Perspectives in Diabetes

From the Periphery of the Glomerular Capillary Wall Toward the Center of Disease

Podocyte Injury Comes of Age in Diabetic Nephropathy

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Nephropathy is a major complication of diabetes. Alterations of mesangial cells have traditionally been the focus of research in deciphering molecular mechanisms of diabetic nephropathy. Injury of podocytes, if recognized at all, has been considered a late consequence caused by increasing proteinuria rather than an event inciting diabetic nephropathy. However, recent biopsy studies in humans have provided evidence that podocytes are functionally and structurally injured very early in the natural history of diabetic nephropathy. The diabetic milieu, represented by hyperglycemia, nonenzymatically glycated proteins, and mechanical stress associated with hypertension, causes downregulation of nephrin, an important protein of the slit diaphragm with antiapoptotic signaling properties. The loss of nephrin leads to foot process effacement of podocytes and increased proteinuria. A key mediator of nephrin suppression is angiotensin II (ANG II), which can activate other cytokine pathways such as transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) systems. TGF-β1 causes an increase in mesangial matrix deposition and glomerular basement membrane (GBM) thickening and may promote podocyte apoptosis or detachment. As a result, the denuded GBM adheres to Bowman's capsule, initiating the development of glomerulosclerosis. VEGF is both produced by and acts upon the podocyte in an autocrine manner to modulate podocyte function, including the synthesis of GBM components. Through its effects on podocyte biology, glomerular hemodynamics, and capillary endothelial permeability, VEGF likely plays an important role in diabetic albuminuria. The mainstays of therapy, glycemic control and inhibition of ANG II, are key measures to prevent early podocyte injury and the subsequent development of diabetic nephropathy. *Diabetes* 54: 1626–1634, 2005

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AGE, advanced glycation end product; ANG II, angiotensin II; CD2AP, CD2-associated protein; GBM, glomerular basement membrane; HSPG, heparan sulfate proteoglycans; RAGE, receptor for AGE; ROS, reactive oxygen species; STZ, streptozotocin; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

Diabetic nephropathy is the leading cause of end-stage renal disease and is clinically characterized by proteinuria and progressive renal insufficiency. Dating back to the first description by Kimmelstiel and Wilson (1), histological analyses have focused on the increase in mesangial matrix as the main lesion of diabetic glomerulopathy. In addition, glomerular basement membrane (GBM) thickening has been considered an important pathophysiological event in the disease. These pathological alterations have been linked to functional consequences, first described in landmark structure-function studies done in patients with type 1 as well as type 2 diabetes (2–4). Specifically, mesangial matrix expansion correlates closely with both proteinuria and deterioration of renal function. It has been proposed that accumulation of matrix in the mesangial area reduces the capillary surface area available for filtration, thereby contributing to the progressive loss of renal function (2). Renal failure may also arise from nephron dropout due to tubulointerstitial fibrosis (5), and this process is aggravated by the harmful downstream effects of proteinuria on the tubules (6). However, the genesis of proteinuria in diabetes is not readily explained by the associated mesangial matrix expansion. Rather, consideration should be given to alterations of the glomerular filtration barrier, which is composed of the glomerular endothelium, the GBM, and the podocyte (glomerular visceral epithelial cell). Widespread endothelial dysfunction is believed to result in proteinuria (7), which is exacerbated by intraglomerular hemodynamic stress (8). Although it is highly fenestrated, the glomerular endothelium might pose some hindrance to protein permeability (9,10). As for the GBM, its conspicuous thickening in diabetes, perhaps due to accumulation of collagen IV and alterations in its architecture and composition (11), would seem to constitute a more effective barrier to the filtration of proteins but is in fact more porous to proteins (12). While loss of charge selectivity in the GBM has been proposed to partly explain the proteinuria (13), a decrease in negatively charged

proteoglycans occurs late in the course of diabetic nephropathy, sometimes long after the appearance of microalbuminuria (14). This leads to the conclusion that the final barrier restricting plasma proteins to the vasculature is the slit diaphragm of the podocyte; little research, however, has been conducted on podocyte biology in diabetes until recently. The advent of improved cell culture techniques, the discovery of key podocyte-specific molecules, and advances in transgenic technology have revolutionized the study of podocytes in health and disease (15). The purpose of this review is to summarize the emerging evidence that podocytopathy plays a pivotal role in the manifestations of diabetic glomerulopathy, expanding upon the "mesangiocentric" dogma of diabetic glomerular disease.

PODOCYTE BIOLOGY

A comprehensive description of the biology of this highly differentiated cell is beyond the scope of this article, but a basic understanding is necessary to appreciate how podocyte malfunction contributes to diabetic nephropathy (15). Podocytes extend long processes toward the GBM to which they affix by cell surface adhesion proteins such as the $\alpha 3\beta 1$ integrin and dystroglycan (16–18). The foot processes of adjacent podocytes interdigitate and are separated by narrow spaces (30–40 nm) that are bridged by a porous membrane called the slit diaphragm. These membranes contain pores that are freely permeable to water and small solutes but relatively impermeable to plasma proteins (19,20). Thus, the integrity of the slit diaphragm is one of the principal determinants of the permselective properties of the glomerular filtration barrier, and knowledge of its molecular architecture will help elucidate the role that the slit diaphragm plays in proteinuria (15). A major advance was the discovery of nephrin, whose gene is mutated in the congenital nephrotic syndrome of the Finnish type, a rare form of hereditary nephrosis characterized by diffuse foot process effacement of the podocytes (21). Nephrin, a transmembrane protein with a large extracellular portion, self-associates in a zipper-like arrangement through homophilic dimerization, forming the molecular substrate of the slit diaphragm (22). Other proteins such as podocin and CD2-associated protein (CD2AP) interact with nephrin in cholesterol-rich regions of the cytoplasm called lipid rafts and anchor nephrin to actin filaments of the podocyte cytoskeleton (15,23). CD2AP links nephrin and podocin to phosphoinositide 3-OH kinase, and this complex has cell-signaling properties that stimulate Akt, a serine-threonine kinase (24). One target of nephrin/CD2AP-induced phosphorylation is Bad, a proapoptotic protein of the Bcl-2 family (24). Upon phosphorylation, Bad is inactivated and apoptosis does not occur. Thus, afferent signaling coming from intact slit diaphragms prevents podocyte apoptosis (24,25), a beneficial effect given that terminally differentiated podocytes fail to proliferate and are not easily replaced (26). The inability of podocytes to proliferate may be secondary to upregulation of cell cycle inhibitory proteins, p57 and p27 (22). Expression of p27 in cultured podocytes can be stimulated by high glucose, and this further prevents cell replication (22).

Because it does not normally divide, the podocyte represents one of the last unexplored frontiers in renal cell culture research. Podocytes in primary culture can be coaxed into reentering the cell cycle, but they invariably lose their differentiated characteristics. Besides, the cell homogeneity in primary culture is questionable because glomerular parietal epithelial cells often contaminate and overgrow the podocytes (27). To circumvent these difficulties, "conditional immortalization" has been developed to obtain a pure population of podocytes that can either proliferate or differentiate (28). The technique involves the introduction of an oncogene whose gene product is temperature sensitive, such as the tsA58 variant of the SV40 large tumor antigen (TAg) (29). The TAg protein complexes with and disables p53 and retinoblastoma proteins, removing two major barriers to cell cycle progression and allowing the podocyte to be propagated in culture. When differentiated podocytes are needed for experimentation, the cells can be "thermoshifted" to a higher temperature that will inactivate tsA58, reverse the immortalization, and permit differentiation to occur.

PODOCYTOPATHY IN HUMAN DIABETIC KIDNEY DISEASE

Experiments from 25 years ago in diabetic rats described foot process widening of podocytes (30), a finding later confirmed in patients with relatively advanced nephropathy due to type 1 diabetes (31). More recent investigations also described an increase in foot process width in microalbuminuric type 1 diabetic subjects, and foot process width correlated directly with the urinary albumin excretion rate (32). In addition to foot process widening, the number and density of podocytes have been reported to be markedly reduced (podocytopenia) in diabetic patients, whether afflicted with type 1 or type 2 diabetes (33–36). While an ideal protocol for counting podocytes in biopsy specimens has not been developed yet, it has been argued that modern morphometric techniques can yield reasonable approximations. For instance, it has been estimated that the podocyte number is significantly reduced, even in diabetes of short duration (33). In Pima Indians with type 2 diabetes, podocyte density is drastically reduced, and the remaining foot processes are widened to maintain GBM coverage (34,37). Among all glomerular morphological characteristics, the decreased number of podocytes per glomerulus was the strongest predictor of progressive renal disease, with fewer cells predicting more rapid progression (38). Additionally, a recent morphometric study (36) in European type 2 diabetic patients has shown that a diminution of podocyte density rather than absolute reduction in podocyte number is more predictive of the presence and magnitude of albuminuria. The numerical density of podocytes per glomerulus was reduced in patients with microalbuminuria and was decreased even further in patients with overt proteinuria (36). A cross-sectional study also reported a significant inverse correlation between proteinuria and both podocyte number and density per glomerulus (39). Consistent with the observed loss of podocytes in diabetes, podocytes were present in the urine in 53% of type 2 diabetic patients with microalbuminuria and in 80% with macroalbuminuria, while normoalbuminuric patients and healthy control subjects had undetectable levels of urinary podocytes (40). Interest-

ingly, treatment with an ACE inhibitor reduced the number of urinary podocytes (40).

Podocytopenia may exacerbate the development of proteinuria because a denuded GBM can come into contact with Bowman's capsule and promote synechiae formation, an initial step in the development of glomerulosclerosis (15). Morphologic changes in the podocyte are also believed to engender proteinuria. With foot process widening, the decrease in slit diaphragm length might impede the filtration of water and lower the glomerular filtration rate. However, if protein permeability remains intact, as postulated in an innovative theory (41), the amount of protein relative to water is increased in the urinary space, and the elevated protein concentration could exceed the tubule's reabsorptive capacity and manifest as proteinuria. The merit of this theory is that it reconciles the paradox of increasing proteinuria in the face of declining renal function, a characteristic of diabetic nephropathy, but the theory remains unproven and controversial (41). Even so, the worsening proteinuria could induce tubular atrophy and interstitial fibrosis, and these irreversible tubulointerstitial changes, together with glomerulosclerosis, then lead to chronic renal insufficiency (6).

To detect earlier functional abnormalities in podocytes, several recent studies have focused on the expression of podocyte-specific proteins in diabetic patients. For instance in type 1 diabetes, nephrin excretion in the urine (nephrinuria), detected by Western blot analysis, was present in 30% of the patients with normoalbuminuria, 17% of those with microalbuminuria, and 28% of those with macroalbuminuria, whereas none of the nondiabetic subjects had nephrinuria (42). These findings were not correlated with biopsies, but they suggest that increased urinary nephrin equates with early podocyte injury, even before the onset of microalbuminuria. Another study (43) found that nephrin staining was extensively reduced in renal biopsy specimens from nephropathic patients with type 1 diabetes. Several studies have evaluated nephrin mRNA and protein expression in type 2 diabetes. In general, nephrin protein production was downregulated in the diabetic subjects compared with the nondiabetic control subjects (44–46), and the decrease in nephrin correlated with the broadening of the foot process widths. In contrast, CD2AP expression was not reduced in podocytes from diabetic patients, suggesting that the reduction in nephrin was not due to widespread podocyte loss or injury (45); this underscores the importance of nephrin in maintaining podocyte integrity. Finally, type 2 diabetic patients who were treated with the ACE inhibitor perindopril for 2 years exhibited a nephrin mRNA expression that was close to normal when compared with nondiabetic control subjects; patients not treated with the ACE inhibitor had significantly reduced nephrin transcripts (46).

PODOCYTE DYSFUNCTION IN EXPERIMENTAL DIABETES

Animal experiments allow for the repeated sampling of kidney tissue over time, furnishing a longitudinal record of the development of diabetic nephropathy and the efficacy of pharmacological interventions. Serial investigations (47) on kidneys from obese Zucker *fa/fa* rats, a model of type 2 diabetes that develops segmental glomerulosclerosis, revealed that nephropathy started with damage to podocytes, manifesting as foot process effacement and cytoplasmic accumulation of lipid droplets. Early podocyte damage antedated the development of glomerulosclerosis and tubulointerstitial damage in this model (48). In the streptozotocin (STZ)-induced diabetic rat, a model of type 1 diabetes, several studies (49,50) have reported a decrease in podocyte number, broadening of the foot processes, and reduction in nephrin expression.

PODOCYTE APOPTOSIS AND/OR DETACHMENT IN HUMANS AND ANIMAL MODELS

The exact etiology for podocyte loss in diabetes remains speculative, but two mechanisms can be suggested: apoptosis and cell detachment. Evidence for a primary role of apoptosis in either human or experimental diabetic nephropathy is scant (51–54). However, podocyte apoptosis can be demonstrated in cell culture. For instance, angiotensin II (ANG II) induces apoptosis in cultured rat glomerular epithelial cells, and this effect is mediated by the transforming growth factor-β (TGF-β) system since it is inhibited by an anti–TGF-β antibody (55). In TGF-β1–overexpressing transgenic mice, albeit nondiabetic, the podocyte undergoes apoptosis in situ shortly after the sclerotic lesion appears in the glomerulus (56). Thus, the heightened intraglomerular activity of TGF-β1 that is characteristic of diabetes may theoretically be responsible for apoptosis (57), a process that can be mediated through Smad7, which inhibits the nuclear translocation of the cell survival factor nuclear factor-κB (56,58).

The other mechanism of podocyte loss in diabetes may relate to the detachment of podocytes from the GBM. This scenario does not exclude a role for apoptosis, since cells may first detach and then undergo apoptosis because of interruption of viability cues deriving from cell-matrix interactions (17). Alternatively, detached cells can be shed in the urine as live podocytes (59). In fact, podocyturia worsens with the progression from normoalbuminuria to microalbuminuria to overt proteinuria (40). Loss of cell anchorage to the GBM may result from downregulation of the α3β1 integrin receptor, the principal adhesion complex that attaches the podocyte to the GBM (16,17,60,61). Several studies (62–64) have shown that the α3β1 integrin is decreased in the podocytes of humans and rats with diabetes. Furthermore, high glucose media in cultured rat or human podocytes decreases the expression of α3β1 integrin (62,64); this downregulation is perhaps mediated by increased levels of TGF-β1 (65,66).

ROLE OF ANG II IN NEPHRIN EXPRESSION AND PODOCYTE INJURY

Pharmacological interventions in animal models have supported the view that an increased ANG II activity is involved in podocyte injury in diabetes. ACE inhibition or AT_1 receptor antagonism attenuated podocyte foot process broadening in rats with STZ-induced diabetes (49). In another study (67) involving the STZ-induced diabetic rat, therapy with an ACE inhibitor, but not an endothelin receptor antagonist, prevented loss of podocytes and podocyte injury. Similarly, an ACE inhibitor, but not aminoguanidine, an inhibitor of advanced glycation end product (AGE) formation, attenuated the diabetes-associated re-

duction in nephrin expression (50). Likewise, an AT_1 receptor antagonist, but not the calcium channel blocker amlodipine, normalized the reduced nephrin expression in podocytes from spontaneously hypertensive rats with superimposed STZ-induced diabetes (68,69). Finally, the renal damage that occurs in obese Zucker rats was more effectively controlled by combined treatment with an ACE inhibitor and an AT_1 receptor antagonist than either monotherapy alone (70). Thus, it may be concluded that suppression of nephrin expression in podocytes can be caused by increased renal ANG II activity in diabetes, but the molecular mechanisms are incompletely understood.

Podocytes express the AT_1 and probably the AT_2 receptors after injury and therefore could respond to stimulation with ANG II (15,71). Transgenic rats with targeted overexpression of the podocyte's AT_1 receptor showed pseudocysts in podocytes, followed by foot process effacement and local detachment, with subsequent progression to focal segmental glomerulosclerosis (72). High glucose concentrations induce ANG II formation in podocytes through upregulation of angiotensinogen expression (15). Furthermore, ANG II production in podocytes can be activated by proteinuria, likely by the transit of proteins through the filtration barrier (73) and by mechanical stretch, which mimics the hemodynamic effects of intraglomerular hypertension (74). Interestingly, ANG II formation as a consequence of mechanical stretch appears to be independent of ACE (74). As an alternative to ACE for the conversion of ANG I to ANG II, chymase has been shown to be upregulated in the glomeruli of patients with nephropathy due to type 2 diabetes (75). Because chymase is not blocked by ACE inhibitors, glomeruli in the diabetic state may still generate ANG II despite ACE inhibition. Some evidence indicates that the increase in angiotensinogen expression is signaled by intracellular reactive oxygen species (ROS) (74). Oxidative stress is a leading initiator of cellular dysfunction in diabetes complications (76,77), and increased ROS generation can induce podocyte dysfunction (78,79).

ROLE OF HEPARAN SULFATE PROTEOGLYCANS

A decrease in the GBM content of negatively charged HSPG contributes to the loss of charge selectivity in the glomerular filtration barrier and to the progression of proteinuria (80,81). One potential mechanism for decreased de novo synthesis of proteoglycans is the increased generation of ROS (81,82). Although the predominant proteoglycan in the GBM was thought to be perlecan, it has become more clear that agrin is the more abundant HSPG (83). Synthesis of proteoglycans occurs in all three glomerular cell types, but podocytes are an especially important source of these negatively charged molecules. Proteoglycan synthesis in podocytes is differentially influenced by high ambient glucose and ANG II (83,84); high glucose suppresses the production of agrin's core protein, whereas ANG II decreases synthesis of the core protein and diminishes the sulfation of its side chains (83,84). Podocytes exposed to ANG II decrease the amount of HSPG on their cell surfaces and in the extracellular matrix (83); these results may partly explain the antiproteinuric effect of ACE inhibitors and angiotensin receptor blockers in diabetic nephropathy.

ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR

Losses of nephrin and proteoglycan are not the only pathological changes fostering proteinuria in diabetic nephropathy. Vascular endothelial growth factor (VEGF), a survival and angiogenic factor with strong microvascular permeabilizing properties (85), may increase the permeability of the glomerular filtration barrier to circulating proteins. The most convincing evidence that VEGF overexpression is involved in the proteinuria of diabetes comes from studies in type 1 diabetic (STZ-induced) rats and type 2 diabetic *db/db* mice. Neutralization of VEGF with a systemically administered anti-VEGF antibody reduced the urinary albumin excretion by at least 50% compared with the untreated diabetic controls (86,87). In addition, VEGF induces endothelial nitric oxide synthase, thereby promoting the vasodilation and hyperfiltration that are typical of early diabetic nephropathy (86,88).

The expression of VEGF in the glomerulus is most pronounced in the podocytes, and experimental diabetes increases VEGF mRNA and protein expression (89,90). In cultured podocytes, VEGF expression can be stimulated by high glucose concentrations (91), by TGF-β1 (91,92), and by ANG II acting via AT_1 and AT_2 receptors (93,94). The gene expression of VEGF is also stimulated by a transcription factor called hypoxia-inducible factor-1 (95). We have demonstrated in PC12 cells that hypoxia-inducible factor-1α expression is stimulated by ANG II in a posttranscriptional mechanism involving AT_2 receptors (96). This increase is caused by downregulation of a prolyl hydroxylase (SM-20/PHD3), the enzyme responsible for initiating hypoxia-inducible factor-1α degradation (96). Upregulation of podocyte-derived VEGF in diabetes may depend on signals downstream of the receptor for AGE, or RAGE (90). RAGE expression is increased in the podocytes of diabetic *db/db* mice (90), and inhibition of AGE-RAGE interactions by soluble RAGE treatment significantly depressed VEGF expression in the kidney and ameliorated albuminuria and glomerulosclerosis (90). Precursors of AGE such as Amadori-glycated serum albumin may mediate diabetic proteinuria (97) and promote glomerular production of hydrogen peroxide (98). Whether increased ROS production stimulates podocyte VEGF expression remains to be investigated.

An intriguing concept that has come to attention is that podocytes not only produce VEGF but are also acted upon by VEGF (92,99). This VEGF autocrine loop may play important roles in podocyte biology since VEGF decreases intracellular calcium concentrations and protects against cytotoxicity (99), possibly via the antiapoptotic actions of nephrin (25). These cytoprotective effects were reversed when endogenous VEGF was blocked by either a class III tyrosine kinase inhibitor PTK787/ZK222584 (99) or a monoclonal anti-VEGF antibody (25).

VEGF also stimulates the podocyte to produce the α3 chain of collagen IV, a principal ingredient of the GBM; this effect may be mediated by VEGFR-1 signaling (92). When endogenous VEGF secretion by podocytes was stimulated by TGF-β1 treatment, the production of α3(IV) collagen increased (92). Blockade of endogenous VEGF action by a specific inhibitor of VEGF receptor kinases, SU5416, reduced the TGF-β1–induced expression of α3(IV) collagen by ~50%, establishing a pivotal role for the

VEGF autocrine system in at least one aspect of the regulation of GBM composition by podocytes (92).

It may be speculated that VEGF-stimulated podocyte production of α3(IV) collagen contributes to diabetic thickening of the GBM and its altered permselectivity. Extrapolating from our in vitro work in cultured podocytes, we treated diabetic *db/db* mice with intraperitoneal injections of SU5416 at 2 mg/kg body wt, given twice a week for 8 weeks (100). SU5416 treatment, which had no adverse effects, significantly prevented GBM thickening and albuminuria without affecting blood glucose levels (100 and S.H. Sung, S.C., F.N.Z., unpublished data). These findings with SU5416 compare favorably with those of the anti-VEGF antibody in *db/db* mice (87); however, that study did not report the effect of anti-VEGF therapy on GBM thickening. That SU5416 particularly benefited the podocyte was also suggested by the preservation of nephrin protein, assayed by immunostaining (S.H. Sung, S.C., F.N.Z., unpublished data). Glomerular nephrin levels, which are typically reduced in the *db/db* mouse, were restored almost to normal by SU5416 treatment (S.H. Sung, S.C., F.N.Z., unpublished data), suggesting that nephrin preservation represents another mechanism whereby VEGF blockade might ameliorate diabetic albuminuria.

ROLE OF TGF-B1

TGF-β plays an integral role in the pathogenesis of diabetic nephropathy (101). This fibrogenic cytokine is stimulated by the diabetic state, is increased in the kidney during diabetes, and is able to recapitulate the hypertrophic and sclerotic features of diabetic renal disease (102). Elevated levels of TGF-β have been measured in the glomeruli of STZ-induced diabetic rats by micropuncture techniques (103,104), and an intracellular transducer of TGF-β signaling, Smad3, has been shown to translocate into the glomerular nuclei of diabetic *db/db* mice (105), attesting to the overactivity of the TGF-β system in diabetic nephropathy. Inhibition of TGF-β by a panselective neutralizing antibody in diabetic *db/db* mice prevented diabetic renal hypertrophy, mesangial matrix expansion, and the development of renal insufficiency (106). However, there was no significant effect on albuminuria in the diabetic mice (106).

A clear role for TGF-β in the pathogenesis of diabetic albuminuria cannot be established at present because of conflicting studies. In nondiabetic models, overexpression of active TGF-β1 in transgenic mice caused mesangial expansion, interstitial fibrosis, renal insufficiency, and progressive proteinuria (107). In addition, an increase in albumin permeability was observed when isolated glomeruli from normal rats were exposed ex vivo to TGF-β1 (108). However, in our studies on the type 2 diabetic *db/db* mouse, we failed to see a significant reduction in albuminuria after 8 weeks of treatment with a neutralizing anti–TGF-β antibody, perhaps because VEGF expression in the kidney cortex was kept elevated by other diabetic factors (106). Interestingly, the work of Benigni et al. (109) in uninephrectomized STZ-induced diabetic rats revealed

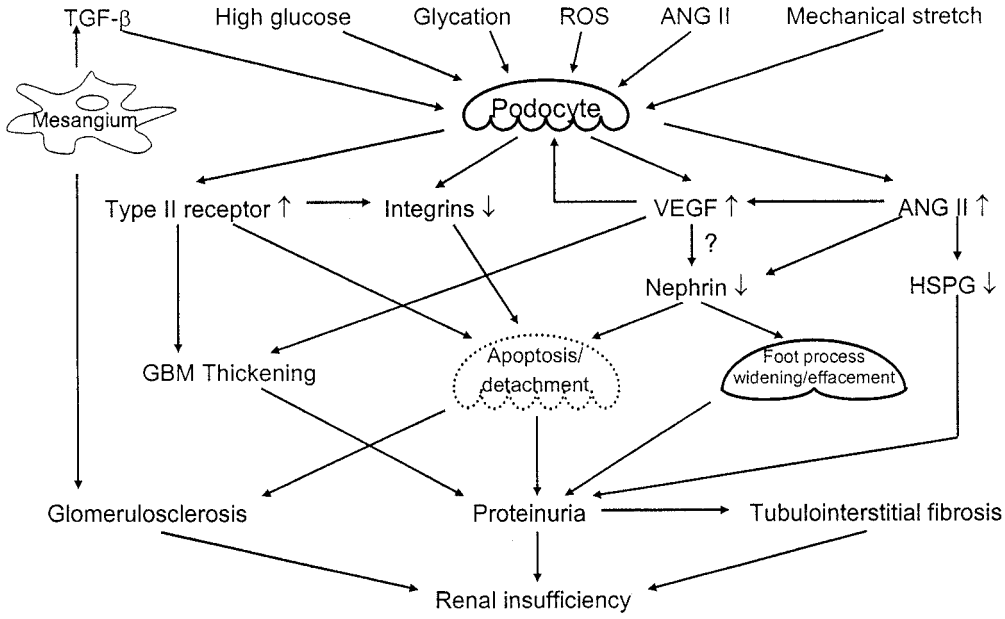

FIG. 1. Overview of the mechanisms of podocyte injury leading to diabetic nephropathy. Metabolic factors in the diabetic milieu (TGF-β, high glucose, glycated proteins, ROS, and ANG II) and hemodynamic factors (via mechanical stretch) converge on the podocyte to increase VEGF and ANG II production. Other effects include upregulation of the TGF-β type II receptor and downregulation of the cell surface α3β1 integrins. Podocyte-derived VEGF operating in an autocrine loop, perhaps via VEGFR-1 signaling, stimulates the production of α3(IV) collagen, leading to GBM thickening, and suppresses the expression of nephrin, favoring apoptosis and foot process widening/effacement. Paracrine actions of VEGF on the endothelium may increase glomerular capillary permeability and relax afferent arteriolar tone (through endothelial nitric oxide synthase), generating hemodynamic forces that can injure podocytes. ANG II also suppresses nephrin expression, and it decreases production of the negatively charged HSPG. ANG II and high glucose upregulate the TGF-β type II receptor and may augment the podocyte's response to paracrine TGF-β, such as that coming from the mesangial cell. The TGF-β/type II receptor interaction stimulates extracellular matrix production by the podocyte (contributing to GBM thickening) and by the mesangium (leading to mesangial matrix expansion). The TGF-β system in the podocyte also promotes apoptosis and decreases integrin expression, which can lead to podocyte detachment and podocyturia. As the results of the above processes, the podocytopenia, foot process widening with loss of nephrin, GBM dysfunction, decreased HSPG, and hemodynamic stress all provoke or exacerbate diabetic proteinuria. Worsening proteinuria coupled with profibrotic stimuli (exemplified by the TGF-β system) induce glomerulosclerosis and tubulointerstitial fibrosis, leading relentlessly to progressive renal insufficiency.

that one but not another species of anti–TGF-β antibody significantly reduced proteinuria when compared with control IgG. Nevertheless, either antibody could potentiate the antiproteinuric effect of an ACE inhibitor when given in combination (109). Other studies showed that when a portion of the TGF-β signaling pathway was selectively inhibited by the creation of a Smad3 knockout mouse, the albuminuria persisted when the mice were rendered diabetic with STZ (100). However, another cohort of Smad3 knockout mice with a different strain background showed significant amelioration of albuminuria in STZ-induced diabetes (110). Nevertheless, both diabetic Smad3 knockout models displayed prevention of mesangial matrix expansion (110 and S.C., S.H. Sung, N.J. Laping, F.N.Z., unpublished data). Thus, it is possible to dissociate albuminuria from mesangial matrix expansion, and the pathogenesis of proteinuria may relate more to VEGF and nephrin while the pathogenesis of glomerulosclerosis may deal more with TGF-β and other fibrogenic pathways.

The podocyte is also subject to the increased levels and overactivity of intraglomerular TGF-β in diabetes. As discussed above, podocyte detachment and/or apoptosis can be caused by increased TGF-β activity (55,58,62). Additionally, TGF-β1 stimulates the podocyte expression of α3(IV) collagen (91), but surprisingly, TGF-β1 suppresses the production of both α1(IV) and α5(IV) collagen (91), important exceptions to the dogma that TGF-β is uniformly profibrotic. TGF-β1 also stimulates the podocyte expression of VEGF (91) that in turn increases the activity of the VEGF autocrine loop, with all of its attendant effects (25,92,99).

Unlike other renal cells, podocytes may not respond to the diabetic state by increasing their expression of TGF-β. The production of TGF-β1 in cultured mouse podocytes was not significantly stimulated by high glucose (91) or by ANG II (S.C., F.N.Z., unpublished data). Instead, these metabolic stimuli significantly upregulated the expression of the TGF-β type II receptor (91 and S.C., F.N.Z., unpublished data), which binds to the TGF-β ligand and initiates the TGF-β signaling cascade (111). In theory, the increase in type II receptor could make the podocyte more sensitive to the elevated levels of TGF-β in the diabetic state, and it could enhance a paracrine interaction between the podocyte and the mesangial and/or glomerular endothelial cell.

CONCLUSIONS

Although the pathophysiology of mesangial cells has long been considered to be the major factor in the development of glomerulosclerosis in type 1 or type 2 diabetes, more recent evidence suggests that podocytes also play a critical role in the early functional and structural changes of diabetic kidney disease. On the one hand, podocytes are the victims of pathogenetic events surrounding the progression of diabetic nephropathy, including the harmful effects of high glucose, AGE, ANG II, ROS, TGF-β, and mechanical stretch. On the other hand, podocytes are the culprits in diabetic nephropathy in that a reduced nephrin expression may lead to podocyte foot process broadening and effacement, while increased VEGF production may exacerbate GBM thickening and proteinuria, leading to tubular atrophy and interstitial fibrosis (Fig. 1). Many of these events are mediated by ANG II, whose local concentration is increased in diabetic kidney disease. ANG II in turn suppresses nephrin and induces TGF-β type II receptor expression in podocytes, perhaps intensifying the paracrine effects of TGF-β derived from the mesangium or glomerular endothelium. Increased TGF-β activity may lead to podocyte apoptosis and/or detachment with podocytopenia and the development of progressive glomerulosclerosis. An additional sequela is VEGF overactivity that could increase glomerular hemodynamic stress, alter the production of GBM components, and repress nephrin expression, all maladaptive events that foster proteinuria (Fig. 1).

The ideal therapy for diabetic nephropathy remains elusive, but excellent glycemic control and ANG II antagonism have become the foundations of current therapy. Promising new treatments that target the roles of VEGF, TGF-β, nephrin, HSPG, glycated proteins, and ROS are beginning to appear on the horizon. In this regard, protection of podocytes will be of paramount importance in preventing the development and progression of diabetic nephropathy.

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