Role of the Microvascular Endothelium in Progressive Renal Disease

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Abstract. The role of the vascular endothelium in progressive renal disease is not well understood. This review presents evidence that progressive renal disease is characterized by a progressive loss of the microvasculature. The loss of the microvasculature correlates directly with the development of glomerular and tubulointerstitial scarring. The mechanism is mediated in part by a reduction in the endothelial proliferative response, and this impairment in capillary repair is mediated by alteration in the local expression of both angiogenic (vascular endothelial growth factor) and antiangiogenic (thrombospondin 1) factors in the kidney. The alteration in balance of angiogenic growth factors is mediated by both macrophage-associated cytokines (interleukin-1β) and vasoactive mediators. Finally, there is intriguing evidence that stimulation of angiogenesis and/or capillary repair may stabilize renal function and slow progression and that this benefit occurs independently of effects on BP or proteinuria. Therefore, angiogenic agents may represent a novel therapeutic approach for slowing the progression of renal disease.

The Classic Paradigm for the Pathogenesis of Progressive Renal Disease

The observation that kidney scarring progresses after sufficient renal injury has occurred (1) has resulted in intense research focusing on the pathogenic mechanisms (2). A major breakthrough in our understanding was the recognition by Brenner *et al.* (3) that the homeostatic mechanisms involved in maintaining GFR in the setting of reduced nephron number have long-term deleterious consequences. Thus, in response to a fall in GFR, there is a stimulation of cyclooxygenase-2 (COX-2) in the macula densa (4), which results in the synthesis of prostaglandins that dilate the afferent arteriole; there is also a COX-2 stimulation of renin, which leads to angiotensin II generation and efferent arteriolar constriction (5). The resultant increase in systemic and glomerular hydrostatic pressure causes glomerular endothelial (6) and mesangial (7) injury, initiating local platelet and macrophage accumulation that releases growth factors and stimulates local factor expression (6). Mesangial cells, driven by local platelet-derived growth factor

(PDGF) and transforming growth factor–β (TGF-β) expression, proliferate and secrete extracellular matrix (7,8). Glomeruli also become hypertrophied in response to angiotensin II and other cytokines (9). The visceral epithelial cell (podocyte) is unable to cover the expanding capillary basement membrane, due in part to the endogenous expression of cell cycle inhibitors (10); hence areas of bared basement membrane bow out and attach to the parietal epithelium, forming synechiae (11). The parietal epithelial cells then grow around the attached capillary loop, collapsing it down to enhance the segmental sclerosing lesion (11). Shortly afterward, the intraglomerular cell proliferation subsides and progressive apoptosis occurs (12) with involution and eventual obsolescence of the glomerulus.

The increase in glomerular pressure may have a role in the induction of proteinuria, which appears to have a critical role in mediating the accompanying tubular and interstitial cell injury (13). The proteinuria contains numerous substances that can activate tubular cells, including cytokines, growth factors, iron proteins that can generate oxidants, and complement components that can be activated in urine (reviewed in reference 14). Cytokines generated within the glomerulus also exit via the circulation into the peritubular capillaries, where they may activate local cells (15). Tubular and interstitial cell activation results in the local expression of chemokines (especially MCP-1) and adhesion molecules (such as ICAM-1 and osteopontin) that lead to monocyte/macrophage and T cell accu-

mulation (16). The inflammatory response results in local growth factor (PDGF, TGF-β) and cytokine (interleukin-1β [IL-1β], TNF-α) expression that leads to proliferation and activation of fibroblasts and tubular cells (17). Marked phenotypic changes in cells can occur, in which fibroblasts and, to a lesser extent, tubular cells express smooth muscle proteins such as alpha actin that are important in wound contraction (18). Similar to the glomerulus, the initial proliferative response is eventually replaced by a progressive apoptosis (19), resulting in an end-stage fibrotic kidney.

Chronic Ischemia in Progressive Renal Disease

An important component of progressive renal disease that is often not considered part of the above classic paradigm is the role of ischemia (20). Ischemia could occur via several mechanisms, such as by intrarenal vasoconstriction (secondary to increased local angiotensin II or endothelin or a local loss of nitric oxide) or via structural lesions that impair blood flow delivery to the tubules. The latter could result from arteriolar disease (such as in diabetes or hypertension), from intraglomerular lesions (such as in rapidly progressive glomerulonephritis) or from loss of the peritubular capillaries. Interstitial fibrosis itself may lead to local ischemia by impairing the diffusion gradient from the capillary to the tubule. The most susceptible region to hypoxia is the juxtamedullary region and outer medulla (21). The tubules in this region are normally in a borderline hypoxic state due to the countercurrent circulation and high oxygen demands of the medullary thick ascending tubules and the S3 segments of the proximal tubules (21). Hence modest reductions in renal blood flow could lead to worsening hypoxia in this region (22,23). In turn, hypoxia can induce tubular and interstitial cell injury and activation, proliferation, cytokine generation, and matrix synthesis associated with an increased expression of hypoxia-inducible factor–1α (HIF-1α) (20,24).

Interestingly, angiotensin-converting enzyme (ACE) inhibitors, which have been shown to be able to slow progression in both diabetic and nondiabetic renal disease, may work in part by blocking renal ischemia. Much of the protection of ACE inhibitors can be ascribed to their ability to reduce systemic and glomerular hypertension and to reduce proteinuria (25,26). However, ACE inhibitors also block intrarenal vasoconstriction secondary to angiotensin II and will increase blood flow (27). This may be one of the mechanisms by which ACE inhibitors are protective in nonproteinuric renal disease, such as urinary obstruction (28), and in cyclosporine nephropathy (29).

The Loss of the Microvasculature in Progressive Renal Disease

The maintenance of the microvasculature would thus appear to be critical for the prevention of progressive renal disease. Maintenance of glomerular capillary number would help maintain GFR, whereas maintaining peritubular capillaries in the interstitium would be essential for providing oxygen and nutrition to the tubules and interstitial cells.

Morphometric studies have documented that the initial re-

sponse to a fall in nephron number is a hypertrophic response in which the glomerular capillaries increase in both number and length (30,31). Shimizu *et al.* (32) and Kitamura *et al.* (33) have also documented an early proliferative response of the glomerular endothelium in rats with either anti-GBM disease or with remnant kidneys, respectively. Unfortunately, the proliferation is not sustained, and there is a progressive loss of the endothelium due to unchecked apoptosis over time. They have posited that the loss of the glomerular endothelium predisposes to activation of platelets and the coagulation system that favors capillary collapse and the development of glomerulosclerosis. Indeed, the loss of glomerular endothelium correlates directly with the development of glomerulosclerosis (32,33). We have also documented a loss of the glomerular endothelium in both the aging kidney (34) and in the remnant kidney model (35) (Figure 1, A and C).

A similar finding also occurs in the interstitium. Bohle *et al.* (36) noted that there was a loss of peritubular capillaries in progressive renal disease in humans, and they posited an essential role for impaired blood flow in the etiology of the interstitial fibrosis. Our group confirmed this observation in a large variety of chronic tubulointerstitial nephritis in humans and further demonstrated that peritubular capillary loss is correlated with interstitial fibrosis and tubular atrophy, independent of the injury to larger blood vessels (37). Likewise, both our group (34,35,38) and Ohashi *et al.* (39) have found that, although there is an early proliferative response of the peritubular capillary endothelium, the proliferation is not sustained, and a progressive capillary loss (rarefaction) occurs in models of progressive renal disease such as aging kidney, the remnant kidney, and chronic cyclosporine A nephropathy (Figure 1, B and D). The degree of glomerular and peritubular capillary loss in models of progressive renal disease correlates with the severity of glomerulosclerosis and interstitial fibrosis (Figure 1, E and F).

On the Mechanism for the Progressive Endothelial Loss: Alterations in the Local Balance of Angiogenic and Endothelial Survival Factors

The endothelial cell is essential to the survival of other cells by virtue of its role in delivering oxygen and nutrients. Accordingly, it is not surprising that a variety of both autocrine and paracrine signals can strongly influence the integrity of the microvasculature in the kidney and in other organs. Several growth factors have been identified to have a critical role in promoting or inhibiting endothelial cell proliferation and survival (Table 1). Growth factors, such as vascular endothelial growth factor (VEGF), have trophic, survival, and angiogenic properties (40), whereas factors such as thrombospondin-1 (TSP-1) not only inhibit endothelial proliferation but also cause endothelial cell death (41). Although several studies have identified important roles of these growth factors in embryogenesis, malignancy, or wound healing, there is still relatively little information regarding the role of many of these growth factors in progressive renal disease. This review will focus on several of the key molecules that we and others have recently
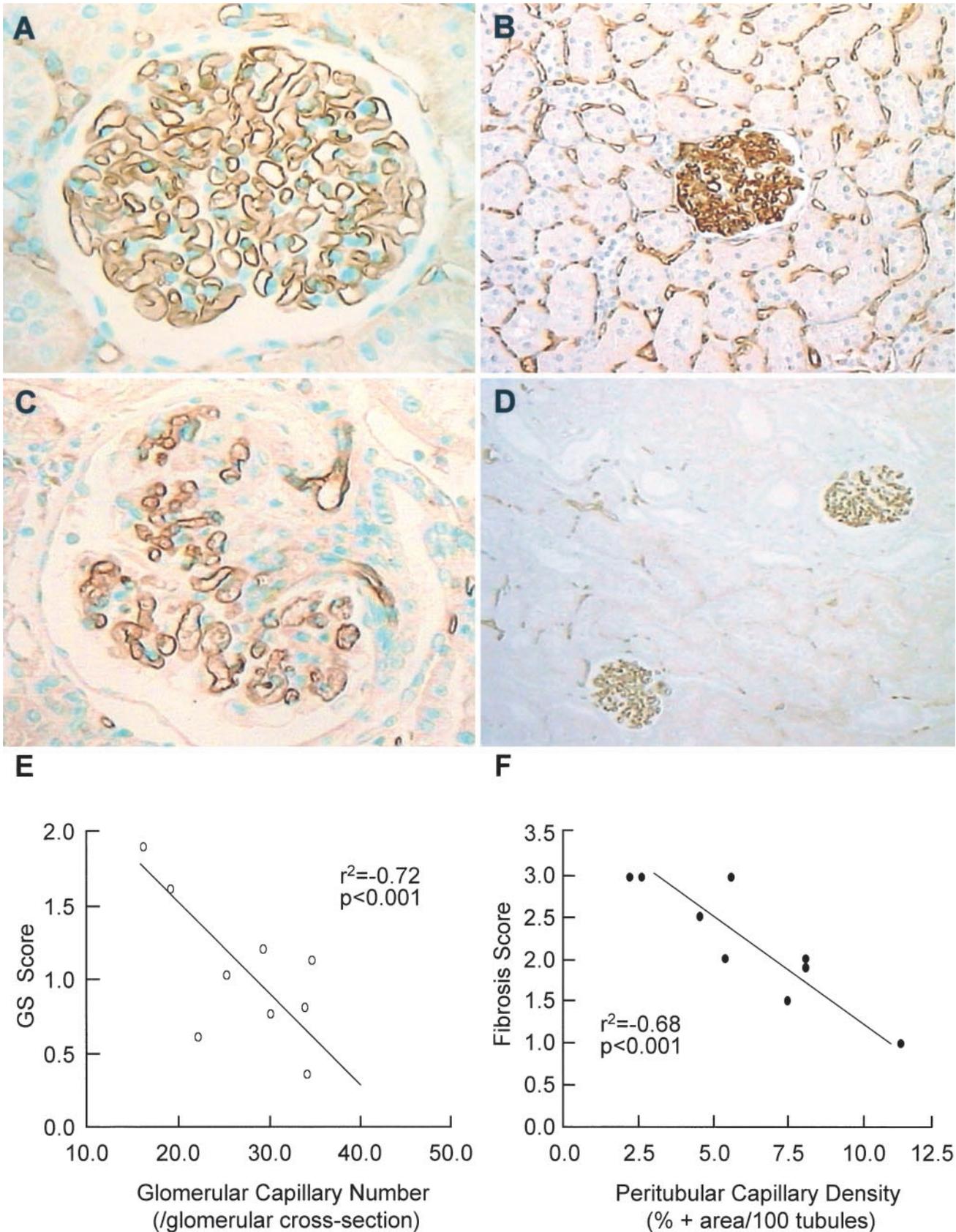
Figure 1. Loss of microvascular endothelium occurs in progressive renal disease. In contrast to the normal glomerular (A) and peritubular (B) endothelium, there is a progressive loss of glomerular capillary loops (C) and peritubular capillaries (D) over time in the remnant kidney model. The capillary loss correlates with both the degree of glomerulosclerosis (E) and interstitial fibrosis (F) in aging kidneys. Panels A through D are reprinted with permission from reference 35, and panels E and F are reprinted with permission from reference 34.

Table 1. Endogenous factors that modulate endothelial cell growth

Proangiogenic Factors	Antiangiogenic Factors
Vascular endothelial growth factor (VEGF)	Angiostatin
Basic fibroblast growth factor (bFGF, FGF2)	Endostatin
Transforming growth factor–β (TGF-β)	Thrombospondin-1
Transforming growth factor–α (TGF-α)	SPARC
Hepatocyte growth factor (HGF)	Vascular endothelial growth inhibitor (VEGI)
Prostaglandin E$_2$ (PGE2)	METH-1, METH-2
Platelet-derived endothelial cell growth factor (PD-ECGF)	
Angiogenin	
Interleukin–8 (IL-8)	
Angiopoietin	

identified as having a critical role in influencing the renal microvasculature in progressive renal disease.

TSP-1: An Antiangiogenic Factor Associated with Progressive Renal Injury.   TSP-1 is a high–molecular weight glycoprotein that is expressed by a variety of cell types, especially platelets and macrophages (42). The function of TSP-1 is complex. As discussed above, TSP-1 is a potent inhibitor of endothelial cell proliferation and will also directly initiate endothelial cell apoptosis (41). TSP-1 also has other functions, including facilitating the uptake of apoptotic cells by macrophages (43). One of the more important functions of TSP-1 is in the nonproteolytic activation of TGF-β from its latent form (44); this may be an important mechanism for the activation of TGF-β in acute glomerular and tubulointerstitial disease (45) and in diabetes (46).

There is increasing evidence that TSP-1 expression is involved in progressive renal disease and that its expression correlates with a loss of the microvascular endothelium. In the normal kidney, expression of TSP-1 is limited to rare parietal epithelial cells in Bowman's capsule (47). However, TSP-1 is markedly induced in models of progressive renal disease. In aging kidneys, TSP-1 expression is increased (34,48), and its expression in glomeruli and in the tubulointerstitium correlate closely with a loss of glomerular and peritubular capillaries. In the remnant kidney model as well, expression of TSP-1 is marked in both glomeruli and in the interstitium (35,49). Within the glomerulus, TSP-1 is expressed primarily by mesangial cells, but some endothelial cells, podocytes, macrophages, and platelets also express TSP-1 in these lesions (49). TSP-1 is also expressed by tubular cells, interstitial fibroblasts, and macrophages within the interstitium (45,49). As in the aging kidney, the degree of TSP-1 expression in the remnant kidney model correlates tightly with the loss of capillaries.

Increased Expression of other Antiangiogenic Growth Factors.   Less is known about the expression of other antiangiogenic growth factors in progressive renal disease. The one exception is SPARC (secreted protein, acidic and rich in cysteine). SPARC is a potent inhibitor of endothelial cell proliferation (50) that is expressed in acute models of glomerular and tubulointerstitial injury (51,52). SPARC expression falls during the renal hypertrophy that occurs in diabetic rats after streptozotocin administration (53). In contrast, SPARC expression is increased in the remnant kidney model (52). It remains unclear whether SPARC has a major role in inhibiting endothelial cell proliferation in these disease states. Studies in which SPARC peptides were infused into rats with acute glomerulonephritis only showed a trend toward less endothelial cell proliferation (54). The major effect of SPARC appears to be stimulation of local TGF-β synthesis (54,55).

A Loss of the Angiogenic Growth Factor, VEGF, in Progressive Renal Disease.   In addition to the increased expression of antiangiogenic factors, there is a loss of angiogenic and endothelial survival factors in progressive renal disease. One of the most important proendothelial survival factors is VEGF, which exists as both secreted (VEGF$_{121}$, VEGF$_{165}$) and cell-associated (VEGF$_{189}$, VEGF$_{206}$) isoforms (40). VEGF is a proliferative, survival and trophic factor for endothelial cells (40), acting primarily through the receptors, VEGFR-2 (KDR/flk-1) and VEGFR-1 (flt-1). VEGFR-2 has a lower affinity for VEGF but demonstrates much higher intracellular kinase activity upon binding. VEGFR-1 may function primarily as a coreceptor, having a very high affinity for VEGF but fewer signaling properties. VEGF also binds to the coreceptors, neuropilin-1 and neuropilin-2, which are also expressed on endothelium (56,57). Many of the actions of VEGF appear to be mediated through endothelial VEGFR-2 and activation of phosphatidylinositol 3-kinase and Akt, leading to increased endothelial nitric oxide synthase (eNOS) activity (58,59). VEGF may also bind mesangial cells (60) and tubular cells (61), although the precise actions on these cells remains unclear. Although VEGF can also stimulate monocyte chemotaxis through monocytic VEGFR-1 *in vitro* (62), studies in which VEGF is inhibited or blocked suggest that the main function of VEGF *in vivo* is on the endothelium (63,64).

VEGF is constitutively expressed in the normal kidney, and its expression is primarily localized to the glomerular podocyte (65) and to tubular cells (66), especially in the outer medulla and medullary rays (65,67). The expression of VEGF in the podocyte contrasts with the expression of VEGF receptors on the endothelial cell, and this paradoxical expression of VEGF and VEGF receptor suggests the possibility of crosstalk, probably mediated via heparan sulfate proteoglycans (glypican-1), which may act to shuttle VEGF across the basement membrane (68). Interestingly, an acute increase in VEGF can be observed in acute glomerulonephritis and acute transplant rejection (69,70); however, VEGF expression is decreased in chronic renal disease. A loss of glomerular VEGF expression has been observed in focal segmental glomerulosclerosis (FSGS) (71), and tubular VEGF expression is reduced with chronic interstitial scarring such as occurs in chronic rejection (70). Our group

demonstrated that VEGF expression was maintained in hypertrophic tubules in human chronic tubulointerstitial nephritis regardless of etiology but that it is lost in atrophic ones (37).

We have investigated the expression of VEGF in models of aging-associated renal disease (34). There is a remarkable loss of VEGF in both the podocyte and in the tubules in the outer medulla, and the loss of tubular VEGF expression correlates with the severity of the peritubular capillary loss ($r^2 = 0.57$; $P < 0.01$). VEGF expression was also inversely correlated with the degree of tubulointerstitial inflammation, reflected by osteopontin expression ($r^2 = -0.55$; $P < 0.05$) and macrophage infiltration ($r^2 = -0.64$; $P < 0.01$). The loss of VEGF was paralleled by a reduction in proliferating endothelial cells. The observation that there is decreased endothelial cell proliferation in the aging kidney is consistent with a study by Reed *et al.* (72), which showed that there is a generalized defect in angiogenesis with aging.

We also examined the expression of VEGF in the remnant kidney model (36,49). In the first few weeks after induction of the model, we observed an increase in endothelial cell proliferation in both the glomerular and peritubular capillaries, which was associated with an early increase or maintenance in VEGF expression in podocytes and tubular cells (35). However, over the subsequent weeks there was a progressive loss of both glomerular and peritubular capillaries (Figure 1), which was paralleled by a progressive loss in glomerular and tubular VEGF expression (Figure 2A). Similar to the aging kidney, the loss of VEGF was associated with a reduction in proliferating endothelial cells to levels below that observed in sham-operated animals (Figure 2, B and C).

Macrophage-Derived Cytokines as a Mechanism for Inhibiting VEGF Expression and Inducing TSP-1 Expression. These studies demonstrate that with progressive renal disease there is a loss of glomerular and peritubular capillaries that is associated with a defect in capillary repair. This involves a local alteration in the balance of angiogenic factors, with an increased expression of the antiangiogenic growth factor, TSP-1, and a loss of expression of the proangiogenic growth
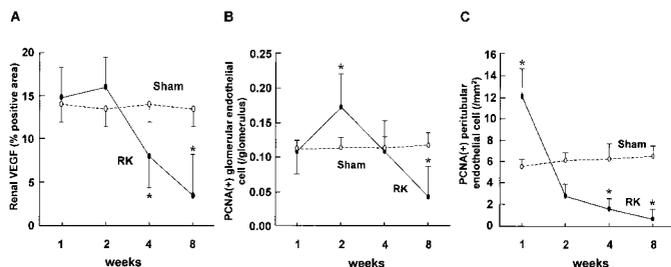
factor, VEGF. But what mediates the changes in these growth factors?

An interesting observation in both the aging and remnant kidney models was that the loss of VEGF correlated with sites of macrophage infiltration (34,35). Furthermore, the number of infiltrating macrophages in individual kidneys correlated with the loss of VEGF ($r^2 = -0.46$; $P < 0.05$). Macrophages are often thought to be proangiogenic (73), but it is important to realize that there are many different macrophage phenotypes (74) and that they can also produce antiangiogenic factors, such as TSP-1 (75).

Given the fact that macrophages correlated both quantitatively and spatially with the sites where VEGF expression was lost, we examined the effect of macrophage-derived cytokines on VEGF expression in renal cells. In this regard, the major type of tubular cell that lost its constitutive VEGF expression in disease was the medullary thick ascending limb (mTAL) epithelial cell. The main cytokines we examined were IL-1β, TNF-α, and IL-6, as these are three of the most important proinflammatory cytokines expressed by macrophages. All three cytokines were able to reduce VEGF mRNA and protein expression in the mTAL cells, and this was shown under normoxic conditions as well as hypoxic conditions such as might be expected to occur *in vivo* in this region of the kidney (Figure 3) (21,35). Furthermore, we have recently found that the mechanism involves accelerated degradation of VEGF mRNA, as opposed to effects on VEGF transcription (D.H. Kang, L. Feng, R. J. Johnson: unpublished observations).

Similarly, we have also found that the number of infiltrating macrophages correlate with the degree of TSP-1 expression in
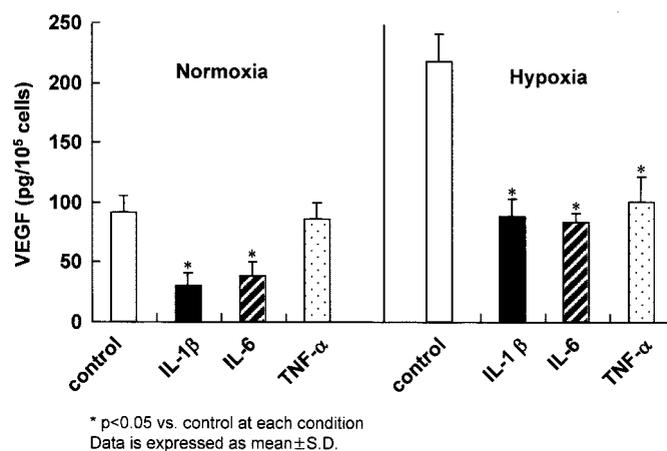

Figure 2. Decreased renal vascular endothelial growth factor (VEGF) expression is associated with decrease in endothelial cell proliferation in progressive renal disease. Renal VEGF expression is gradually decreased in the remnant kidney model (A), which was associated with decreased endothelial cell proliferation in glomeruli (B) and peritubular capillaries (C). Early in the course of progressive renal disease, there is characteristic proliferation of glomerular and peritubular endothelial cells, which is not maintained thereafter. Reprinted with permission from reference 35.

* p<0.05 vs. control at each condition
Data is expressed as mean±S.D.

Figure 3. Effect of macrophage-derived cytokines on VEGF mRNA expression by medullary thick ascending limb (mTAL) cells. Exposure of mTAL cells to macrophage-associated cytokines (10 ng/ml of interleukin–1β [IL-1β] and IL-6) for 24 h results in reduced VEGF protein secretion both in normoxic and hypoxic conditions. The studies examining the effects of hypoxia were performed to simulate the *in vivo* conditions present in this region of the kidney (21). Tumor necrosis factor–α (TNF-α) does not decrease VEGF secretion at the concentration of 10 ng/ml in normoxic condition but it induces a significant decrease under hypoxic conditions. Data is expressed as mean ± SD. Reprinted with permission from reference 35.

both the aging and remnant kidney models. Furthermore, macrophage cytokines such as IL-1β and TNF-α appear to stimulate TSP-1 mRNA expression in the mTAL cells (D. H. Kang, A. H. Joly, R. J. Johnson: unpublished observations); in contrast, they inhibit VEGF expression in the same condition. Thus, these data support a major role for macrophages and for proinflammatory cytokines (such as IL-1β and TNF-α) in altering the expression of angiogenic factors within the kidney.

Vasoactive Mediators as Modulators of Renal VEGF Expression. Other important modulators of renal VEGF expression are the vasoactive factors. We have recently found that nitric oxide (NO) modulates VEGF expression in tubular cells. Indeed, incubation of mTAL cells with L-NAME (an inhibitor of NO synthesis) blocks the increase in VEGF expression that occurs with hypoxia (76). L-NAME may also block some of the proliferative effects of VEGF in the endothelial cells (77). Thus, it was not surprising to find that L-NAME treatment of rats with remnant kidneys was associated with more severe glomerular and peritubular capillary endothelial cell loss, with a greater impairment in capillary endothelial cell proliferation, and with more severe glomerulosclerosis and tubulointerstitial fibrosis (76).

Although blockade of NO inhibits tubular VEGF expression, it had opposite effects on vascular smooth muscle cells (SMC). L-NAME–treated vascular SMC markedly increased hypoxia-induced VEGF expression (76). Interestingly, VEGF was expressed *de novo* in the SMC of preglomerular arterial vessels in L-NAME–treated remnant kidneys, and this was associated with preservation and occasionally hyperplasia of the adjacent endothelium (76). Thus, there appears to be marked differences between the effects of NO on mesenchymal (*e.g.*, SMC) and epithelial (*e.g.*, mTAL) cells, with blockade of NO-inhibiting tubular VEGF expression but stimulating SMC VEGF expression.

A similar phenomenon may exist for angiotensin II regulation of VEGF. Williams *et al.* (78) have shown that angiotensin II is a potent stimulus of VEGF in vascular SMC, yet our group has found that angiotensin II is a potent inhibitor of VEGF expression in mTAL cells (Figure 4). Furthermore, we have found that ACE inhibition is associated with preservation of capillaries in the aging kidney (Figure 5) (D. H. Kang, S. Anderson, L. Ferder, R. J. Johnson: unpublished observations). Theoretically, this could provide an additional mechanism by which ACE inhibition could slow the progression of renal disease.

Can Administration of Angiogenic Agents Prevent Renal Progression?

Progressive renal disease is associated with loss of the glomerular and peritubular capillary microvasculature, and this is associated with defective capillary repair due in part to an imbalance in the renal expression of angiogenic factors. A major question is whether stimulation of angiogenesis, by virtue of its ability to increase capillary density, might slow progression. If true, this would provide the strongest evidence for the role of ischemia in progressive renal injury (20) and
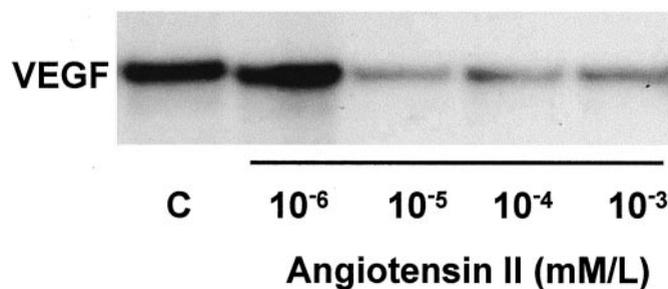

Figure 4. Angiotensin II reduces VEGF protein secretion by mTAL cells. Stimulation of cultured mTAL cells with angiotensin II for 24 h significantly reduces VEGF protein secretion from the concentration of 10^{-5} mM/L. Representative Western blot is shown. C, control without angiotensin II. Reprinted with permission from reference 35.

would potentially pave the way for new therapies for progressive renal disease.

There have been several lines of evidence suggesting that stimulation of endothelial proliferation might be beneficial in kidney disease. We have previously reported that glomerular endothelial cell proliferation plays a key role in the repair of capillaries and microaneurysms in the Thy-1 model of glomerulonephritis (79). Ostendorf *et al.* (80) later showed that blockade of the capillary repair in this model with an aptamer that inhibits VEGF leads to progressive renal damage. In addition, Masuda *et al.* (81) have shown that VEGF administration enhances capillary repair and improves renal function in Thy 1 nephritis. A key role for VEGF in glomerular capillary formation has also been suggested from studies of glomerular development in neonatal rats and mice (63,64).

Additional evidence was generated in a model of thrombotic microangiopathy in rats (82,83). In this model, the renal infusion of an antibody to glomerular endothelial cells results in complement-dependent killing (apoptosis) of the glomerular and peritubular capillary endothelium associated with local thrombosis and acute renal failure. Shortly after the injury, there is a pronounced proliferative response of the glomerular and peritubular capillary endothelium (84). However, whereas the glomerular capillaries undergo dramatic repair, the peritubular capillary endothelium does not completely recover, and this is associated with a loss of VEGF from the mTAL and other tubular cells (82). Administration of VEGF$_{121}$ to rats with established injury was able to increase peritubular capillary density, and this was associated with the development of less fibrosis and better preserved renal function (82). Furthermore, VEGF was shown to reduce the endothelial cell apoptosis and renal infarction that occurs in this model (83).

These studies suggested that VEGF might provide benefit in progressive renal disease, because as discussed earlier, VEGF expression is reduced in this condition and impaired angiogenesis can be shown. We therefore performed a study in which rats underwent the remnant kidney procedure and were then randomized at 4 wk to receive VEGF$_{121}$ or vehicle (85). The therapy was continued for an additional 4 wk at which time animals were sacrificed. Vehicle-treated rats developed pro-
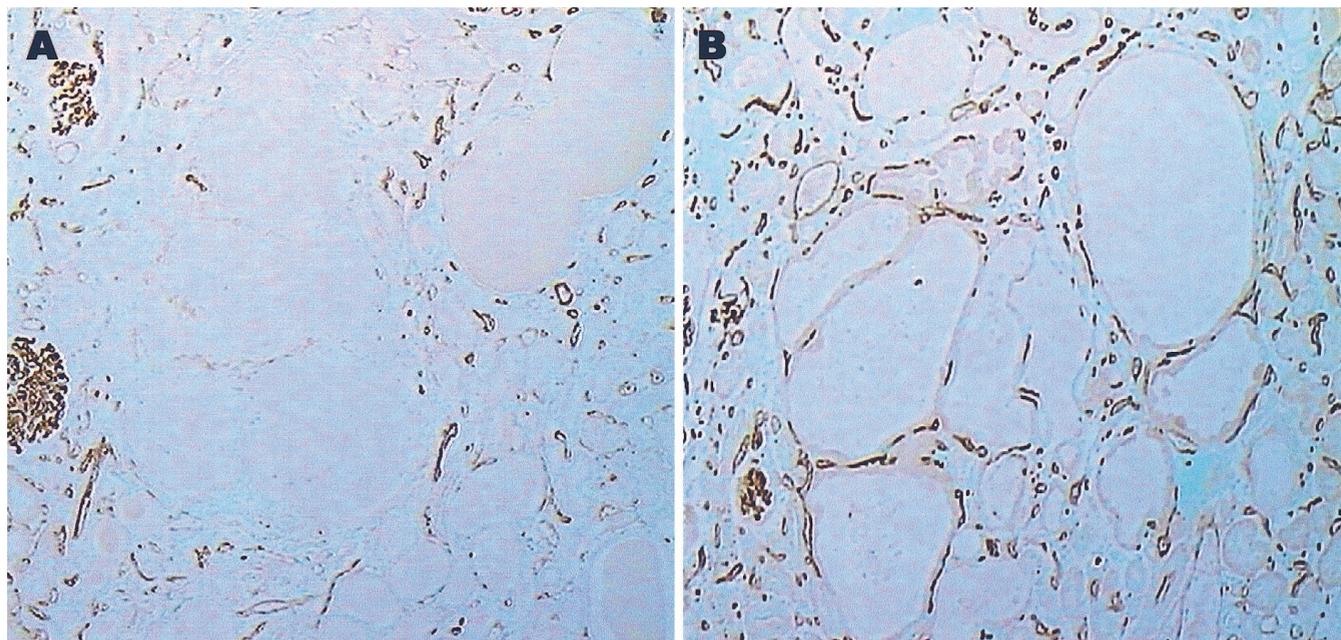
Figure 5. Inhibition of angiotensin II is associated with capillary preservation in aging kidneys. Treatment with angiotensin-converting enzyme (ACE) inhibitor in aging rats results in impressive preservation of peritubular capillaries (B) compared with untreated aging kidneys (A), despite being of comparable tubular damage.

gressive capillary loss and impaired glomerular and peritubular capillary endothelial cell proliferation in association with the development of glomerulosclerosis and tubulointerstitial fibrosis. However, VEGF-treated rats had relative preservation of the glomerular and peritubular capillary endothelium with an enhanced endothelial proliferative response (Figure 6). Remarkably, there was stabilization of renal function in the VEGF-treated rats, and this correlated both with a preservation of glomerular capillary loop number and with a reduction in the severity of the tubulointerstitial fibrosis (Figure 6).

An important finding was that the beneficial effect of VEGF occurred independently of most of the main mediators of progression. For example, VEGF did not affect systolic BP, proteinuria, glomerular hypertrophy, or macrophage infiltra-

tion. Even glomerulosclerosis was not significantly altered by VEGF treatment. Although micropuncture studies need to be performed, the observation that systemic BP, proteinuria, glomerulosclerosis, and glomerular hypertrophy were not altered suggests that glomerular hypertension was also not prevented by VEGF administration in this model. Therefore, the best explanation for the preservation of renal function appears to be due to the ability of VEGF to stimulate capillary endothelial cell proliferation and preserve glomerular capillary loops. This may help preserve GFR by maintaining glomerular capillary filtration surface area. The improvement in peritubular capillary density was also associated with less tubulointerstitial fibrosis, and this could provide a second mechanism for the improvement in GFR.
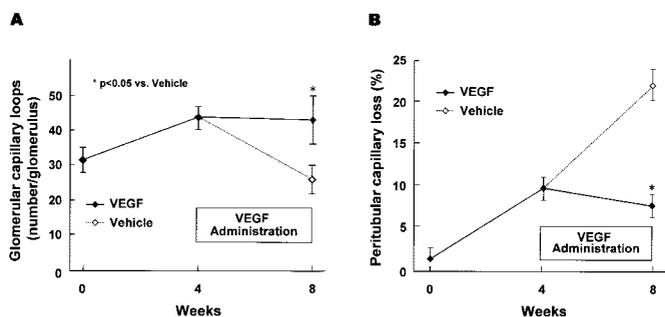

Figure 6. Overall time course of renal microvascular changes in VEGF/vehicle-treated remnant kidney rats. VEGF administration maintains the glomerular capillary loop number (A) and prevents progressive peritubular capillary rarefaction (B) in the remnant kidney rats. Data is expressed as mean ± SD. Reprinted with permission from reference 85.

Controversial Areas Related to Angiogenesis and the Kidney

Not all studies would support the view that increased angiogenesis prevents renal disease progression. The role of angiogenesis in the progression of diabetic renal disease is unclear at present. In a recent study, the administration of a neutralizing VEGF monoclonal antibody was shown to decrease the early hyperfiltration, albuminuria, and glomerular hypertrophy in diabetic rats (86). The VEGF antibody treatment prevented eNOS upregulation by glomerular endothelium, suggesting that this may be the underlying mechanism for the improvement in renal dysfunction. There is also evidence that VEGF may play a role in the neovascularization that occurs in the retina of diabetic subjects. In contrast, a lack of VEGF has been proposed to play a role in ischemic heart disease and peripheral vascular disease in diabetic patients (87). We think that the role

of angiogenic factors in diabetes may depend on the organ involved and also on the stage of diabetic nephropathy. Another recent study identified increased angiogenesis and VEGF expression in the cysts of adult polycystic kidney disease (88). The authors postulated a role for VEGF in increasing vascular permeability and promoting cyst formation in this disorder. Finally, a recent study in mice concluded that peritubular capillary growth and proliferation may be enhanced with nephron reduction (89). Whether the difference in findings compared with our study relate to species differences remains unclear. However, the conclusion that the vascular endothelium was increased was largely based on staining for CD34, which is also expressed by lymphatic endothelium (90). We have recently found that, in contrast to the vascular endothelium, there is a marked increase in lymphatics in the rat remnant kidney (D. Kerjaschki, D. H. Kang, *et al.*: unpublished observations). Thus, it is possible that the increase in endothelial staining in that study might reflect a marked increase in lymphatics with renal injury.

Conclusion

While the classic paradigm initiated by Brenner *et al.* (3) over 20 years ago remains valid, there is new and emerging evidence that loss of the microvasculature may also contribute to progressive renal disease, as well as to the aging-associated decline in renal function. We have presented evidence that this is due in part to an imbalance in the expression of angiogenic factors, with a loss of intrinsic VEGF expression, and an increase in local TSP-1 expression. Both macrophage-derived cytokines and vasoactive mediators appear to be responsible for this imbalance in angiogenic factors that favors the development of the 'impaired angiogenic' state that characterizes progressive renal disease. Perhaps most excitingly, new data suggests that preservation of stressed endothelium and/or stimulation of angiogenesis may stabilize renal function and slow histologic progression and that this may act independently of other factors that may also prevent renal deterioration. However, because angiogenic stimulation may also increase the risk for tumors (91) and the risk for retinal angiogenesis in diabetic subjects (92), it remains controversial as to whether angiogenic agents will be useful in human disease. Nevertheless, further studies on the role of the microvasculature in progressive renal disease are warranted, and the insights may lead to new therapeutic approaches for the treatment of this serious medical condition.

Acknowledgments

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