Angiogenesis in Diabetic Nephropathy

Roy Zent, MD, PhD, and Ambra Pozzi, PhD

Summary: Angiogenesis, the formation of new blood vessels from pre-existing vasculature, plays a key role in both physiologic and pathologic events, including wound healing, cancer, and diabetes. Neovascularization has been implicated in the genesis of diverse diabetic complications such as retinopathy, impaired wound healing, neuropathy, and, most recently, diabetic nephropathy. Diabetic nephropathy is one of the major microvascular-associated complications in diabetes and is the leading cause of end-stage renal disease worldwide. In this review we describe the major factors involved in the pathologic glomerular microvascular alterations in response to hyperglycemia and the possible use of anti-angiogenic therapies for the treatment of diabetic nephropathy.
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Diabetic nephropathy represents a major cause of morbidity and mortality in type 1 and type 2 diabetic subjects and has become the leading cause of end-stage renal disease worldwide. Currently there is no specific therapy for this condition, which almost invariably progresses to end-stage renal failure. One of the hallmarks of diabetic nephropathy is glomerular microvascular injury, which potentially may be a therapeutic target for this devastating medical condition. In this review we describe (1) the major steps involved in angiogenesis, (2) the pathologic glomerular vascular changes observed in diabetic nephropathy, and (3) the possible use of anti-angiogenic therapy for the treatment of diabetic-induced renal vascular damage.

ANGIOGENESIS

Angiogenesis is the formation of new blood vessels from pre-existing vasculature. This process plays a key role in both physiologic and pathologic events, including embryonic development, menstruation, wound healing, tumor growth, and diabetes. Angiogenesis is a multistep process that requires at least 4 independent events by endothelial cells, including detachment from basement membranes, proliferation, migration, and maturation.[1] Normally these events are regulated tightly by both pro-angiogenic and anti-angiogenic factors, however, in pathologic events such as diabetes there is increased synthesis of pro-angiogenic factors with concomitant down-regulation of anti-angiogenic molecules. This leads to increased proliferation and migration of endothelial cells, resulting in the formation of immature and leaky vessels.

Proangiogenic Factors

The soluble molecules vascular endothelial growth factor (VEGF) and angiopoietins (Ang 1 and Ang 2), are the best-characterized growth factors that play a role in angiogenesis. The VEGF family consists of at least 4 members, VEGF-A, -B, -C, and -D.[2] VEGF-A, the most predominant, consists of at least 8 isoforms, with VEGF165 the major form expressed in humans (VEGF 164 in mouse). VEGF was first described as a vascular permeability factor because of its ability to induce leaky vessels. It exerts its actions by binding 3 different receptors selec-

Department of Research Medicine, Veterans Affairs Hospital, Nashville, TN, and Department of Medicine, Division of Nephrology, Medical Center North, Vanderbilt University, Nashville, TN.
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Address reprint requests to Ambra Pozzi, PhD, Department of Medicine, Division of Nephrology, Vanderbilt University, Medical Center North, B3109, Nashville, TN, 37232. E-mail: ambra.pozzi@vanderbilt.edu
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tively expressed on endothelial cells, VEGF receptor I (ie, VEGFR1, Flt-1), VEGF receptor 2 (ie, VEGFR2, Flk-1), and VEGF receptor 3 (VEGFR3).[3] Flt-1 is required for the recruitment of hematopoietic precursors and the migration of monocytes and macrophages, whereas Flk-1 and VEGFR3 are essential for the functions of vascular endothelial and lymphendothelial cells, respectively.[3] VEGF is probably the most potent angiogenic factors and its up-regulation often is observed in pathologic conditions, including cancer, rheumatoid arthritis, and diabetes. Up-regulated VEGF synthesis is accompanied by increased endothelial cell migration, proliferation, and formation of immature vessels characterized by leakiness and decreased vascular resistance.

The angiopoietins belong to a family of at least 4 members, with Ang 1 and Ang 2 being the most predominant.[4] Both Ang 1 and Ang 2 exert their action by binding the same receptor Tie-2, selectively expressed on endothelial cells. Interestingly, Ang 1 and Ang 2 exert opposite effects on endothelial cell function. Although Ang 1–mediated signaling via Tie-2 leads to vessel maturation, quiescence, and reduced leakage,[5,6] Ang 2 blocks the Ang 1/Tie-2 signal resulting in increased angiogenesis, vessel instability, and consequent leakage.[7,8] Moreover, although Ang 1 promotes endothelial cell adhesion, spreading, and formation of focal contacts, Ang 2 enhances endothelial cell migration and tubulogenesis. Ang 1 and Ang 2, unlike VEGF, are not considered complete angiogenic factors because they cannot trigger angiogenic responses by themselves, but rather they positively or negatively modulate VEGF-induced endothelial cell function.[9] Interestingly, Ang 2 expression can be up-regulated by VEGF[10] and it enhances VEGF-mediated angiogenesis.[4] In pathologic events, such as cancer or diabetes, increased VEGF synthesis often is accompanied by increased Ang 2 levels with decreased and/or unchanged levels of Ang 1.

Anti-angiogenic Factors

To ensure that there is not an overproduction of blood vessels there are endogenous inhibitors of angiogenesis that can be classified into 2 major categories: proteolytic fragments and gene products.[11] Among the proteolytic fragments, extracellular matrix–derived and plasminogen-derived fragments have been shown to inhibit angiogenesis by inhibiting endothelial cell migration, proliferation, and tubulogenesis. Some of these fragments include angiostatin (a cleavage product of circulating plasminogen), endostatin (a cleavage product of collagen XVIII), the α1NC1 domain of collagen IV,[12] and the α3NC1 domain of collagen IV.[11,13] In contrast, most of the gene product inhibitors have pleiotropic effects that are not necessarily related to the regulation of angiogenesis. For example, thrombospondin-1 and pigment epithelium–derived factor (PEDF), which are well-described inhibitors of angiogenesis in both physiologic and pathologic conditions, can promote endothelial cell apoptosis by inducing the Fas ligand.[14] PEDF originally was isolated as a protein secreted by cultured pigment epithelial cells of fetal human retina,[15] but later was shown to possess plural effects, including neuronal cell differentiation, protection of neurons from various neurotoxic agents, and, most importantly, angiogenesis inhibition.[16] Moreover, in retinal endothelial cells PEDF down-regulates the levels of VEGF, thus preventing vascular permeability and angiogenesis.[17] Finally, there are 2 inhibitors, soluble VEGF receptor 1 and vasohibin, which are expressed only in endothelial cells, and have selective activities against endothelial cells themselves.[11] Soluble VEGF receptor 1 selectively blocks VEGF signaling and only inhibits VEGF-mediated effects, including angiogenesis and vascular permeability. Vasohibin is proposed to be the first negative feedback regulator of angiogenesis and it works by interacting with specific endothelial cell intracellular signaling pathways.

Fig. 1 summarizes the major pro-angiogenic and anti-angiogenic factors that contribute to the homeostasis of blood vessel formation.

DIABETIC NEPHROPATHY AND VASCULAR DAMAGE

The clinical entity of diabetic nephropathy, the most common cause of end-stage renal disease in the developed world, is characterized ini-

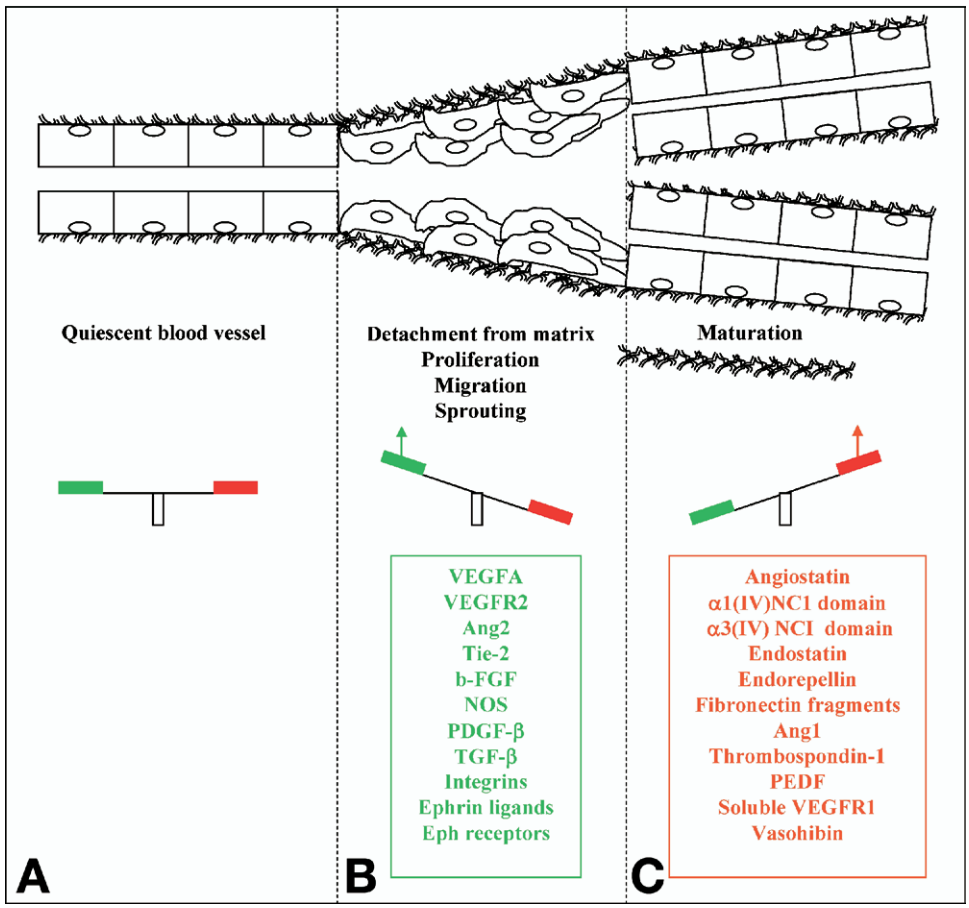

Figure 1. Schematic representation of the major pro- and anti-angiogenic factors involved in the control of angiogenesis. In quiescent blood vessels (panel A) there is a tight balance between the production of pro-angiogenic (see list in panel B) and anti-angiogenic (see list in panel C) factors. Increased production of pro-angiogenic factors controls specific events in new blood vessel formation, including detachment from matrix, proliferation, migration, and sprouting (panel B). In contrast, maturation of blood vessels is accompanied by increased production of anti-angiogenic factors (panel C).

tially by glomerular hyperfiltration, glomerular and tubular epithelial hypertrophy, and microalbuminuria. In established diabetic nephropathy, the glomeruli show basement membrane thickening, mesangial matrix expansion, arteriolar hyalinosis, and sclerosis, and there is evidence of interstitial fibrosis.[18,19] A principal mechanism whereby hyperglycemia induces diabetic nephropathy is by stimulating excessive production of reactive oxygen species (ROS) in multiple cell types, including mesangial cells[20] and podocytes.[21] ROS in turn can up-regulate the expression of profibrotic molecules such as connective tissue growth factor (CTGF) and transforming growth factor-β (TGF-β), thus increasing the glomerular extracellular matrix deposition.[22,23] Hyperglycemia also increases the production of advanced glycation end-products (AGEs) of extracellular matrix components in the mesangium and glomerular basement membrane, resulting in changes in permeability of the filtration barrier.[24] Finally, mechanical stress associated with intraglomerular hypertension causes podocyte damage, which is associated with down-regulation of nephrin, an important protein of the slit diaphragm with anti-apoptotic signaling properties. The loss of nephrin correlates with foot process effacement of podocytes and increased proteinuria.[25]

Although the mesangial cells and podocytes are proposed as the major mediators of diabetic nephropathy, diabetic-induced microvasculature injury also plays a key role in the pathogenesis. Similar to diabetic retinopathy, an in-

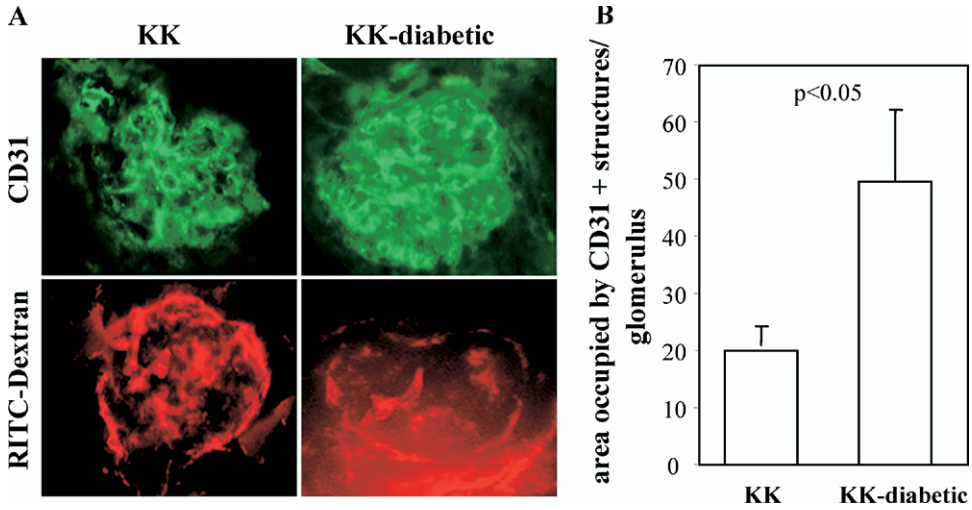

Figure 2. Increased glomerular vascularity and permeability in KK diabetic mice. (A) Frozen sections of kidneys derived from control (KK) and 25-week-old diabetic mice (KK-diabetic) were stained with anti-mouse CD31 (upper panel) to determine the degree of vascularity. Ten minutes before death the mice were injected intravenously with RITC-dextran (mw, 60 kd) (lower panel) to visualize the integrity of the blood vessels. Note the diffuse RITC-dextran pattern in KK-diabetic mice compared with the KK mice, which is an indication of leaking vessels. (B) The area occupied by CD31-positive structures per glomerulus was quantified using Scion Image analysis (Scion Corporation, Frederick, MD), as previously described.[77] Thirty glomeruli/group were analyzed (Breyer and Pozzi, unpublished data).

creased density of glomerular capillaries, resulting from glomerular neovascularization, and an increased number of efferent arterioles at the glomerular vascular pole have been seen in biopsy specimens of patients with type 1 diabetes[26] as well as in rats and db/db mice with diabetic nephropathy.[27,28] Increased glomerular vascular density accompanied by increased vessel leakage also was noted in diabetic KK mice compared with nondiabetic controls (Fig. 2).

The mechanism whereby increased angiogenesis of abnormal vessels occurs in diabetic nephropathy is understood poorly. One possibility is that the balance between pro-angiogenic and anti-angiogenic factors, critical for the regulation of vascular permeability and angiogenesis, is altered in the course of diabetes. In this context, increased expression of pro-angiogenic factors and decreased expression of anti-angiogenic molecules within the glomerulus of diabetic patients or rodents has been documented.[29,30] The major angiogenic and nonangiogenic factors involved in the pathogenesis of diabetic nephropathy are summarized in Fig. 3.

Pro-angiogenic Factors in Diabetic Nephropathy

Among the pro-angiogenic factors, VEGF is probably the most potent permeability factor up-regulated in diabetes.[31] This growth factor is produced predominantly by the podocyte in the glomerulus and high glucose increases VEGF synthesis in cultured podocytes[32] and tubular cells.[31] Moreover, a recent study performed on renal biopsy specimens of diabetic patients showed increased expression of VEGF and increased VEGF-receptor activation in mildly injured glomeruli.[33] This was accompanied by increased endothelial cell proliferation, suggesting that VEGF activation in mildly affected diabetic kidney might lead to increased glomerular angiogenesis.[33] This study also parallels the observation that VEGF gene expression correlates with glomerular neovascularization in human diabetic nephropathy[29] and increased expression of VEGF 121 isoform is up-regulated in the kidneys of patients with type 2 diabetes.[34] The mechanism whereby VEGF contributes to glomerular vascular damage is not clear. On one hand, it is required for the induction and maintenance of the fenestrae

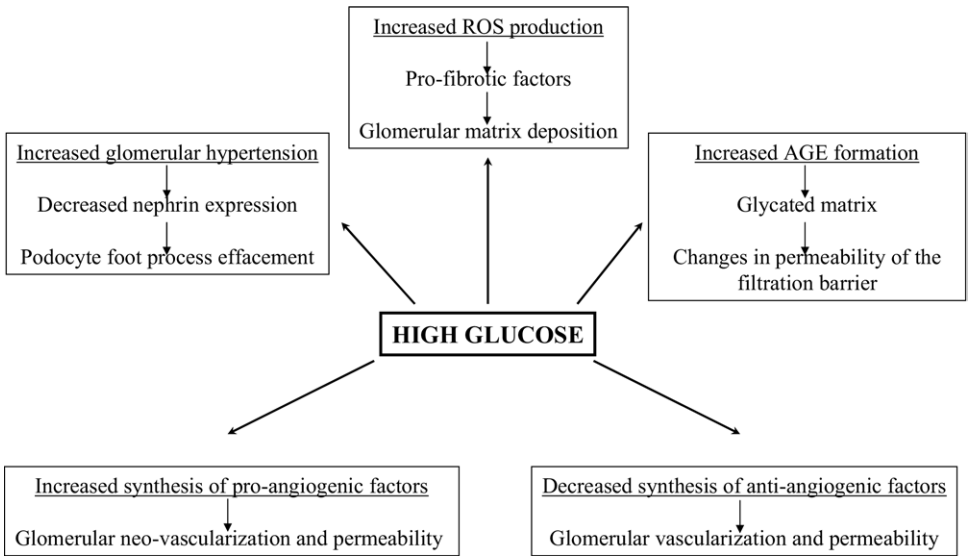

Figure 3. Major factors involved in the pathogenesis of diabetic nephropathy. Hyperglycemia can lead to increased ROS production, glycation of glomerular matrix, intraglomerular pressure, and synthesis of pro-angiogenic factors with concomitant decreased synthesis of anti-angiogenic molecules. These changes significantly contribute to the glomerular damage, neovascularization, matrix deposition, and altered filtration observed in diabetes.

in glomerular capillary endothelial cells.[35] On the other hand, podocyte-specific overexpression of the VEGF164 isoform leads to collapsing glomerulopathy.[36] The cause of up-regulated VEGF synthesis in diabetes also remains speculative. Of the known pathways up-regulated by hyperglycemia, protein kinase C,[37] ROS,[38] and AGEs[39] have been shown to increase VEGF production in both glomerular and nonglomerular compartments.

As mentioned previously, Ang 1 and Ang 2 belong to a family of both pro-angiogenic and anti-angiogenic factors that exert their cellular functions by binding to the Tie2 receptor. During kidney development, Ang 1, Ang 2, and Tie2 are highly expressed and play pivotal roles in the maturation of glomeruli and renal blood vessels,[40] with Ang 2 promoting angiogenesis and the establishment of leaky vessels, and Ang 1 enhancing endothelial cell stability and reducing vessel leakage. In the kidneys, Ang 1 counteracts the action of VEGF[41] and increases transendothelial electrical resistance in cultured glomerular endothelial cells.[42] In contrast, up-regulation of Ang 2 has been documented in a type 1 mouse diabetic nephropathy model[43,44] and in mesangial cells exposed to high glucose levels. In addition, high glucose levels significantly increased the levels of secreted Ang 2 by mesangial cells,[45] suggesting the involvement of this angiogenesis-associated factor in the progression of diabetic nephropathy.

Anti-angiogenic Factors in Diabetic Nephropathy

The proteolytic fragments of both non-extracellular and extracellular matrix have been shown to improve diabetic nephropathy. These are discussed later in the section on enhancing the generation and/or action of anti-angiogenic factors because they most likely only exert their protective effects when given in pharmacologic doses and play a minimal role in the normal pathophysiology of this disease process.

The anti-angiogenic factor PEDF has been shown to be involved in both diabetes-mediated retinal and renal vasculature complications. Interestingly, there appears to be an inverse correlation between the levels of PEDF and VEGF in both physiologic and pathologic angiogenesis. PEDF down-regulates the levels of VEGF in retinal endothelial cells[17] and decreased PEDF levels are associated with diabetic retinopathy in human beings.[46] Moreover, the levels of this anti-angiogenic factor are decreased at both the messenger RNA and protein levels in the kidney of diabetic rats.[30] In this context, high glucose levels and AGEs decrease

PEDF production in human mesangial cells[30] and in microvascular endothelial cells.[47] Finally, PEDF prevents high glucose level–induced overexpression of the profibrotic growth factor, TGF-β.[48] Taken together, these data suggest that decreased expression of PEDF in diabetic kidneys contributes to extracellular matrix overproduction, increased angiogenesis, and consequent development of diabetic nephropathy.

Renin-Angiotensin System in Diabetic Nephropathy

The renin-angiotensin system (RAS) plays a key role in the progression of diabetic nephropathy and inhibition of this system is one of the major therapeutic strategies used to slow the progression of this disease process. There is convincing evidence from multiple large trials that blocking the RAS decreases the progression of diabetic nephropathy and overall proteinuria. This is proposed to be induced primarily by altering glomerular hemodynamics, resulting in a decrease in intraglomerular pressure. There is, however, some new and interesting evidence suggesting that the RAS might in part contribute to the diabetic-induced vascular damage by affecting glomerular endothelial cell functions. In this context, angiotensin II contributes to glomerular vascular growth and leakage by promoting VEGF synthesis by podocytes.[49] Another interesting mechanism, currently described in tumor angiogenesis, is that angiotensin II can promote endothelial cell migration and tubulogenesis, thus directly contributing to angiogenesis in pathologic settings.[50-53]

ANTI-ANGIOGENIC THERAPY IN DIABETIC NEPHROPATHY

Because the pathologic consequences of diabetes, including diabetic nephropathy, are in large part caused by both microvascular and macrovascular complications, new strategies are required to prevent these complications. As mentioned previously, increased generation of pro-angiogenic factors, decreased expression of anti-angiogenic factors, hypertension, and high glucose level–mediated AGE formation represent the major mediators of diabetes-induced vascular damage. It is therefore apparent that altering the activity of one and/or many of theses mediators might be beneficial in treating and ideally preventing the glomerular vascular complications found in diabetes types 1 and 2.

Blocking Traditional Pro-angiogenic Factors

Increased expression of pro-angiogenic factors and their receptors has been largely documented in diabetes.[54-56] Because most of these factors might contribute to the glomerular vascular damage by increasing endothelial cell proliferation and permeability, many studies have been performed to block these pathways in animal models of diabetes. Anti-VEGF antibodies administered to type 1 diabetic rats immediately after induction of diabetes have been shown to decrease hyperfiltration, albuminuria, and glomerular hypertrophy.[57] VEGF blockade also prevented the up-regulation of endothelial nitric oxide synthase expression in glomerular capillary endothelial cells, suggesting that anti-VEGF therapy might be beneficial in preventing vascular damage in the early events of diabetic nephropathy.[57] Similarly, anti-VEGF therapy attenuated increases in kidney weight and glomerular volume in the diabetic db/db mouse, a model of obese type 2 diabetes,[58] and inhibition of VEGF prevented early glomerular hypertrophy in the Zucker diabetic fatty rat, another model of obese type 2 diabetes.[59] In contrast to these studies, anti-VEGF antibodies did not lead to any beneficial effects to the kidney when used in the Goto-Kakizaki rat, a lean type 2 diabetes model.[60] Although very promising in animal studies, anti-VEGF therapy has not yet been used in human subjects with diabetes, which makes it difficult to appreciate its potential therapeutic efficacy. However, when this therapy was administered for colorectal cancer, frequent adverse effects, including hypertension, bleeding episodes, thrombotic events, and proteinuria, were reported,[61] suggesting that these antibodies will be difficult to administer in patients with diabetic nephropathy.

Enhancing the Generation and/or Action of Anti-angiogenic Factors

Decreased expression of anti-angiogenic factors, including PEDF, non-extracellular matrix, and extracellular-matrix cleavage products has been documented in diabetic nephropathy,[30,43,44,62] suggesting that the administration of these anti-angiogenic factors might be beneficial in the treatment of diabetic nephropathy. Because the levels of the anti-angiogenic PEDF are decreased in kidneys of streptozotocin-induced diabetic rats[30] and PEDF down-regulates the production of high glucose level–mediated TGF-β synthesis by mesangial cells,[30] diabetic rats were treated with an adenovirus expressing PEDF to evaluate its effects on diabetic complications.[48] Enhanced expression of renal PEDF significantly alleviated microalbuminuria in early stages of diabetes and prevented the expression of the profibrotic agents TGF-β and CTGF. These results suggested that exogenous expression of PEDF is beneficial in a rat model of diabetic nephropathy.

Among the non-extracellular matrix–derived cleavage products, angiostatin recently was shown to ameliorate the glomerular vascular damage induced by diabetes.[62] Angiostatin is generated from circulating plasminogen by different enzymes, including matrix metalloproteinase (MMP)2, 3, 7, 9, and 12.[13] Angiostatin is a potent inhibitor of endothelial cell functions both in vivo and in vitro and it has been shown to ameliorate pathologic angiogenesis, particularly tumor-associated angiogenesis.[63] Based on the observation that angiostatin inhibits retinal vascular permeability in diabetic retinopathy,[64] the effect of angiostatin was analyzed in diabetic nephropathy.[62] In this study the levels of angiostatin and one of its major generators, MMP2, were found to be significantly decreased in the kidneys of streptozotocin-treated rats.[62] When exogenous angiostatin was delivered by using an adenoviral delivery, albuminuria and glomerular hypertrophy was alleviated significantly and there was a reduction in the high glucose level–induced expression of VEGF and TGF-β.[62] Although this study strongly suggests that treatment with angiostatin might be beneficial in diabetic nephropathy, a recent study suggested that use of this therapy may lead to potential complications. In this study performed on human subjects, mammary artery capillary density and VEGF expression were reduced significantly in diabetic patients compared with nondiabetic patients.[65] This was correlated with an up-regulation in arterial expression and the release of active MMP-2 and MMP-9, as well as angiostatin.[65] Therefore, in contrast to the glomerular vasculature, the increased angiostatin production and reduced VEGF formation in the diabetic arterial vasculature might lead to decreased and impaired angiogenesis.[65] This paradigm highlights the interesting concept that endothelial cells and their responses to specific stimuli differ within tissues and organs. Moreover, anti-angiogenic therapy might be beneficial in one organ (ie, kidney), but deleterious in others.

Among the extracellular matrix–derived products, endostatin (a cleavage product of collagen XVIII) and the α3-NC1 domain of collagen IV (α3-NC1[IV]) have been shown to ameliorate the glomerular vascular complications induced by diabetes.[43,44] Treatment of streptozotocin-induced diabetic mice with either α3-NC1(IV) domain[43] or endostatin[44] reduced the vascular damage, glomerular hypertrophy, hyperfiltration, albuminuria, and the number of glomerular endothelial cells in the early stage of diabetic nephropathy.

Interestingly, increased podocyte production of collagen IV (particularly the α3 chain) driven by angiotensin II, VEGF, and TGF-β has been observed in diabetes.[66] Although this chain may contribute to the glomerular damage by promoting glomerulosclerosis, it also might play a role as a physiologic inhibitor of angiogenesis if its cleavage product, α3-NC1(IV), is generated proteolytically in this pathologic process.

Blocking AGEs and Their Receptors

AGEs are proteins or lipids that are non-enzymatically glycated after exposure to sugars.[67] AGEs are prevalent in the diabetic vasculature and contribute to microvascular and macrovascular complications because they form cross-links between molecules in the extracellular matrix milieu. In addition, they alter cellular function by interacting with the RAGE recep-

tors.[67] In the endothelium, AGEs cause the production of ROS[68] and in human glomerular endothelial cells they regulate the expression of both VEGF and VEGFR2.[69,70] Despite the recognition of their deleterious effects in diabetes, the pharmacologic treatment currently available for either type 1 or type 2 diabetic patients does not directly address the excess accumulation of AGEs. However, studies performed on animal models of diabetes strongly suggest that prevention of AGE formation and/or blocking their cellular actions would be beneficial in slowing diabetic nephropathy. Treatment with anti-RAGE antibodies in obese type 2 diabetic mice[71] or mice with type 1 diabetes[72] reduced kidney weight, albumin excretion, and collagen IV production, thus supporting the hypothesis that RAGE is an important pathogenic factor in the renal changes found in type 1 and type 2 diabetes. Moreover, the suppression of AGE formation by aminoguanidine decreases albuminuria and the severity of glomerular lesions in diabetic rodents.[73] Another compound, pyridoxamine, which inhibits the glycoxidative breakdown of Amadori products to AGE,[74] also prevents the increase of plasma creatinine levels, albuminuria, and glomerular hypertrophy in streptozotocin-diabetic rats[75] and obese type 2 diabetic mice.[76] Interestingly when pyridoxamine was administered together with the angiotensin II receptor blocker valsartan, both mortality and the progression of diabetic nephropathy were reduced significantly compared with the single treatments,[76] strongly suggesting that combination therapy might be beneficial for the treatment of established type II diabetes.

CONCLUSIONS

Diabetic nephropathy is a significant medical problem because of its increasing incidence, morbidity, and mortality. One of the principal pathogenic mechanisms of this disease is aberrant angiogenesis. In this review we have highlighted the major pro-angiogenic and anti-angiogenic factors that potentially could affect the glomerular vascular damage in the setting of type 1 and type 2 diabetes. A better understanding of the role of angiogenesis in diabetes is critical for the development of successful inhibitors to this process. Because inhibition of the renin-angiotensin system is the only therapy currently available to retard the progression of this incurable disease, targeting the pathologic angiogenesis associated with diabetic nephropathy might be viewed as a valid tool to decrease and ideally halt the morbidity and mortality associated with this disease.

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