comparison between consecutive serial sections. Tubular, interstitial, and vascular stainings were semiquantitatively evaluated on a scale from 0 to 3+ (0, absent staining; 1+, moderately strong staining in less than 25% of cells; 2+, strong staining in 25% to 50% of the cells; and 3+, very strong staining in >50% of cells).

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Statistical difference was assessed by analysis of variance (ANOVA) followed by *t* test or Mann-Whitney U for nonparametric data as appropriate. A P value <0.05 was considered to be significant.

RESULTS

Glomeruli

Immunohistochemical analysis in normal control kidneys showed localization of FGF-1 in mesangial cells (0.67 ± 0.58), glomerular endothelial cells (0.67 ± 0.58), podocytes (2.30 ± 0.58), and parietal epithelial cells (1.67 ± 0.58). FGFR-1 showed a similar localization pattern of staining (mesangial cells 1.33 ± 0.58; glomerular epithelial cells 1.33 ± 0.58; podocytes 2.33 ± 0.58; and parietal epithelial cells 2.33 ± 0.58) (Table 2). FGF-1 expression in parenchymal cells in diseased kidneys was significantly increased compared to normal controls only in podocytes (2.30 ± 0.58 vs. 3.00 ± 0.00) ($P < 0.05$) and parietal epithelial cells (1.67 ± 0.58 vs. 2.25 ± 0.50) ($P < 0.05$) in CAN (Fig. 1). Other glomerular parenchymal cells of diseased kidneys showed no significant change in the expression of FGF-1 or FGFR-1 compared to normals.

Diseased glomeruli, especially those from PLN, had increased intraglomerular infiltrating inflammatory cells (mostly macrophages) expressing FGF-1 and FGFR-1 (Fig. 2). The localization of FGF-1 and FGFR-1 in macrophages and T lymphocytes was confirmed by double staining for CD68, and by serial sections analysis with separate staining for CD4 and CD8.

FSP-1 was localized within organizing crescents and occasional podocytes in PLN (Fig. 3). Double staining revealed that FSP-1+ cells did not stain with CD68.

Table 2. FGF-1 and FGFR-1 expression in glomerular parenchymal cells

Cell type	Groups	FGF-1 (Mean ± SD)	FGFR-1 (Mean ± SD)
Mesangial cell	AIN	1.20 ± 0.83	2.20 ± 1.09
	CAN	1.50 ± 0.58	1.00 ± 0.81
	PLN	1.29 ± 1.10	1.67 ± 1.15
	NPLN	1.60 ± 1.14	1.40 ± 1.14
	Normal	0.67 ± 0.58	1.33 ± 0.58
Glomerular endothelial cell	AIN	1.60 ± 1.34	2.40 ± 0.89
	CAN	1.00 ± 0.00	0.50 ± 1.00
	PLN	1.08 ± 1.08	1.67 ± 1.15
	NPLN	1.40 ± 1.14	1.40 ± 0.89
	Normal	0.67 ± 0.58	1.33 ± 0.58
Podocyte	AIN	2.40 ± 0.55	2.40 ± 0.89
	CAN	3.00 ± 0.00[a]	1.50 ± 1.29
	PLN	2.50 ± 0.80	2.17 ± 0.83
	NPLN	2.40 ± 0.89	2.00 ± 1.00
	Normal	2.30 ± 0.58	2.33 ± 0.58
Parietal epithelial cell	AIN	2.20 ± 0.83	2.60 ± 0.54
	CAN	2.25 ± 0.50[a]	2.00 ± 0.81
	PLN	1.90 ± 0.79	2.54 ± 0.52
	NPLN	2.20 ± 0.83	2.80 ± 0.44
	Normal	1.67 ± 0.58	2.33 ± 0.58

The expression pattern of FGF-1 and its receptor did not change in glomeruli of diseased kidneys with varying inflammatory processes. There was increased expression of FGF-1 only in CAN in podocytes and parietal epithelial cells.

[a] $P < 0.05$ compared to normal.

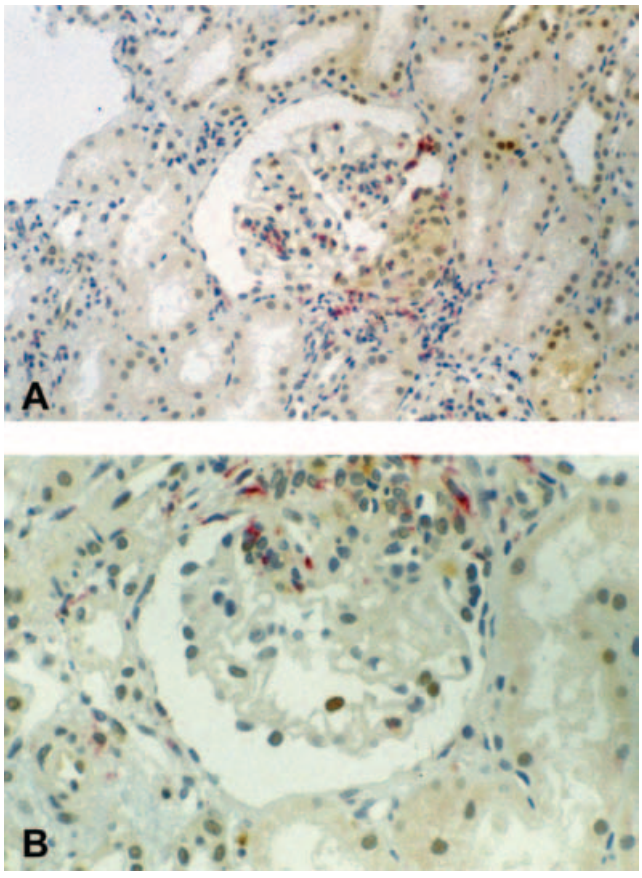

Fig. 3. Fibroblast-specific protein-1 (FSP-1) (brown) showed positivity within crescents (*A*) and occasional podocytes (*B*) in proliferative lupus nephritis (PLN) [anti-FSP-1+ CD68 antibodies, 400× (A) and 200× (B)].

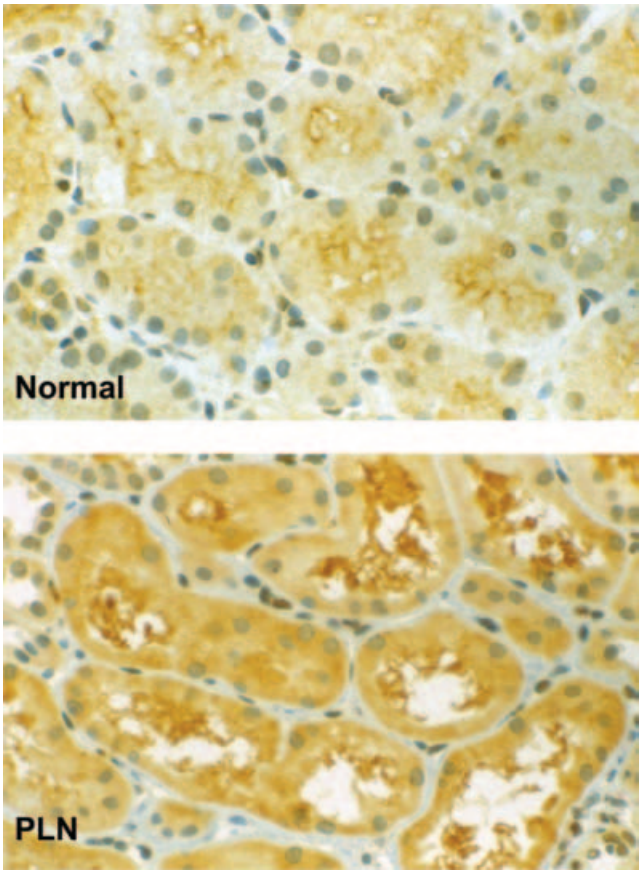

Fig. 4. Tubular cell expression of fibroblast growth factor receptor-1 (FGFR-1) was slightly increased in diseased kidney, illustrated by proliferative lupus nephritis (PLN) (*B*) vs. normal (*A*) (anti-FGFR-1 antibody, both 400×].

Tubulointerstitial compartment

In normal kidneys, FGF-1 was not expressed in tubular epithelial cells. In contrast, FGFR-1 was normally localized in tubular epithelium, and also in arterial endothelium and smooth muscle. In kidneys with inflammatory conditions, tubular epithelial cells expressed slightly increased FGFR-1 compared to normal controls (Fig. 4), whereas FGF-1 remained negative in tubules.

Double staining for CD 68 and serial sections analysis with separate staining for CD4 and CD8 showed that interstitial macrophages, CD4+ and CD8+ T lymphocytes frequently expressed FGF-1 and FGFR-1 in inflammatory infiltrates of diseased kidneys, most prominently in PLN, AIN, and CAN with rare positive inflammatory cells in NPLN (Fig. 5). These inflammatory cell infiltrates were closely associated with areas of interstitial fibrosis.

FSP-1 expression was prominent in the interstitium of all kidneys with interstitial inflammation, and most prominent in CAN (Fig. 6). Occasional atrophic tubules showed FSP-1 positivity. Interstitial FSP-1+ cells were consistent with a myofibroblast-type morphology. Double staining, again, revealed that FSP-1+ cells did not

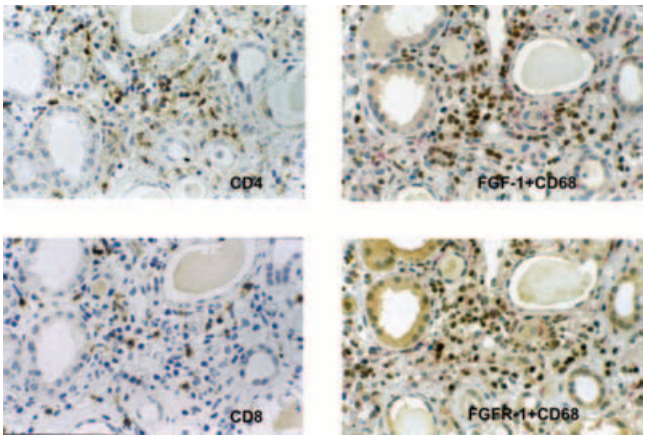

Fig. 5. CD4/CD8 T lymphocytes in interstitium of proliferative lupus nephritis (PLN) kidneys (left panel) also expressed both fibroblast growth factor-1 (FGF-1)/FGF receptor-1 (FGFR-1), as did CD68+ macrophages (right panel, serial section of left panel) (all 200×).

stain with CD68. In the interstitium, FSP-1 expression was closely associated with the presence of fibrosis and of inflammatory cells (i.e., macrophages, CD4 and CD8 T lymphocytes) expressing FGF-1 and its receptor FGFR-1.

DISCUSSION

FGF-1 and FGF-2 are members of a family of heparin-binding growth factors that stimulate proliferation, migration, and differentiation of cells of neuroectodermal and mesodermal origin. FGF-2 has been proven to play a role in the pathogenesis of renal disease by inducing fibrosis and contributing to renal damage in immune-mediated injury [8–10]. The role of FGF-1 in kidney disease in less known. FGF-1 is widely expressed during normal embryonic development and there is evidence suggesting that it may also play a role in adult tissues at sites of injury in different phases of the wound healing process [4].

FGF-1 expression is increased in some immune-mediated disorders, including rheumatoid arthritis and cardiac and renal allograft undergoing chronic rejection. FGF-1 and its receptor FGFR-1 are overexpressed in synovial cells of patients with rheumatoid arthritis. In addition, a subset of FGFR-1–bearing CD4+ T-lymphocytes was found in the blood of these patients [11]. For CD4+ T cells that express FGFR-1, FGF-1 may provide a costimulatory signal required for interleukin (IL)-2 production [22, 23]. Increased FGF-1 and FGFR-1 have been found to correlate with accelerated graft arteriosclerosis (AGA) in human heart allografts. Further, soluble FGFR-1, capable of binding both FGF-1 and FGF-2, inhibited AGA in rats undergoing aortic transplant [24].

The potential role of FGF-1 role in kidney disease has been explored in only a few studies, mainly in transplants. Whether this growth factor also could play a role in other

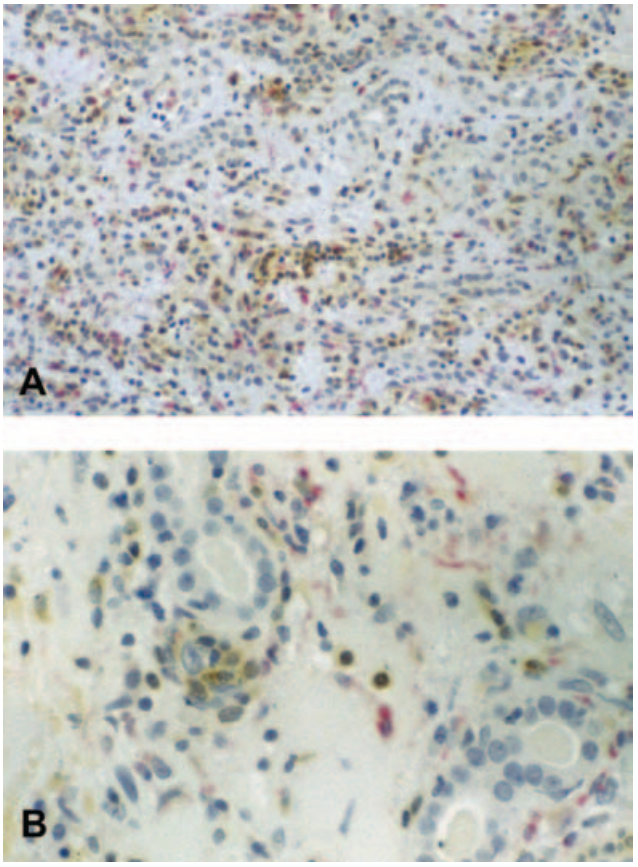

Fig. 6. Fibroblast-specific protein-1 (FSP-1) expression was most prominent in the interstitium of chronic allograft nephropathy (CAN) in cells with myofibroblast-like morphology. FSP-1+ cells did not stain with CD68 [anti-FSP-1+ CD68 antibodies, 200× (*A*) and 400× (*B*)].

inflammatory conditions is not known. Previous studies found increased FGF-1 and FGFR-1 expression in transplanted kidneys with CAN in blood vessels and tubulointerstitial compartment compared to controls, particularly in area of intimal proliferation and inflammation [17, 18]. In the glomeruli, increased of FGF-1 was observed only in visceral and parietal epithelial cells [25], similar to the current study. Further, in a murine model of autoimmune nephritis there was no change in FGF-1 [26].

In the present study, we examined the expression of FGF-1 and its receptor in various inflammatory renal diseases, LN, AIN, and acute rejection superimposed on CAN, and examined whether there was any linkage to type of inflammatory infiltrate and fibrosis. We found that renal inflammation, whether immune complex, hypersensitivity or rejection type, did not alter expression patterns of FGF-1 or its receptor in glomeruli. There was significantly increased glomerular parenchymal FGF-1 expression only in CAN, in visceral and parietal epithelial cells, confirming previous studies in transplants [25]. We also observed that infiltrating cells, including macrophages, CD4- and CD8-positive T lymphocytes, expressed FGF-1

and its receptor FGFR-1. In the interstitium the inflammatory cell infiltrates and corresponding FGF-1 and FGFR-1 expression were mainly associated with areas of fibrosis. In addition, compared to normal controls, FGFR-1 expression was also slightly increased in tubules of diseased kidneys, where FGF-1 remained negative. These findings suggest the possibility that FGF-1 produced by inflammatory infiltrating cells might have both autocrine and paracrine functions in renal inflammation and associated fibrosis, but do not prove causality of this hypothesis. These results also confirm the work of others suggesting that infiltrating cells, a source of FGF-1, might play a role through their mitogenic properties in tubulointerstitial remodeling after injury [27, 28].

We also examined FSP-1 expression and any possible link with the FGF-1/FGFR-1 axis. FSP-1 is a fibroblast-specific protein and is expressed by tubular epithelial cells that undergo EMT, a process in which the tubular epithelial cell changes its phenotype and migrates into the interstitium where it can participate in the scarring process [1]. Our study shows that FGFR-1 was expressed in the normal tubular epithelium and that its expression was slightly increased in tubules of diseased kidneys. We also found that expression of FSP-1 was prominent in the interstitium of all kidneys with interstitial inflammation, a process often associated with fibrosis. Further, we found that interstitial FSP-1+ cells did not stain with CD68, a macrophage marker, and by morphology FSP-1 appeared to be positive in myofibroblast type cells and not lymphocytes. The interstitial inflammatory cells (i.e., macrophages, CD4 and CD8 T lymphocytes) expressed FGF-1 and its receptor FGFR-1. Of note, increased expressions of molecules in human renal disease do not prove causality of these proteins in the disease process. However, this data support the hypothesis that the FGF-1/FGFR-1 axis could play a role in the EMT and fibrosis evidenced by colocalized FSP-1 expression. Further specific manipulations in experimental systems are necessary to explore this possibility. These data also support that FSP-1 is a marker of fibrogenesis in human tissue as well as in rodents. In addition, FSP-1 also showed positivity within organizing crescents and occasional podocytes in PLN, suggesting the hypothesis that not only tubular cells, but also injured podocytes in inflammatory diseases may transition to a profibrotic state. Podocyte dedifferentiation occurs in a severe glomerular injury seen in collapsing glomerulopathy or human immunodeficiency virus (HIV)-associated nephropathy [29]. However, the expression of FSP-1, a marker of EMT, has not to our knowledge been documented in podocytes before. FGF-1 and its receptor FGFR-1 might also play a role in this process since they are expressed in those glomerular parenchymal cells. More studies are needed to investigate this hypothesis and to specifically determine whether the FGF-1/FGFR-1 axis is linked to the EMT.

CONCLUSION

Our results show that the localization of FGF-1 and its receptor is not modified in glomerular parenchymal cells of inflammatory renal disease. However, FGF-1 and FGFR-1 are increased in inflammatory diseases in macrophages, CD4- and CD8-positive T lymphocytes infiltrating both glomeruli and interstitium. In the interstitium FGF-1 and FGFR-1–bearing inflammatory cells are mainly localized in areas of interstitial fibrosis, in areas with increased FSP-1. Whether the FGF-1/FGFR-1 axis also plays a role in EMT in podocytes is a hypothesis that merits further investigation. FGFR-1, but not FGF-1, is increased and expressed in tubular epithelial cells of diseased kidneys. Our data thus suggest that FGF-1 produced by infiltrating inflammatory cells might play an autocrine and paracrine role in tubulointerstitial remodeling and EMT after injury.

Reprint requests to Agnes B. Fogo, M.D., Department of Pathology, MCN C-3310, Vanderbilt University Medical Center, Nashville, TN 37232.
E-mail: agnes.fogo@vanderbilt.edu

REFERENCES

1. Iwano M, Plieth D, Danoff TM, *et al*: Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J Clin Invest* 110:341–350, 2002
2. Kalluri R, Neilson EG: Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest* 112:1776–1784, 2003
3. Cancilla B, Davies A, Cauchi JA, *et al*: Fibroblast growth factor receptors and their ligand in the adult rat kidney. *Kidney Int* 60:147–155, 2001
4. Powers CJ, McLeskey SW, Wellstein A: Fibroblast growth factors, their receptors and signaling. *Endocr Relat Cancer* 7:165–197, 2000
5. Boulet AM, Moon AM, Arenkiel BR, Capecchi MR: The roles of Fgf4 and Fgf8 in limb bud initiation and outgrowth. *Dev Biol* 273:361–372, 2004
6. Jung J, Zheng M, Goldfarb M, Zaret KS: Initiation of mammalian liver development from endoderm by fibroblast growth factors. *Science* 284:1998–2003, 1999
7. Sil AK, Maeda S, Sano Y, *et al*: IkappaB kinase-alpha acts in the epidermis to control skeletal and craniofacial morphogenesis. *Nature* 428:660–664, 2004
8. Floege J, Kriz W, Schulze M, *et al*: Basic fibroblast growth factor augments podocyte injury and induces glomerulosclerosis in rats with experimental membranous nephropathy. *J Clin Invest* 96:2809–2819, 1995
9. Shankland SJ, Floege J, Thomas SE, *et al*: Cyclin kinase inhibitors are increased during experimental membranous nephropathy: Potential role in limiting glomerular epithelial cell proliferation in vivo. *Kidney Int* 52:404–413, 1997
10. Strutz F, Zeisberg M, Ziyadeh FN, *et al*: Role of basic fibroblast growth factor-2 in epithelial-mesenchymal transformation. *Kidney Int* 61:1714–1728, 2002
11. Byrd V, Zhao X-M, McKeehan WL, *et al*: Expression and functional expansion of fibroblast growth factor receptor T cell in rheumatoid synovium and peripheral blood of patients with rheumatoid arthritis. *Arthritis Rheum* 39:914–922, 1996
12. Thomas JW, Thieu T-H, Byrd VM, Miller GG: Acidic fibroblast growth factor in synovial cells. *Arthritis Rheum* 43:2152–2159, 2000
13. Barrios R, Pardo A, Ramos C, *et al*: Upregulation of acidic fibroblast growth factor during development of experimental lung fibrosis. *Am J Physiol* 273:L451–L458, 1997
14. Ichimura T, Maier JA, Maciag T, *et al*: FGF-1 in normal and regenerating kidney: Expression in mononuclear, interstitial, and regenerating epithelial cells. *Am J Physiol* 269:F653–F662, 1995

15. Cancilla B, Davies A, Cauchi JA, *et al*: Fibroblast growth factor receptors and their ligands in the adult rat kidney. *Kidney Int* 60:147–155, 2001
16. Floege J, Hudkins KL, Eitner F, *et al*: Localization of fibroblast growth factor-2 (basic FGF) and FGF receptor-1 in adult human kidney. *Kidney Int* 56:883–897, 1999
17. Kerby JD, Luo KL, Ding Q, *et al*: Immunolocalization of acidic fibroblast growth factor and receptors in the tubulointerstitial compartment of chronically rejected human renal allograft. *Transplantation* 63:988–995, 1997
18. Kerby JD, Verran DJ, Luo KL, *et al*: Immunolocalization of FGF-1 and receptors in human renal allograft vasculopathy associated with chronic rejection. *Transplantation* 62:467–475, 1996
19. Weening JJ, D'Agati VD, Schwartz MM, *et al*: The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int* 65:521–530, 2004
20. Colvin RB, Cohen AH, Saiontz C, *et al*: Evaluation of pathologic criteria for acute renal allograft rejection: Reproducibility, sensitivity, and clinical correlation. *J Am Soc Nephrol* 8:1930–1941, 1997
21. Dikov MM, Reich MB, Dworkin L, *et al*: A functional fibroblast growth factor-1 immunoglobulin fusion protein. *J Biol Chem* 273:15811–15817, 1998
22. Byrd VM, Ballard DW, Miller GG, Thomas JW: Fibroblast growth factor-1 (FGF-1) enhances IL-2 production and nuclear translocation of NF-kappaB in FGF receptor-bearing Jurkat T cells. *J Immunol* 162:5853–5859, 1999
23. Byrd VM, Kilkenny DM, Dikov MM, *et al*: Fibroblast growth factor receptor-1 interacts with the T-cell receptor signalling pathway. *Immunol Cell Biol* 81:440–450, 2003
24. Luo W, Liu A, Chen Y, *et al*: Inhibition of accelerated graft arteriosclerosis by gene transfer of soluble fibroblast growth factor receptor-1 in rat aortic transplants. *Arterioscler Thromb Vasc Biol* 24:1081–1086, 2004
25. Kerby JD, Verran DJ, Luo KL, *et al*: Immunolocalization of FGF-1 and receptors in glomerular lesions associated with chronic human renal allograft rejection. *Transplantation* 62:190–200, 1996
26. Madiai F, Hackshaw KV: Lack of FGF-1 overexpression during autoimmune nephritis in the kidneys of MRL lpr/lpr mice. *Res Commun Mol Pathol Pharmacol* 103:37–44, 1999
27. Anderson RJ, Ray CJ: Potential autocrine and paracrine mechanisms of recovery from mechanical injury of renal tubular epithelial cells. *Am J Physiol* 274:F463–F472, 1998
28. Zinn KR, Kelpke S, Akhi K, *et al*: Glomerular targeting of acidic fibroblast growth factor-1 in renal transplanted rats. *Transplantation* 73:1447–1454, 2002
29. Barisoni L, Kriz W, Mundel P, D'Agati V: The dysregulated podocyte phenotype: A novel concept in the pathogenesis of collapsing idiopathic focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol* 10:51–61, 1999