

# Expression of Vascular Permeability Factor (VPF/VEGF) Is Altered in Many Glomerular Diseases<sup>1</sup>

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## ABSTRACT

Vascular permeability factor (VPF), also known as vascular endothelial growth factor (VEGF), is a potent enhancer of microvascular permeability and a selective endothelial cell growth factor. In normal human kidney, VPF/VEGF mRNA and protein are strongly expressed by visceral glomerular epithelial cells, and VPF/VEGF may be an important regulator of glomerular endothelial cell function. This study examined 47 renal biopsies from patients with a variety of glomerular diseases for expression of VPF/VEGF mRNA and protein by *in situ* hybridization and immunohistochemistry. In many glomerular diseases, VPF/VEGF-expressing cells were decreased in number or absent in areas of focal or global glomerular sclerosis. Decreased numbers of VPF/VEGF-expressing cells in glomeruli were also noted in amyloidosis, diabetes, crescentic glomerulonephritis, and diffuse endocapillary proliferative glomerulonephritis associated with systemic lupus erythematosus. Normally, release of VPF/VEGF must be under strict control because it is some 50,000 times more potent than histamine as an inducer of microvascular permeability. Damage to visceral epithelial cells in a variety of glomerular diseases has the potential for releasing relatively large amounts of VPF/VEGF locally, leading to increased glomerular permeability. In addition, because VPF/VEGF is also an endothelial growth factor, the loss of normal, controlled secretion of VPF/VEGF after damage to visceral epithelial cells could lead to important alterations in glomerular endothelial cell function.

**Key Words:** Vascular endothelial growth factor, renal disease, glomerular permeability

**V**ascular permeability factor (VPF), also known as vascular endothelial growth factor (VEGF), is both a potent enhancer of microvascular permeability

(1-3) and a selective endothelial cell growth factor (4-8). VPF/VEGF interacts with at least two specific tyrosine kinase receptor proteins found on endothelial cells, *flt-1* and *KDR* (9,10). VPF/VEGF is thought to play an important role in the increased permeability and angiogenesis associated with malignancy (11-18), wound healing (19), and certain inflammatory conditions (20-24). Strong VPF/VEGF expression has also been demonstrated in several normal tissues, including kidney (16,25-27). Immunoperoxidase and *in situ* hybridization studies have shown that, in the normal human kidney, VPF/VEGF protein and mRNA are localized predominantly to visceral glomerular epithelial cells (25). The precise role of VPF/VEGF in normal renal function is uncertain, but the known biologic properties of VPF/VEGF and its localization to podocytes strongly suggest an important role for VPF/VEGF in the regulation of glomerular endothelial cell function.

Many glomerular diseases are characterized by epithelial alterations that might result in changes in VPF/VEGF expression. To test this hypothesis, we used *in situ* hybridization and immunohistochemistry to study VPF/VEGF mRNA and protein expression in renal biopsies from patients with a variety of diseases.

## METHODS

### Material Studied

Forty-seven renal biopsies from patients with a variety of renal diseases were selected from our tissue bank of frozen renal biopsy material (Table 1). Twenty-nine biopsies were studied with both immunohistochemistry (IH) and *in situ* hybridization (ISH), and 18 with IH only.

### Immunohistochemistry

IH was performed on 4-micron sections using affinity-purified rabbit antibodies raised to a synthetic peptide corresponding to amino acid residues 1 through 26 of human VPF/VEGF as previously described (25). This anti-peptide antibody binds VPF/VEGF in ELISA assays and, on immunoblots, blocks VPF/VEGF activity and binds VPF/VEGF in solution (28). Normal rabbit immunoglobulin G (IgG) diluted to an equivalent protein concentration was substituted for the primary antibody as a control.

### *In Situ* Hybridization

Four-micron frozen sections were fixed for 15 min in 4% paraformaldehyde in phosphate-buffered saline, and were subsequently subjected to *in situ* hybridization as previously described (29), using <sup>35</sup>S-labeled, 204-base pair, single-stranded antisense and control-sense RNA probes. The antisense probe recognizes all known VPF/VEGF splicing variants.

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TABLE 1. Renal biopsies<sup>a</sup>

Cases	Disease	VPF/VEGF Expression in Glomerular Epithelium
11	Nil disease/FSS	Decreased in sclerotic areas
3	Membranous GN	Decreased in sclerotic areas
3	Amyloidosis	Decreased with marked amyloid deposition
3	Arteriosclerosis	Decreased in sclerotic areas
8	IgA nephropathy	Decreased in sclerotic areas
5	Diabetes	Decreased in sclerotic areas and in relation to matrix nodules
9	Crescentic GN	Decreased in glomeruli compressed by crescents
4	SLE	Decreased in two cases with diffuse endocapillary proliferation and no change in two cases with minimal light microscopic changes
1	Acute tubular necrosis	No change

<sup>a</sup> VPF/VEGF, vascular permeability factor/vascular endothelial growth factor; FSS, focal segmental sclerosis; GN, glomerulonephritis; SLE, systemic lupus erythematosus.

## RESULTS

Forty-seven biopsies were studied: twenty-nine biopsies with both immunohistochemical staining (IH) for VPF/VEGF protein and *in situ* hybridization (ISH) for VPF/VEGF mRNA, and 18 with IH only (Table 1). In agreement with previous studies, both VPF/VEGF mRNA and protein were localized predominantly to visceral epithelial cells in glomeruli. Focal tubular epithelial expression of VPF/VEGF mRNA was also noted by ISH, but the level of expression was much lower and less consistent than that found in glomeruli. No specific cellular labeling was seen by ISH with sense-control probes and background was low. No glomerular staining was seen by IH when control IgG was substituted for the primary antibody.

Eleven cases of nil disease/focal segmental sclerosis were studied by IH and ISH. The pathology ranged from cases with no light microscopic abnormalities to cases with end-stage focal and segmental glomerulosclerosis. VPF/VEGF mRNA (Figure 1A,B) and protein (Figure 2A) were strongly expressed by visceral epithelial cells in glomeruli that appeared normal by light microscopy. Expression was similar in both pattern and degree to the strong expression described in normal glomeruli. In contrast, in glomeruli with segmental or global sclerosis, cells expressing VPF/VEGF mRNA and protein were not present in the sclerotic areas. Because the study was limited to the examination of frozen sections, histologic detail was suboptimal and "early," lesions of focal segmental sclerosis

could not be identified with certainty and evaluation of expression in such areas could not be performed.

Four cases of SLE glomerulonephritis were studied by IH and ISH, including two cases of diffuse endocapillary proliferative glomerulonephritis, one case of membranous glomerulonephritis with a mild increase in thickness of capillary loops, and one case with a slight increase in the number of mesangial cells. In the two cases with diffuse endocapillary proliferative glomerulonephritis, cells expressing VPF/VEGF mRNA (Figure 1C,D) and protein (Figure 2C) were markedly reduced in number in the hypercellular glomeruli. Strong expression of VPF/VEGF mRNA was present in the case of membranous glomerulonephritis and the case with a mild increase in mesangial cells, but in these cases, glomerular pathology was minimal by light microscopy.

Nine cases of pauci-immune crescentic glomerulonephritis were studied: two cases by IH and ISH, and seven cases by IH alone. Expression of VPF/VEGF mRNA (Figure 1E,F) and protein (Figure 2D) was markedly decreased in glomeruli that were compressed by crescents. Neither VPF/VEGF mRNA nor protein was expressed by the cells comprising the crescents. VPF/VEGF-expressing cells were also decreased in number or absent in areas of sclerosis. Expression of VPF/VEGF was strong in glomeruli that appeared relatively normal by light microscopic criteria.

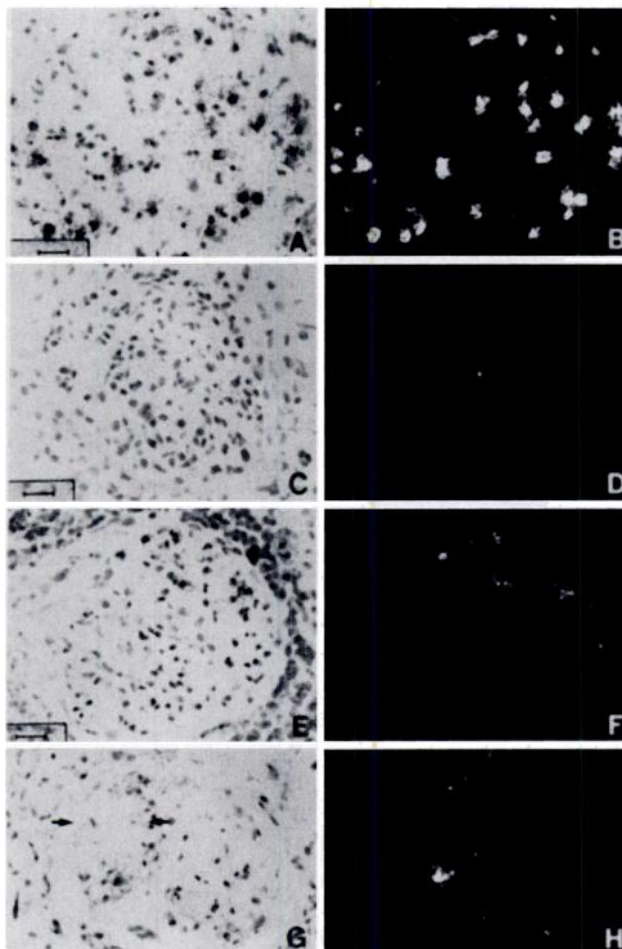
Five cases of diabetes were studied: one case by IH and ISH and four cases by IH alone. Varying amounts of glomerular sclerosis were noted and three of the cases had mesangial matrix nodules. VPF/VEGF mRNA and protein expression were strong in glomeruli with relatively preserved architecture, but the normal interlacing pattern of visceral epithelial cells expressing VPF/VEGF mRNA (Figure 1G,H) and protein (Figure 2B) was disrupted by matrix nodules, and VPF/VEGF expressing cells were markedly decreased in number or absent in sclerotic areas.

Three cases of membranous glomerulonephritis were studied by IH and ISH. Each case contained some glomeruli with relatively preserved architecture and others with segmental or global sclerosis. VPF/VEGF protein and mRNA expression were strong in glomeruli with relatively preserved architecture but were markedly decreased or absent in sclerotic areas.

Eight cases of IgA nephropathy were studied: five by IH and ISH, and three by IH only. Pathologic changes ranged from mild to end-stage disease. VPF/VEGF protein and mRNA expression were strong in glomeruli with relatively preserved architecture but markedly decreased or absent in sclerotic areas.

Three cases of amyloidosis were studied by IH. The normal interlacing pattern of visceral epithelial cells expressing VPF/VEGF protein was disrupted by the amyloid deposits, and expression was decreased overall in glomeruli with marked amyloid deposition.

Three cases of arteriosclerosis with variable degrees of global and segmental glomerular sclerosis were



**Figure 1.** *In situ* hybridization for VPF/VEGF mRNA. (A, C, E, G) Brightfield photomicrographs, and (B, D, F, H) corresponding darkfield photomicrographs of the same field. (A, B) Minimal change disease: diffuse strong labeling of visceral epithelial cells for VPF/VEGF mRNA. (C, D) SLE, diffuse endocapillary proliferative glomerulonephritis: markedly decreased glomerular epithelial labeling for VPF/VEGF mRNA. (E, F) Crescentic glomerulonephritis: decreased labeling for VPF/VEGF mRNA in residual compressed glomerulus (upper right of glomerular space). Crescent does not label for VPF/VEGF mRNA. (G, H) Diabetes: decreased labeling for VPF/VEGF mRNA in a glomerulus with sclerosis and a matrix nodule (arrows). (Original magnification for all figures,  $\times 330$ .)

studied: two cases by ISH and IH, and one case by IH alone. VPF/VEGF protein and mRNA expression were strong in glomeruli with relatively preserved architecture but expressing cells were markedly decreased in number or absent in sclerotic areas.

One case of acute tubular necrosis was studied by IH and ISH. VPF/VEGF mRNA and protein were strongly expressed in visceral epithelial cells in the glomeruli.

## DISCUSSION

VPF/VEGF is both a potent enhancer of microvascular permeability (1–3) and a selective endothelial cell growth factor (4–8). Immunoperoxidase and *in situ* hybridization studies have shown that, in the normal human kidney, VPF/VEGF protein and mRNA are localized predominantly to visceral glomerular epithelial cells (25). The precise role of VPF/VEGF in

the normal glomerulus is uncertain, but the known biologic properties of VPF/VEGF, its strong expression, and its localization to podocytes suggest an important role for VPF/VEGF in the regulation of glomerular endothelial cell function.

In this study, we examined 47 renal biopsies from patients with a variety of renal diseases for expression of VPF/VEGF mRNA and protein by *in situ* hybridization and immunohistochemistry. Expression of VPF/VEGF protein paralleled expression of VPF/VEGF mRNA in all cases. Expression of VPF/VEGF was significantly altered in a wide variety of glomerular diseases. Strong expression of VPF/VEGF, similar to that seen in normal kidney, was present in visceral epithelial cells in glomeruli with preserved architecture that appeared relatively normal by light microscopy.

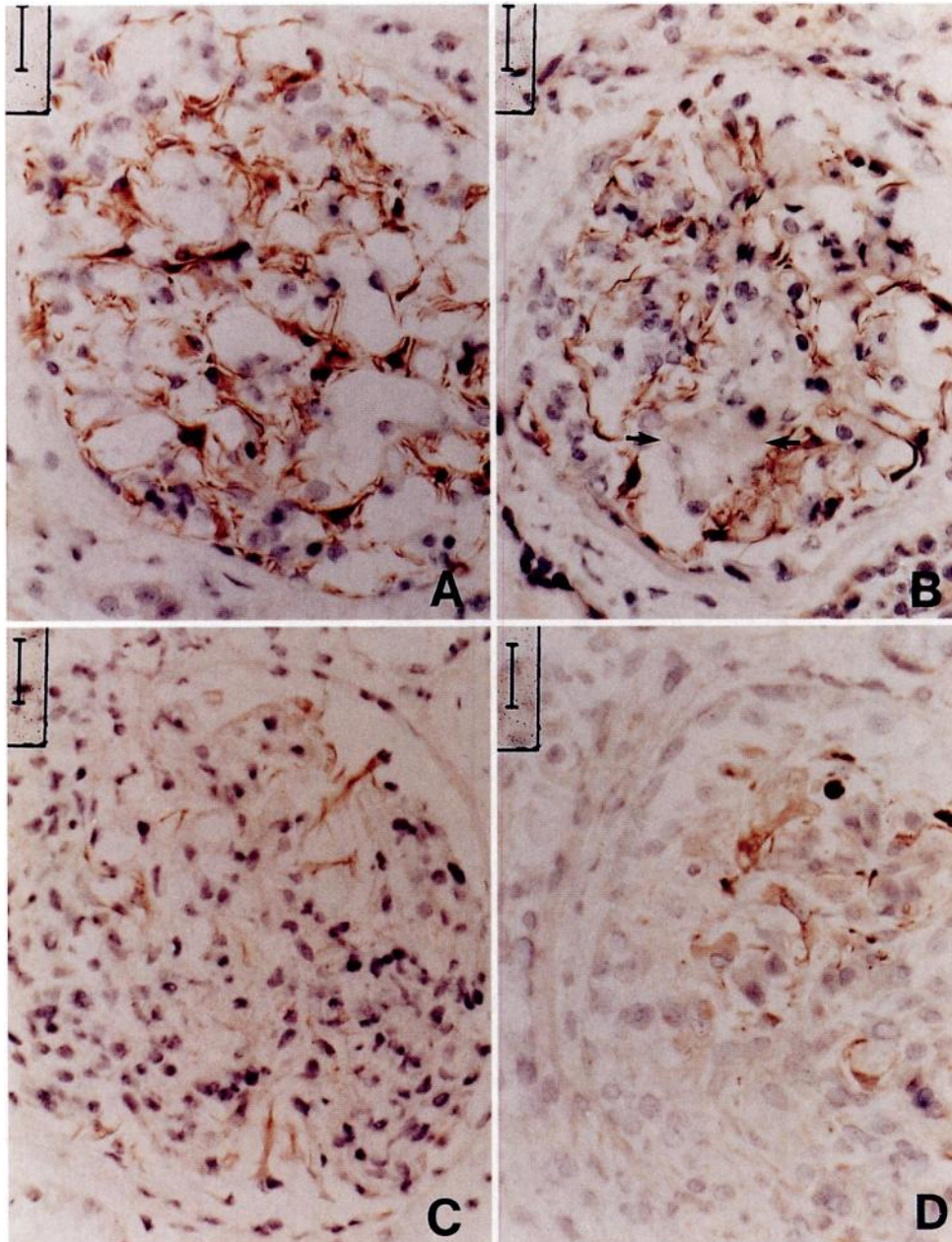


Figure 2. Immunohistochemical staining for VPF/VEGF. (A) Minimal change disease: diffuse strong staining of visceral epithelial cells for VPF/VEGF. (B) Diabetes: focally decreased staining for VPF/VEGF in a glomerular matrix nodule (arrows). (C) SLE, diffuse endocapillary proliferative glomerulonephritis: markedly decreased glomerular epithelial staining for VPF/VEGF. (D) Crescentic glomerulonephritis: decreased staining for VPF/VEGF in compressed glomerulus. Crescent does not stain for VPF/VEGF. (Original magnification for all figures,  $\times 430$ .)

Several patterns of altered expression of VPF/VEGF were noted in glomerular diseases with pathologic light microscopic changes. In glomeruli with segmental or global sclerosis, cells expressing VPF/VEGF were markedly decreased in number or absent in the sclerotic areas. This pattern was noted in cases of focal segmental sclerosis, membranous glomerulone-

phritis, IgA nephropathy, arteriosclerosis, and diabetes. Decreased expression of VPF/VEGF was also noted in relation to deposits of amyloid and in association with matrix nodules in diabetes. In pauci-immune crescentic glomerulonephritis, a marked decrease in expression of VPF/VEGF was noted in damaged glomeruli compressed by crescents. The



cells comprising the crescents did not express VPF/VEGF. In diffuse endocapillary proliferative glomerulonephritis associated with SLE, cells expressing VPF/VEGF were markedly reduced in number in the hypercellular glomeruli.

In normal glomeruli, visceral epithelial cells strongly express VPF/VEGF mRNA and protein (25). However, because VPF/VEGF increases microvascular permeability with a potency some 50,000 times that of histamine (3), its release must be under strict control. The decrease in the number of VPF/VEGF-expressing cells noted in the glomerular diseases studied is likely a result of epithelial cell damage or destruction. Damage to visceral epithelial cells has the potential for releasing relatively large amounts of stored VPF/VEGF, which may contribute to the increased glomerular permeability associated with many of these diseases. In addition, if VPF/VEGF is indeed a critical trophic factor for glomerular endothelial cells, its loss after the destruction of visceral epithelial cells could lead to alterations in endothelial cell growth, integrity, or function, which might also contribute to glomerular pathology. VPF/VEGF has a strong affinity for certain proteoglycans. VPF/VEGF released by normal or damaged glomerular epithelial cells may bind to stromal or basement membrane proteoglycans, keeping it in the vicinity of endothelial cells even in the presence of filtration forces that might tend to move it away from endothelial cells.

Glomeruli that were relatively unremarkable by light microscopic criteria (*i.e.*, minimal change disease, *etc.*) strongly expressed VPF/VEGF in a pattern similar to that seen in normal glomeruli. However, this does not exclude the possibility that the regulation of VPF/VEGF synthesis or release might be altered in these diseases. It is conceivable that VPF/VEGF expression is increased, but it is not possible to identify the increase by ISH or IH in a cell type with such strong baseline expression. A more quantitative technique, such as Northern analysis, would be necessary, but is impractical given the small amount of tissue in a renal biopsy. Several spliced variants of VPF/VEGF have been described (30). The larger variants may remain cell-associated and the smaller forms are more likely to be secreted (31). It is possible that the relative proportions of the various spliced forms of VPF/VEGF change in glomerular disease with corresponding effects on permeability. Our probe recognizes all spliced variants and we cannot at present differentiate between them by ISH. In addition, many factors other than VPF/VEGF could play an important role in increasing permeability in glomerular disease. The precise role of VPF/VEGF in glomerular disease remains to be determined.

In summary, expression of VPF/VEGF by glomerular visceral epithelial cells is altered in a wide variety of glomerular diseases, and this may have important consequences for glomerular endothelial cell function.

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