Abnormal Angiogenesis in Diabetic Nephropathy

Takahiko Nakagawa,[1] Tomoki Kosugi,[1] Masakazu Haneda,[2] Christopher J. Rivard,[1] and David A. Long[3]

Diabetic retinopathy is the leading cause of blindness in the Western world (1) and is characterized by abnormal angiogenesis driven by several factors, including tissue ischemia and hyperglycemia. This abnormal angiogenesis results in new vessels that are often immature and play a pathological role in retinopathy, contributing to both vitreous hemorrhage and fibrosis (2). In addition, increased vascular permeability leading to plasma leakage accounts for the development of macula edema, disrupting visual function (2). These evidences have led to the development of several therapeutic strategies targeting angiogenesis in diabetic retinopathy (3).

Abnormal angiogenesis also occurs in diabetic nephropathy; therefore, the overriding question is whether new vessel formation in the kidney plays a pathological role in diabetic nephropathy similar to that observed in retinopathy. Intriguingly, the progression of both diabetic retinopathy and nephropathy is altered by vascular growth factor signaling through receptor tyrosine kinases, specifically involving the vascular endothelial growth factor (VEGF)-A and angiopoietin families. This review discusses abnormal angiogenesis and the role of both VEGF-A and angiopoietins in diabetic nephropathy.

Evidence of abnormal angiogenesis in diabetic nephropathy. In 1987, Osterby and Nyberg (4) described abnormal blood vessels in glomeruli of patients with long-term type 1 diabetes, and later these findings were shown to occur in type 2 diabetic patients (5,6) (Fig. 1A). The abnormal vessels occupied 1–5% of glomerular capillary area, they were occasionally dilated, and the glomerular basement membrane adjacent to them was found to be focally extremely thin. Abnormal vessels were also present in Bowman's capsule or in the glomerular vascular pole, the latter of which could often be detected as an "extra efferent arteriole" (4,7). Min and Yamanaka (8) then performed detailed analyses of computer-generated three-dimensional images in 94 patients with diabetic nephropathy and found the presence of extravessels. Intriguingly, in this study the abnormal vessels anastomosed to the lobular structure of the intraglomerular capillary network, mainly to afferent branches through the widened vascular hilus, while the distal end of the vessels connected to the peritubular capillary. In these vessels, native endothelial cell function was likely impaired, with the endothelial cells initially swollen and endothelial thickness gradually decreasing as diabetes progressed (9,10). It was also documented that the vascular wall was thickened, owing to an accumulation of matrix in these arterioles (10). Of importance was the finding that these vessels were observed in diabetic patients during the first 2 years of disease (8), which supports the contention that the development of these vessels occurs even in the early phases of diabetic nephropathy.

In diabetic animals, Nyengaard and Rasch (11) identified abnormal glomerular capillaries in an animal rat model induced by streptozotocin. They determined that after both 10 and 50 days following injection, the average total surface area, length, and numbers of glomerular capillaries were elevated compared with those of controls. Similarly, *db/db* mice were found to exhibit increased endothelial cell number and elongation of capillaries in their glomeruli (12,13). Recently, the occurrence of excessive blood vessel formation in diabetes has been demonstrated by immunohistochemistry using endothelial cell markers. As shown in Fig. 1B, endothelial cell staining was increased in streptozotocin-induced diabetic animals (13–16). However, it should be noted that the later stages of diabetic nephropathy are accompanied by capillary loss and rarefaction in both humans and animal models, a concept that is discussed below (5,15,17).

The pathological role of abnormal angiogenesis in diabetic nephropathy. While the pathological role of abnormal vessels remains unclear, it has been demonstrated that neovascularization is associated with glomerular hypertrophy in diabetic nephropathy. Morphological changes in capillaries such as elongation and increased number contribute to glomerular hypertrophy in both humans and animals with diabetes, whereas changes in mean capillary diameter do not correlate with alterations in glomerular volume (7,12). Interestingly, the development of abnormal vessels was observed in the extraglomerular area and associated with glomerular hypertrophy in both diabetic animals and patients (6,10). Osterby et al. (7) performed a series of studies using electron microscopy and found that abnormal vessels in the vascular pole were associated with enhanced glomerular hypertrophy and increased frequency of glomerular capillary occlusion, fibrinoid lesions, tubulointerstitial injury, and urinary albumin excretion (6,10,18). Additionally, recent evidence has indicated that blocking angiogenesis attenuated glomerular basement membrane thickening, mesangial expansion, and transforming growth factor (TGF)-β1 expression in diabetic animals (13,14,16), suggesting that these vessels have a causal role in the development of early features of diabetic nephropathy.

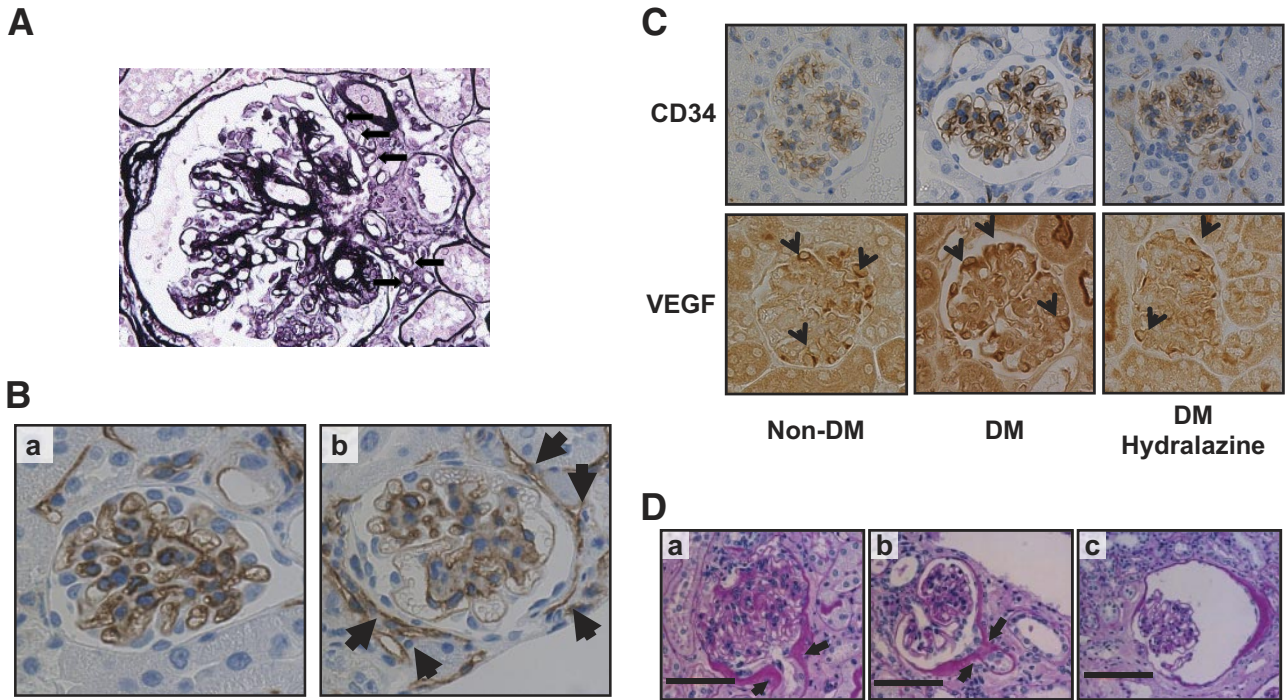The abnormal additional vessels found in diabetes pos-

FIG. 1. Abnormal angiogenesis in diabetic nephropathy. *A*: Extraglomerular neovascularization (black arrows) are found in type 2 diabetic patients. Reprinted with permission from ref. 6. *B*: Similarly, immunohistochemistry for CD34, a marker for endothelial cells, indicates the normal glomerular capillary pattern (brown) in nondiabetic C57BL6 mice (*a*). Alternatively, abnormal capillary formation is observed around glomerulus in diabetic mice lacking endothelial nitric oxide synthase (*b*), resembling abnormal angiogenesis in human diabetic nephropathy. Reprinted with permission from ref. 33. *C*: The association of abnormal angiogenesis with VEGF expression in diabetic eNOSKO mice. In contrast to glomerulus in normal mice, glomerular endothelial staining is increased along with VEGF expression. However, hydralazine treatment attenuates the increase in glomerular capillary number and VEGF expression in diabetic eNOSKO mice. *D*: Glomerular filtrate and atubular glomerulus in diabetic eNOSKO mice. Glomerular filtrates are delivered to the outside of the glomerulus (arrow in *a*) and spread to the glomerulotubular junction (arrows in *a* and *b*). This filtrate may lead to a disconnection between glomerulus and proximal tubules (*b*). Ballooning of Bowman's capsule is observed in *c*. Glomeurlar filtrate can be observed outside of Bowman's capsule in the tubular pole, where the proximal tubulus is completely disconnected from the glomerulus. Bar = 40 μm. (A high-quality digital representation of this figure is available in the online issue.)

sess a thin wall at the basement membrane, while endothelial cells are swollen, suggesting that they are structurally immature and capable of causing an increase in vascular permeability (4,9,19). An increase in capillary permeability often results in the extravasation of plasma protein as well as the forming of lesions in diabetic nephropathy. For example, the capsular drop, fibrin cap, and arteriolar hyalinosis, all of which characterize human diabetic nephropathy, can be considered a consequence of plasma leakage (20). Furthermore, recent studies documented that leakage of plasma protein results in the development of atubular glomeruli in type 1 diabetic patients (21). A potential mechanism for this observation could be due to the glomerular and peritubular filtrate spreading into the glomerulo-tubular junction to eventually separate proximal tubules from the glomerulus (22). Given these facts, the extravasations from immature vessels could have a causal role in the development of diabetic nephropathy.

Mechanisms for the development of abnormal angiogenesis in the diabetic kidney. Angiogenesis is often associated with an increase in endothelial number caused by an imbalance in cell proliferation and apoptosis. Recently, Hohenstein et al. demonstrated that in type 2 diabetic patients, an increased endothelial number was observed and early glomerular lesions were caused by a combination of increased proliferation and decreased apoptosis in glomerular endothelial cells (5). A major driver in this process appears to be VEGF-A expression, which is induced by high glucose levels in the early phases of

diabetes and can stimulate endothelial cell proliferation and inhibit apoptosis. In addition, high glucose levels alone can enhance endothelial cell proliferation (23). Therefore, the beneficial effect of insulin treatment to block the progression of extravessels in patients with type 1 diabetes (7) could be attributed to reduced blood glucose levels and inhibition of VEGF-A expression (15).

Glomerular hypertension may be another important driver in the progression of abnormal angiogenesis in diabetes. Osterby et al. (7) demonstrated that treatment with ACE inhibitors or β-blockers for 8 years to reduce hypertension in diabetic patients suppressed progression of glomerular lesions and extravessel formation. In a similar fashion, we found that lowering blood pressure in a novel animal model of diabetic nephropathy using endothelial nitric oxide synthase (eNOS) knockout (eNOSKO) mice and steptozotocin injection led to attenuated progression of abnormal angiogenesis. These mice developed abnormal vessels accompanied by advanced lesions including nodular lesions and mesangiolysis (15). As shown in Fig. 1*C*, lowering blood pressure in these animals using hydralazine blocked the development of abnormal angiogenesis and inhibited glomerular VEGF-A expression (24). These data suggest that the beneficial effect of lowering blood pressure could be mediated by VEGF-A inhibition. Alternatively, one could postulate that these vessels function as a by-pass to reduce intraglomerular pressure given that abnormal vessels were found to connect intraglomerular capillaries to peritubular capillaries (8). Hence, reducing intraglomerular pressure as a consequence of
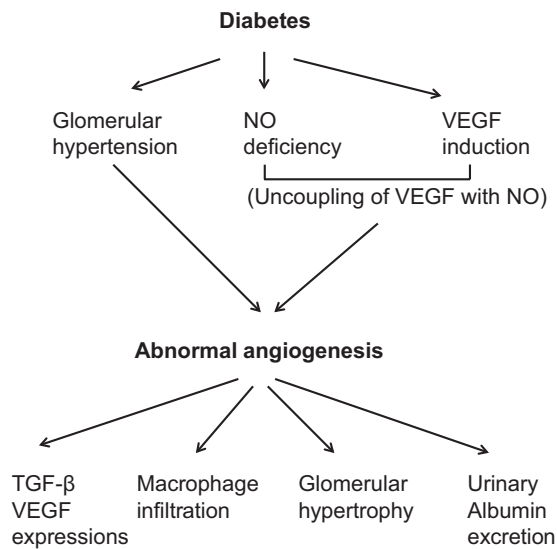
Diabetes

Glomerular hypertension — NO deficiency — VEGF induction

(Uncoupling of VEGF with NO)

Abnormal angiogenesis

TGF-β VEGF expressions — Macrophage infiltration — Glomerular hypertrophy — Urinary Albumin excretion

FIG. 2. **Mechanism and pathogenic role of abnormal angiogenesis in diabetic nephropathy.**

lowering systemic blood pressure might reduce the need for the development of by-pass vessels. A depiction of the factors affecting abnormal angiogenesis and their pathological effects is shown in Fig. 2.

VEGF as a mediator of abnormal angiogenesis in diabetic nephropathy. The VEGF-A family has a role in the development, maintenance, and remodeling of the vasculature, acting through the receptor tyrosine kinases VEGFR-1 and VEGFR-2 (25). The VEGF-A family is very complex with several isoforms generated by alternative splicing of exons 6 and 7. In diabetes, the VEGF-A$_{164}$ and VEGF-A$_{188}$ isoforms are increased and can be reduced by insulin treatment (26). Additional isoforms with anti-angiogenic properties termed VEGF-A$_{xxx}$b occur due to exon 8 distal splice site selection (25), leading to an unique carboxy-terminal sequence.

Several studies have examined the expression pattern of the VEGF-A family in diabetic animals and patients. Cooper et al. (27) examined VEGF-A and VEGFR-2 in short- and long-term diabetic rats (3 and 32 weeks following streptozotocin injection, respectively). Short-term diabetes led to elevated VEGF-A and VEGFR-2 mRNA, whereas in long-term diabetic animals VEGF-A remained elevated and VEGFR-2 was unaltered. VEGF-A was localized to podocytes and, to a lesser extent, tubular epithelial cells, whereas VEGFR-2 was expressed in glomerular and peritubular capillaries. Elevated VEGF-A has been confirmed in our animal model of diabetic nephropathy using eNOSKO mice (15). The mice developed excessive vessels in glomeruli and tubulointerstitium that were associated with upregulation of glomerular VEGF-A expression. Elevation of VEGF-A has also been observed in human biopsy samples where the number of extravessels around the glomerular vascular pole was associated with upregulation of VEGF-A expression in the kidney (6). Finally, examination of urinary VEGF-A showed significant elevations in type 2 diabetic patients (28), compared with healthy control subjects, that positively correlated with urinary albumin-to-creatinine ratio and negatively correlated with creatinine clearance.

A potential consequence of high levels of VEGF-A will be enhanced vascular permeability in the glomerulus (29). In addition, low NO (nitric oxide) bioavailability observed

in diabetes (30,31) could be an additional contributor to the increased vascular permeability. Predescu et al. (32) documented that low levels of endothelial-derived NO altered the integrity of interendothelial junctions in capillaries, resulting in an increase in vascular permeability. As such, a low NO bioavailability along with high VEGF-A expression (we term this condition "uncoupling of VEGF-A with NO") observed in the diabetic milieu of eNOSKO mice could potentiate the vascular permeability in the glomerulus and cause glomerular injury in diabetic nephropathy (15,33). Intriguingly, this uncoupling condition could also cause the development of abnormal angiogenesis. This notion can be supported by recent evidences from our laboratory and other groups that NO can negatively regulate VEGF-A–induced endothelial proliferation (34), whereas NO deficiency enhances VEGF-A activity, leading to endothelial proliferation (35). We have extensively reviewed a causal role of this uncoupling condition in other types of vascular diseases, including coronary artery disease, remnant kidney, and angiotensin II–induced renal injury in previous work (33). It should be noted that while the aforementioned studies indicate low NO contributing to capillary hyperpermeability, Tilton et al. (36) demonstrated that supra-physiological NO positively mediates hyperpermeability in response to exogenous VEGF-A in several different nondiabetic tissues. Therefore, it is likely that physiological levels of NO are required to maintain low vascular permeability and that NO levels that are either too high or too low (depending on the biological situation) may lead to hyperpermeability.

VEGF-A is lowered in the advanced stage of diabetic nephropathy. The study by Cooper et al. (27) suggested that although VEGF-A may be elevated in the initial phases of diabetic nephropathy, it may not be maintained as more chronic fibrotic changes occur in the kidney. Indeed, in many animal models of chronic kidney disease, VEGF-A levels are reduced, correlating with the progression of renal damage (37,38). To examine this in diabetic nephropathy, Baelde et al. (17) used laser-capture microdissection to determine gene expression in glomeruli from 28 diabetic patients. They observed a reduction of 2.5-fold in VEGF-A expression in severely injured glomeruli as evidenced by a loss of endothelial cells and a reduction in podocyte markers (WT-1, nephrin, and podocin mRNAs) (17). Given that podocytes and tubular epithelial cells are the primary source of VEGF-A in the kidney, the mechanism for a reduction in VEGF-A expression in severe renal injury could be attributed to the inability of these cells to produce VEGF-A due to advanced stages of cellular injury. Other studies have found that VEGF-A expression was decreased in sclerotic areas and in nodular lesions of diabetic nephropathy (39,40). In addition, Zucker diabetic fatty rats exhibited a decline in renal VEGF-A expression in advanced stages of diabetic nephropathy (41,42). This interesting concept was highlighted in an elegant study by Hohenstein et al. (5) where they used specific antibodies to examine not only VEGF-A expression but also receptor-bound VEGF-A as a marker of bioactivity in diabetic patients. In their study, although VEGF-A expression was increased in all diabetic glomeruli by many cell types, VEGF-A activity was only increased in the endothelium of mildly injured glomeruli and significantly decreased in more severe glomeruli (5). This data suggests that the upregulation of VEGF-A in early stages of diabetic nephropathy may provide a mechanism for the initial progression of the disease, leading to excessive blood vessel

TABLE 1
Reduction of VEGF expression in diabetic nephropathy

	Renal VEGF expression	Endothelium	Diabetes history	Stage of nephropathy	Ref.
Human	Decreased (mRNA, protein)	NE	Not mentioned	Sclerotic glomerulus	40
Human	Decreased (mRNA)	NE		Sclerotic glomerulus with heavy proteinuria	70
Human type 2 diabetes	Decreased (mRNA)	NE	>5 years	Glomerulosclerosis (24–33%) and tubulointerstitial injury (25–50%)	71
Human type 2 diabetes	Decreased mRNA in VEGF165, increased mRNA in VEGF121	NE	>2 years	Microalbuminuria, 10 of 17 patients; macroalbuminuria, 7 of 17 patients	72
Human type 2 diabetes	Decreased (protein)	Decreased EC proliferation, increased EC apoptosis	12.8 ± 7.8 years	Advanced lesions	5
Human type 2 diabetes	Decreased (mRNA, protein)	Decreased CD31 (+) EC	>5 years	Interstitial fibrosis, podocyte loss	17
Human type 1 and 2 diabetes	Decreased (mRNA, protein)	Decreased CD31 (+) EC	Established diabetes or early diabetes	EC loss in peritubular capillary or tubulointerstitial injury	73
Zucker rat	Decreased (mRNA, protein)	NE	22 weeks old	Advanced renal injury	41

EC, endothelial cell; NE, not examined.

formation. The decline of VEGF-A in the later phase of diabetic nephropathy may reflect a loss of endogenous VEGF-A due to the disruption of podocytes and tubular cells in chronic kidney damage (Table 1).

Alterations in angiopoietin balance as a molecular mechanism of diabetic nephropathy. A second family of growth factors implicated in the progression of diabetic nephropathy are the angiopoietins, which are critical for the normal vascular differentiation, maintenance, and turnover of blood vessels in mature animals (43). Angiopoietin-1 and -2 are ligands for the Tie-2 receptor tyrosine kinase, expressed mainly by endothelia; angiopoietin-1 stimulates receptor activation, leading to promotion of endothelial survival and stabilization. Angiopoietin-2 is considered a natural antagonist of angiopoietin-1 (44), although other data suggest that high concentrations of angiopoietin-2 may activate Tie-2 (45). Alterations in the expression of the angiopoietins have been implicated in the progression of diabetic nephropathy (rev. in 43). In addition, transgenic mice with inducible overexpression of angiopoietin-2 in podocytes in otherwise normal healthy adult animals develop significant increases in albuminuria (46), a parameter that correlates with, and can predict, the progression of renal damage in diabetes (47). Collectively, these observations suggest that a decreased ratio of angiopoietin-1 to angiopoietin-2 might play a role alongside VEGF-A in the pathobiology of diabetic nephrology. Importantly, the biological effects of angiopoietin-2 are context dependent and, in vivo, depend on ambient levels of VEGF-A, such that vessel regression occurs if VEGF-A is lacking, whereas vessel destabilization followed by angiogenesis occurs if the local milieu is rich in VEGF-A (44). It could be postulated that the increased levels of angiopoietin-2 alongside a VEGF-A–rich milieu in glomeruli during the initial phases of diabetes will lead to the destabilization of blood vessels and hence excessive angiogenesis. Therefore, it is possible that modulation of the balance between angiopoietin-1 and -2 may have therapeutic potential in diabetic nephropathy.

Targeting angiogenesis to treat diabetic nephropathy. Given the evidence above, there is a rationale for targeting angiogenic pathways to prevent diabetic nephropathy, and several studies have now blocked VEGF-A activity as a therapy to prevent abnormal angiogenesis. An elegant genetic approach was recently taken by Gnudi and colleagues (48) by blocking VEGF-A signaling in mice administered streptozotocin through overexpressing soluble VEGFR-1, specifically in podocytes. Diabetic mice that overexpressed soluble VEGFR-1 had attenuated albumin excretion, mesangial expansion, glomerular basement membrane thickening, podocyte foot process fusion, and TGF-β1 expression (48). de Vriese's group (49) examined the effect of treatment with a monoclonal anti–VEGF-A antibody in the early phase of diabetes induced by streptozotocin. Administration of the antibody decreased hyperfiltration, albuminuria, and glomerular hypertrophy in diabetic rats. Although the effect on angiogenesis was not specifically examined in this study, VEGF-A blockade prevented the upregulation of eNOS associated with this model (49). Other studies were performed in *db/db* mice and the Zucker diabetic fatty rat (50,51). In *db/db* mice, VEGF-A antibody treatment resulted in a reduction in kidney weight, glomerular volume, basement membrane thickness, and urinary albumin excretion (50); in the Zucker diabetic fatty rat, VEGF-A antibody treatment prevented glomerular hypertrophy. However, neither of

TABLE 2
VEGF inhibitors

Function	Drug name
Inhibition of VEGF secretion	Iressa*
	Tarceva*
Sequestration of VEGF	Bevacizumab*
	Ranibizumab*
	Pegaptanib*
Blocking binding of VEGF to VEGFR	DC101
Inhibitior of receptor tyrosine kinase	Sunitinib*
	Sorafenib*

*Approved by the U.S. Food and Drug Administration.

these studies examined the effect of reducing VEGF-A on abnormal angiogenesis. Similarly, Sung et al. (52) blocked the phosphorylation of the VEGF-A receptors using the pharmacological kinase inhibitor SU5416 in *db/db* mice and found that this approach prevented the development of albuminuria and glomerular basement membrane thickening. Interestingly, blocking VEGF-A activation prevented the loss of nephrin and improved structural changes in podocyte foot processes in *db/db* mice. These results suggest that VEGF-A could impair podocyte function, which may be an additional mechanism by which VEGF-A causes urinary protein excretion. However, since these studies did not examine the process of abnormal angiogenesis per se, further experiments are required to determine whether this beneficial effect of anti–VEGF-A therapy could be due to the blocking of VEGF-associated angiogenesis.

Currently VEGF-A inhibitors are classified into four groups (Table 2) and have been used in clinical practice. Importantly, the efficacy of these individual compounds is not identical. For instance, the tyrosine kinase inhibitors have greater anti-tumor efficacy only at early stages of cancer progression (53), whereas monoclonal antibodies are capable of regressing tumor growth (54). In the kidney, VEGF-A function is also complicated given that it has been found to exhibit both deleterious and beneficial effects (rev. in 33). In fact, VEGF-A is found to be deleterious in diabetic nephropathy but largely beneficial in nondiabetic animal models of renal disease. Hence, we need to be cautious before using VEGF-A inhibitors in the diseased kidney. Previously, the beneficial effect of anti–VEGF-antibodies was shown in two diabetic animal models: streptozotocin-induced diabetic rats and *db/db* mice (49,55). On the contrary, it has been postulated that a potential adverse effect with VEGF-A inhibitors could be endothelial injury because endothelial cells require VEGF-A in physiological conditions. Eremina et al. (56) demonstrated that bevacizumab, the anti–VEGF-antibody, causes renal thrombotic microangiopathy partly due to endothelial injury in patients. Similarly, Advani et al. (57) demonstrated that VEGFR-2 tyrosine kinase inhibitors exacerbated hypertension and renal disease in hypertensive rats. Likewise, systemic overexpression of soluble VEGFR-1 in normal animals was found to cause endotheliosis and podocyte injury, leading to proteinuria and hypertension (58,59). In addition, the deleterious effect of anti-VEGF antibodies could be attributed to the deposition of VEGF–anti-VEGF complex, C3 deposition, and endothelial swelling (54). However, in some experiments, it was also shown that normal kidneys did not have any side effects from VEGF inhibitors treatment (60,61).

Since endothelial cells require VEGF-A in physiological conditions, substantial inhibition can cause endothelial injury. In this regard, it may not be adequate to use VEGF-A inhibitors in patients with normal kidney function or in nondiabetic renal injury in which VEGF-A expression is downregulated. In contrast, Gnudi and colleagues (48) succeeded in treating diabetic nephropathy using podocyte-specific overexpression of soluble VEGFR-1. In this study, neither VEGF-A expression nor VEGFR-2 phosphorylation was significantly blocked by overexpression of soluble VEGFR-1 in the diabetic kidney, suggesting that VEGF-A function was partially inhibited. Thus, the "partial" inhibition might be a means to treat diabetic nephropathy without any adverse effects. Further clarification on the adverse effects of VEGF-A inhibitors is required before they may be used to treat diabetic patients.

Angiopoietins have been used therapeutically in several diabetes situations. Administration of angiopoietin-1 has been shown to suppress diabetic retinopathy by preventing leukocyte adhesion, endothelial cell injury, and blood-retinal barrier breakdown (62). With regard to diabetic nephropathy, Lee et al. (63) demonstrated that systemic adenoviral delivery of COMP-Ang-1 (a modified form of angiopoietin-1) reduced renal fibrosis in *db/db* mice. However, this strategy also caused a significant improvement in hyperglycemia, an event possibly related to the systemic administration of angiopoietin-1, which could itself, at least partly, account for the amelioration of diabetic nephropathy. Therefore, further experiments are required to examine whether modulation of this pathway could be a future treatment for patients with diabetic nephropathy.

Several studies have attempted to block angiogenesis using other anti-angiogenic molecules in animal models, as shown in Table 3 (13,14,16,64). Angiostatin is a potent angiogenic inhibitor that blocks proliferation, induces apoptosis, and prevents migration of endothelial cells in vitro. In addition, angiostatin has anti-inflammatory actions by inhibiting leukocyte recruitment and both neutrophil and macrophage migration. In streptozotocin-induced diabetic nephropathy, adenoviral-mediated delivery of angiostatin was found to alleviate albuminuria and glomerular hypertrophy (64). We also found a similar advantage of angiostatin treatment in the remnant kidney model (65). Similarly, endostatin, a potent inhibitor of angiogenesis derived from type XVIII collagen (14), and tumstatin, an angiogenic inhibitor derived from type IV collagen (16), were both able to prevent glomerular hypertrophy, hyperfiltration, and albuminuria in type 1 diabetic mice. Interestingly, these treatments were shown to prevent mesangial expansion and inflammation and also to attenuate the increase in levels of VEGF-A and angiopoietin-2 normally observed in this model (14,16) independent of blood pressure and blood glucose levels (Fig. 2). Similar observations were made with 2-(8-hydroxy-6-methoxy-1-oxo1H-2-benzopyran-3-yl) propionic acid (a small molecule with anti-angiogenic activity) in *db/db* mice (13). These novel treatments to prevent angiogenesis could be considered for patients in early stages of diabetic nephropathy.

What prospects are there for other novel therapies for diabetic nephropathy? One area of interest may be in examining the anti-angiogenic isoforms of VEGF-A$_{xxx}$b in models of diabetic nephropathy, which may open new avenues of treatment strategies. Another therapy could be the use of RNA aptamers, which are oligonucleotidue ligands that bind with high-affinity to molecular targets.

TABLE 3
Anti-angiogenic therapy in diabetic nephropathy

	Anti-VEGF antibody Blocking VEGF			SU5416	Angiostatin	Endostatin	Tumstatin	NM-3	PEDF
	STZ Wister rat*	db/db mouse*	GK rat*	Pan-VEGFR kinase inhibitor	Proteolytic fragment of plasminogen	NC1 domain of type 18 collagen	NC domain of the α3 chain of type 4 collagen	2-(8-hydroxy-6-methoxy-1-oxo-1H-2-benxopyran-3-yl) propionic acid	Pigment epithelium-derived factor
				db/db mouse	STZ Brown Norway rat	STZ-C57BL6 mouse*	STZ-C57BL6 mouse*	db/db mouse*	STZ Brown Norway rat
Age or weight	250–280 g	8 weeks	8 weeks	8 weeks	8 weeks	7–8 weeks	7–8 weeks	7–8 weeks	8 weeks
Treatment duration	≤6 weeks	60 days	6 weeks	8 weeks	2–3 weeks	4 weeks	2–3 weeks	8 weeks	3 weeks
Increase in CD31(+) endothelial cell in glomeruli	NE	NE	NE	NE	NE	Blocked	Blocked	Blocked	NE
Renal hypertrophy	NE	Blocked	NE	NE	NE	Blocked	Blocked	Not blocked	NE
Glomerular hypertrophy	Blocked	Blocked	No effect	NE	Blocked	Blocked	Blocked	Blocked	NE
Mesangial expansion	NE	Tended to be lowered	NE	Blocked	NE	Blocked	Blocked	Blocked	NE
Glomerular basement membrane thickening	NE	Blocked	NE	Blocked	NE	NE	NE	NE	NE
Hyperfiltration	Blocked	Blocked	No effect	NE	NE	Blocked	Blocked	Blocked	NE
Urinary albumin	Decreased	Decreased	No effect	Decreased	Decreased	Decreased	Decreased	Decreased	Decreased
Podocyte injury or Nephrin expression	NE	NE	NE	Improved	NE	Recovered (Nephrin expression)	Recovered (Nephrin expression)	Recovered (Nephrin expression)	NE
Macrophage infiltration	NE	NE	NE	NE	NE	Blocked	Blocked	Blocked	NE
VEGF expression	NE	NE	NE	NE	Decreased	Decreased	Decreased	Decreased	NE
TGF-β1 expression	NE	NE	NE	NE	Decreased	Decreased	NE	Decreased	Decreased
Reference	49	50	51	52	64	14	16	13	74, 75

NE, not examined; STZ, streptococotin. *Female.

One such aptamer that targets VEGF-A$_{165}$ has been used successfully in clinical trials to block ocular neovascularization (66). Promising results have also been obtained using small-molecule tyrosine kinase inhibitors to treat type 1 diabetic mice (67); however, the kidneys were not examined in these studies. Finally, other molecules involved in angiogenic pathways such as the Notch family (68) may provide interesting information in the pathobiology and treatment of diabetic nephropathy. In this regard, studies by Niranjan et al. (69) have already demonstrated that lack of the Notch1 transcriptional partner *Rbpj* in podocytes is able to modulate the progression of albuminuria in diabetic mice.

In conclusion, while the presence of abnormal angiogenesis was demonstrated more than a decade ago, we are only beginning to unravel the pathophysiological importance of this event. Anti-angiogenic treatments can prevent the progression of animal models of diabetic nephropathy, but further studies are required before these treatments can be used in a clinical setting. The fact that diabetic nephropathy is currently still the leading cause of end-stage renal disease points to the need for additional treatment strategies. Thus, novel therapies that target other angiogenic pathways such as the angiopoietin and Notch families could be an attractive option to block diabetic nephropathy in the future.

REFERENCES

1. Mohamed Q, Gillies MC, Wong TY. Management of diabetic retinopathy: a systematic review. JAMA 2007;298:902–916
2. Aiello LP, Gardner TW, King GL, et al. Diabetic retinopathy. Diabetes Care 1998;21:143–156
3. Afzal A, Shaw LC, Ljubimov AV, Boulton ME, Segal MS, Grant MB. Retinal and choroidal microangiopathies: therapeutic opportunities. Microvasc Res 2007;74:131–144
4. Osterby R, Nyberg G. New vessel formation in the renal corpuscles in advanced diabetic glomerulopathy. J Diabet Complications 1987;1:122–127
5. Hohenstein B, Hausknecht B, Boehmer K, et al. Local VEGF activity but not VEGF expression is tightly regulated during diabetic nephropathy in man. Kidney Int 2006;69:1654–1661
6. Kanesaki Y, Suzuki D, Uehara G, et al. Vascular endothelial growth factor gene expression is correlated with glomerular neovascularization in human diabetic nephropathy. Am J Kidney Dis 2005;45:288–294
7. Osterby R, Bangstad HJ, Nyberg G, et al. On glomerular structural alterations in type-1 diabetes: companions of early diabetic glomerulopathy. Virchows Arch 2001;438:129–135
8. Min W, Yamanaka N. Three-dimensional analysis of increased vasculature around the glomerular vascular pole in diabetic nephropathy. Virchows Arch A Pathol Anat Histopathol 1993;423:201–207
9. Wehner H, Nelischer G. Morphometric investigations on intrarenal vessels of streptozotocin-diabetic rats. Virchows Arch A Pathol Anat Histopathol 1991;419:231–235
10. Osterby R, Hartmann A, Bangstad HJ. Structural changes in renal arterioles in type 1 diabetic patients. Diabetologia 2002;45:542–549
11. Nyengaard JR, Rasch R. The impact of experimental diabetes mellitus in rats on glomerular capillary number and sizes. Diabetologia 1993;36:189–194
12. Guo M, Ricardo SD, Deane JA, et al. A stereological study of the renal glomerular vasculature in the db/db mouse model of diabetic nephropathy. J Anat 2005;207:813–821
13. Ichinose K, Maeshima Y, Yamamoto Y, et al. 2-(8-hydroxy-6-methoxy-1-oxo-1h-2-benzopyran-3-yl) propionic acid, an inhibitor of angiogenesis, ameliorates renal alterations in obese type 2 diabetic mice. Diabetes 2006;55:1232–1242
14. Ichinose K, Maeshima Y, Yamamoto Y, et al. Antiangiogenic endostatin peptide ameliorates renal alterations in the early stage of a type 1 diabetic nephropathy model. Diabetes 2005;54:2891–2903
15. Nakagawa T, Sato W, Glushakova O, et al. Diabetic eNOS knockout mice develop advanced diabetic nephropathy. J Am Soc Nephrol 2007;18:539–550
16. Yamamoto Y, Maeshima Y, Kitayama H, et al. Tumstatin peptide, an inhibitor of angiogenesis, prevents glomerular hypertrophy in the early stage of diabetic nephropathy. Diabetes 2004;53:1831–1840
17. Baelde HJ, Eikmans M, Lappin DW, et al. Reduction of VEGF-A and CTGF expression in diabetic nephropathy is associated with podocyte loss. Kidney Int 2007;71:637–645
18. Osterby R, Asplund J, Bangstad HJ, et al. Neovascularization at the vascular pole region in diabetic glomerulopathy. Nephrol Dial Transplant 1999;14:348–352
19. Osterby R. Glomerular structural changes in type 1 (insulin-dependent) diabetes mellitus: causes, consequences, and prevention. Diabetologia 1992;35:803–812
20. Stout LC, Kumar S, Whorton EB. Insudative lesions—their pathogenesis and association with glomerular obsolescence in diabetes: a dynamic hypothesis based on single views of advancing human diabetic nephropathy. Hum Pathol 1994;25:1213–1227
21. Najafian B, Kim Y, Crosson JT, et al. Atubular glomeruli and glomerulotubular junction abnormalities in diabetic nephropathy. J Am Soc Nephrol 2003;14:908–917
22. Kriz W, Hosser H, Hahnel B, et al. From segmental glomerulosclerosis to total nephron degeneration and interstitial fibrosis: a histopathological study in rat models and human glomerulopathies. Nephrol Dial Transplant 1998;13:2781–2798
23. McGinn S, Saad S, Poronnik P, et al. High glucose-mediated effects on endothelial cell proliferation occur via p38 MAP kinase. Am J Physiol Endocrinol Metab 2003;285:E708–E717
24. Kosugi T, Heinig M, Nakayama T, Connor T, Yuzawa Y, Li Q, Hauswirth WW, Grant MB, Croker BP, Campbell-Thompson M, Zhang L, Atkinson MA, Segal MS, Nakagawa T. Lowering blood presure blocks mesangiolysis and mesangial nodules, but not tubulointerstitial injury, in diabetic eNOS knockout mice. Am J Pathol 2009;174:1221–1229
25. Harper SJ, Bates DO. VEGF-A splicing: the key to anti-angiogenic therapeutics? Nat Rev Cancer 2008;8:880–887
26. Chou E, Suzuma I, Way KJ, et al. Decreased cardiac expression of vascular endothelial growth factor and its receptors in insulin-resistant and diabetic states: a possible explanation for impaired collateral formation in cardiac tissue. Circulation 2002;105:373–379
27. Cooper ME, Vranes D, Youssef S, et al. Increased renal expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 in experimental diabetes. Diabetes 1999;48:2229–2239
28. Kim NH, Oh JH, Seo JA, et al. Vascular endothelial growth factor (VEGF) and soluble VEGF receptor FLT-1 in diabetic nephropathy. Kidney Int 2005;67:167–177
29. Weis SM, Cheresh DA. Pathophysiological consequences of VEGF-induced vascular permeability. Nature 2005;437:497–504
30. Daimon M, Sugiyama K, Saitoh T, et al. Increase in serum ceruloplasmin levels is correlated with a decrease of serum nitric oxide levels in type 2 diabetes. Diabetes Care 2000;23:559–560
31. Giugliano D, Marfella R, Coppola L, et al. Vascular effects of acute hyperglycemia in humans are reversed by L-arginine: evidence for reduced availability of nitric oxide during hyperglycemia. Circulation 1997;95:1783–1790
32. Predescu D, Predescu S, Shimizu J, et al. Constitutive eNOS-derived nitric oxide is a determinant of endothelial junctional integrity. Am J Physiol Lung Cell Mol Physiol 2005;289:L371–L381
33. Nakagawa T. Uncoupling of the VEGF-endothelial nitric oxide axis in

diabetic nephropathy: an explanation for the paradoxical effects of VEGF in renal disease. Am J Physiol Renal Physiol 2007;292:F1665–F1672

34. Bussolati B, Dunk C, Grohman M, et al. Vascular endothelial growth factor receptor-1 modulates vascular endothelial growth factor-mediated angiogenesis via nitric oxide. Am J Pathol 2001;159:993–1008

35. Nakagawa T, Sato W, Sautin YY, et al. Uncoupling of vascular endothelial growth factor with nitric oxide as a mechanism for diabetic vasculopathy. J Am Soc Nephrol 2006;17:736–745

36. Tilton RG, Chang KC, LeJeune WS, et al. Role for nitric oxide in the hyperpermeability and hemodynamic changes induced by intravenous VEGF. Invest Ophthalmol Vis Sci 1999;40:689–696

37. Kang DH, Joly AH, Oh SW, et al. Impaired angiogenesis in the remnant kidney model. I. Potential role of vascular endothelial growth factor and thrombospondin-1. J Am Soc Nephrol 2001;12:1434–1447

38. Yuan HT, Li XZ, Pitera JE, et al. Peritubular capillary loss after mouse acute nephrotoxicity correlates with down-regulation of vascular endothelial growth factor-A and hypoxia-inducible factor-1 alpha. Am J Pathol 2003;163:2289–2301

39. Grone HJ, Simon M, Grone EF. Expression of vascular endothelial growth factor in renal vascular disease and renal allografts. J Pathol 1995;177:259–267

40. Shulman K, Rosen S, Tognazzi K, et al. Expression of vascular permeability factor (VPF/VEGF) is altered in many glomerular diseases. J Am Soc Nephrol 1996;7:661–666

41. Gealekman O, Brodsky SV, Zhang F, et al. Endothelial dysfunction as a modifier of angiogenic response in Zucker diabetic fat rat: amelioration with Ebselen. Kidney Int 2004;66:2337–2347

42. Hoshi S, Shu Y, Yoshida F, et al. Podocyte injury promotes progressive nephropathy in Zucker diabetic fatty rats. Lab Invest 2002;82:25–35

43. Woolf AS, Gnudi L, Long DA. Roles of angiopoietins in kidney development and disease. J Am Soc Nephrol 2009;20:239–244

44. Maisonpierre PC, Suri C, Jones PF, et al. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 1997;277:55–60

45. Yuan HT, Khankin EV, Karumanchi SA, et al. Angiopoietin 2 is a partial agonist/antagonist of Tie2 signaling in endothelium. Mol Cell Biol 2009;29:2011–2022

46. Davis B, Dei Cas A, Long DA, et al. Podocyte-specific expression of angiopoietin-2 causes proteinuria and apoptosis of glomerular endothelia. J Am Soc Nephrol 2007;18:2320–2329

47. Viberti GC, Hill RD, Jarrett RJ, et al. Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. Lancet 1982;1:1430–1432

48. Ku CH, White KE, Dei Cas A, et al. Inducible overexpression of sFlt-1 in podocytes ameliorates glomerulopathy in diabetic mice. Diabetes 2008;57:2824–2833

49. de Vriese AS, Tilton RG, Elger M, et al. Antibodies against vascular endothelial growth factor improve early renal dysfunction in experimental diabetes. J Am Soc Nephrol 2001;12:993–1000

50. Flyvbjerg A, Dagnaes-Hansen F, De Vriese AS, et al. Amelioration of long-term renal changes in obese type 2 diabetic mice by a neutralizing vascular endothelial growth factor antibody. Diabetes 2002;51:3090–3094

51. Schrijvers BF, De Vriese AS, Tilton RG, et al. Inhibition of vascular endothelial growth factor (VEGF) does not affect early renal changes in a rat model of lean type 2 diabetes. Horm Metab Res 2005;37:21–25

52. Sung SH, Ziyadeh FN, Wang A, et al. Blockade of vascular endothelial growth factor signaling ameliorates diabetic albuminuria in mice. J Am Soc Nephrol 2006;17:3093–3104

53. Bergers G, Song S, Meyer-Morse N, et al. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. J Clin Invest 2003;111:1287–1295

54. Gerber HP, Wu X, Yu L, Wiesmann C, et al. Mice expressing a humanized form of VEGF-A may provide insights into the safety and efficacy of anti-VEGF antibodies. Proc Natl Acad Sci U S A 2007;104:3478–3483

55. Flyvbjerg A, Bennett WF, Rasch R, et al. Inhibitory effect of a growth hormone receptor antagonist (G120K-PEG) on renal enlargement, glomerular hypertrophy, and urinary albumin excretion in experimental diabetes in mice. Diabetes 1999;48:377–382

56. Eremina V, Jefferson JA, Kowalewska J, et al. VEGF inhibition and renal thrombotic microangiopathy. N Engl J Med 2008;358:1129–1136

57. Advani A, Kelly DJ, Advani SL, et al. Role of VEGF in maintaining renal structure and function under normotensive and hypertensive conditions. Proc Natl Acad Sci U S A 2007

58. Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest 2003;111:649–658

59. Sugimoto H, Hamano Y, Charytan D, et al. Neutralization of circulating vascular endothelial growth factor (VEGF) by anti-VEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induces proteinuria. J Biol Chem 2003;278:12605–12608

60. Ostendorf T, Kunter U, Eitner F, et al. VEGF(165) mediates glomerular endothelial repair. J Clin Invest 1999;104:913–923

61. Kamba T, Tam BY, Hashizume H, et al. VEGF-dependent plasticity of fenestrated capillaries in the normal adult microvasculature. Am J Physiol Heart Circ Physiol 2006;290:H560–H576

62. Joussen AM, Poulaki V, Tsujikawa A, et al. Suppression of diabetic retinopathy with angiopoietin-1. Am J Pathol 2002;160:1683–1693

63. Lee S, Kim W, Moon SO, et al. Renoprotective effect of COMP-angiopoietin-1 in db/db mice with type 2 diabetes. Nephrol Dial Transplant 2007;22:396–408

64. Zhang SX, Wang JJ, Lu K, et al. Therapeutic potential of angiostatin in diabetic nephropathy. J Am Soc Nephrol 2006;17:475–486

65. Mu W, Long DA, Ouyang X, et al. Angiostatin overexpression is associated with an improvement in chronic kidney injury by an anti-inflammatory mechanism. Am J Physiol Renal Physiol, 2009;296:F145–F152

66. Ng EW, Shima DT, Calias P, et al. Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. Nat Rev Drug Discov 2006;5:123–132

67. Louvet C, Szot GL, Lang J, et al. Tyrosine kinase inhibitors reverse type 1 diabetes in nonobese diabetic mice. Proc Natl Acad Sci U S A 2008;105:18895–18900

68. Gaengel K, Genove G, Armulik A, et al. Endothelial-mural cell signaling in vascular development and angiogenesis. Arterioscler Thromb Vasc Biol 2009;29:630–638

69. Niranjan T, Bielesz B, Gruenwald A, et al. The Notch pathway in podocytes plays a role in the development of glomerular disease. Nat Med 2008;14:290–298

70. Bailey E, Bottomley MJ, Westwell S, et al. Vascular endothelial growth factor mRNA expression in minimal change, membranous, and diabetic nephropathy demonstrated by non-isotopic in situ hybridisation. J Clin Pathol 1999;52:735–738

71. Baelde HJ, Eikmans M, Doran PP, et al. Gene expression profiling in glomeruli from human kidneys with diabetic nephropathy. Am J Kidney Dis 2004;43:636–650

72. Bortoloso E, Del Prete D, Dalla Vestra M, et al. Quantitave and qualitative changes in vascular endothelial growth factor gene expression in glomeruli of patients with type 2 diabetes. Eur J Endocrinol 2004;150:799–807

73. Lindenmeyer MT, Kretzler M, Boucherot A, Berra S, Yasuda Y, Henger A, Eichinger F, Gaiser S, Schmid H, et al. Interstitial vascular rarefaction and reduced VEGF-A expression in human diabetic nephropathy. J Am Soc Nephrol 2007;18:1765–1776

74. Wang JJ, Zhang SX, Mott R, et al. Anti-inflammatory effects of pigment epithelium-derived factor in diabetic nephropathy. Am J Physiol Renal Physiol 2008;294:F1166–F1173

75. Wang JJ, Zhang SX, Mott R, et al. Salutary effect of pigment epithelium-derived factor in diabetic nephropathy: evidence for antifibrogenic activities. Diabetes 2006;55:1678–1685