From Fibrosis to Sclerosis

Mechanisms of Glomerulosclerosis in Diabetic Nephropathy

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Progression of diabetic nephropathy to end-stage kidney disease is mediated by a host of processes, but none is as important as the gradual, inexorable scarring of the renal glomerulus, known as glomerulosclerosis. Hence, a host of studies over the decades have attempted to elucidate the molecular mechanisms that lead to this chronic sclerosing condition so that effective therapies and preventative strategies can be developed. Over the past several years, the general understanding of the pathogenic factors that lead to this important feature of diabetic nephropathy has improved considerably. Glomerulosclerosis in diabetic nephropathy is caused by accumulation of extracellular matrix (ECM) proteins in the mesangial interstitial space, resulting in fibrosis manifested by either diffuse or nodular changes (1). The most common matrix proteins detected are collagen types I, III, and IV and fibronectin (2). These accumulate both due to increased synthesis by mesangial cells and reduced degradation by mesangial matrix metalloproteinases (3). Over 20 years ago, Mauer et al. (4) established the clear link between mesangial matrix expansion and progression of diabetic kidney disease by demonstrating that measures of mesangial expansion strongly predicted the clinical manifestations of diabetic nephropathy. Since then, the critical charge to investigators has been to elucidate the mechanisms that promote glomerulosclerosis in diabetic nephropathy. In this brief perspective, we will review pathogenic processes that appear to be critical in the development of diabetic glomerulosclerosis, emphasizing newer findings and insights.

CLASSICAL VIEW OF DIABETIC GLOMERULOSCLEROSIS

During the 1990s, a general consensus emerged about major signaling mechanisms involved in stimulating mesangial cell synthesis of ECM proteins (Fig. 1). In this consensus view, high extracellular glucose induces an increase in glucose uptake via increased expression of the facilitative glucose transporter GLUT1 (5,6). The resultant enhancement in glucose metabolic flux leads to activation of a number of metabolic pathways that result in increased advanced glycation end product and oxidative stress generation (7–9), which in turn activate a number of signaling pathways that lead to enhanced ECM production directly via protein kinase β stimulation (10,11) of AP-1 transcriptional activation, extracellular signal–related kinase (ERK) pathways, and, critically, transforming growth factor (TGF)-β1 synthesis (12,13), which in an autocrine and paracrine fashion stimulates its signaling pathways to stimulate ECM protein synthesis (Fig. 1). These responses triggered by TGF-β1 appear to be the final common pathway by which nephrosclerosis occurs. While of critical importance in the development of nephrosclerosis in diabetes and in most fibrotic diseases of the kidney, the role of TGF-β and its signaling mechanisms have been the subject of several recent reviews on diabetic nephropathy (14,15) and will not be further detailed in this review, which focuses on mechanisms that have been recently described, many of which trigger or participate in TGF-β responses.

While hugely oversimplified, and ignoring many interactions, the paradigm shown in Fig. 1 includes most of the factors that have been shown to predominate in the mesangial cell in vitro, in animal models, and, as much as can be verified, in human studies. Implicit in this pathogenesis schema is the notion that increased glucose uptake and metabolic flux in mesangial cells can directly or indirectly lead to the activation of the entire program. This is probably not correct in vivo and is certainly not so even in cultured cell systems. The inability of increased glucose uptake and metabolism to alone account for all downstream changes was nicely demonstrated by Weigert et al. (16) who found that overexpression of GLUT1 in mesangial cells exposed to physiologically occurring glucose concentrations and exposure of mesangial cells to high extracellular glucose did not induce the same biochemical pathways. Specifically, the authors found that increased GLUT1 failed to directly induce ERK activation or TGF-β1 synthesis. Thus, elevation of extracellular glucose levels must elicit responses in mesangial cells that are independent of glucose uptake and glucose metabolic flux to turn on these important (and perhaps other) glomerulosclerosis mechanisms. Equally, if not more importantly, there exists a host of factors in vivo that are not derived from mesangial cells and that stimulate glomerulosclerosis independently from hyperglycemia per se.

While the role of the other cells in the glomerulus is outlined in the following section, it is critical to recognize the effects of multiple extraglomerular and sys-

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APC, activated protein C; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal–related kinase; JAK, Janus kinase; STAT, signal transducers and activation of transcription; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

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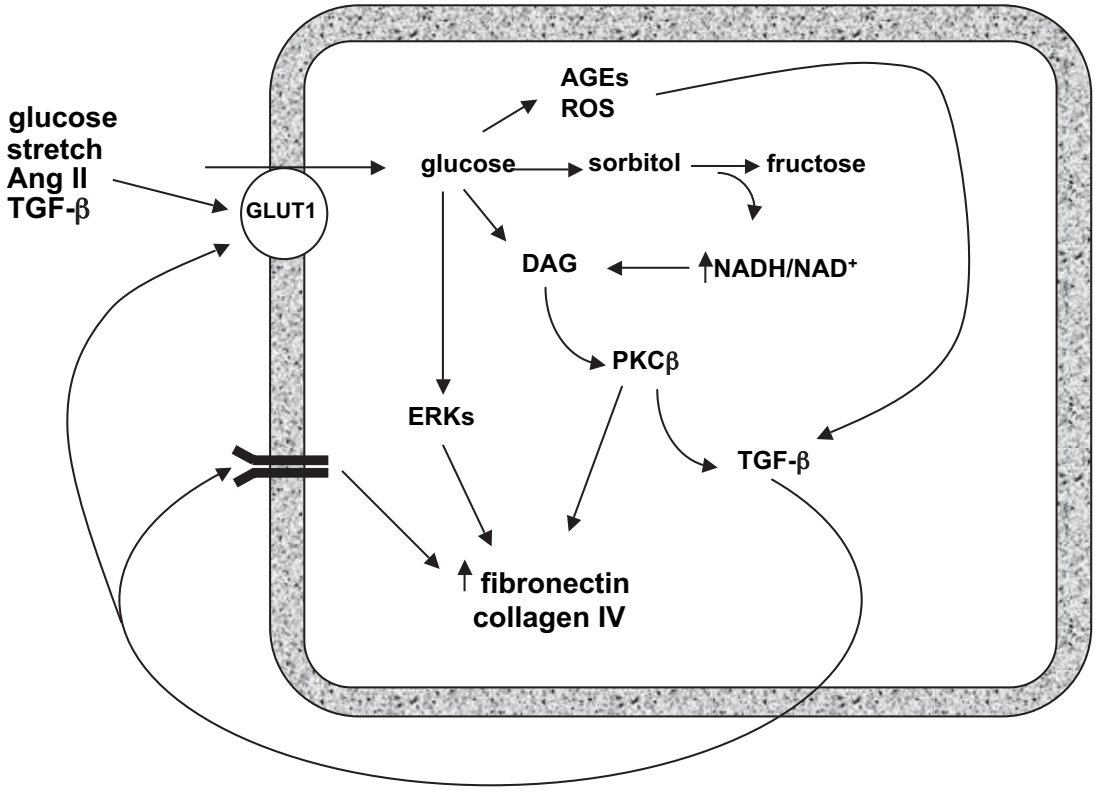

FIG. 1. Simplified, "classic" model of the pathogenesis of glomerulosclerosis. High extracellular glucose leads to increased mesangial cell glucose uptake via enhanced expression of the facilitative glucose transporter GLUT1, activating metabolic pathways that result in increased ROS and AGE generation, which in turn activate a number of signaling pathways that augment ECM production directly via protein kinase Cβ stimulation of AP-1 transcriptional activation, ERK pathways, and, critically, TGF-β1 synthesis, which in an autocrine and paracrine fashion stimulates its signaling pathways to further enhance ECM protein synthesis. Ang II, angiotensin II; DAG, diacylglycerol; PKC, protein kinase C; ROS, reactive oxidant species.

temic effects that stimulate fibrotic responses in the glomerular tuft in diabetes. While these effects are too complex to adequately address in this short review, glomerular and systemic hypertension (17) and activation of the renin-angiotensin-aldosterone system (18) may be among the most important. Several reports suggest that glomerular hypertension and resultant mesangial cell stretch can lead to enhanced expression of the GLUT1 facilitative glucose transporter (19) that would in turn trigger the set of intracellular responses, noted above, leading to glomerulosclerosis.

ALL GLOMERULAR CELL TYPES ARE INVOLVED IN DIABETIC GLOMERULOSCLEROSIS

While it would be natural to assume that the mesangial cell is front and center in mediating the mesangial sclerosis in diabetic nephropathy, this is not exclusively true. Both podocytes and endothelial cells, and crosstalk between all three glomerular cell types, appear to play important roles in the evolution of diabetic glomerulosclerosis, and a comprehensive approach to understanding this pathologic feature requires an analysis of all three glomerular cell types. Most studies have focused on regulation of mesangial cell ECM production, and the involvement of these cells is clear (13). Signaling mechanisms that directly enhance mesangial cell matrix protein expression or reduce matrix metalloproteinase expression are clearly implicated in diabetic glomerulosclerosis. However, more recent reports have indicated that isolated podocyte damage and loss leads to glomerulosclerosis (20) and that podocyte loss appears to be a requisite early event in diabetic nephropathy (21). Indeed, podocyte loss in humans with type 2 diabetes accurately predicts progressive nephropathy, as well as mesangial expansion (22). Presumably, signals to the mesangium from damaged podocytes, or the hemodynamic factors triggered by podocyte loss, could provide stimulus to the mesangial cell to react by increases in ECM synthesis or decreases in ECM degradation. Similarly, attention to glomerular endothelial cells in the pathogenesis of glomerulosclerosis has been revived in the last few years. Increasingly, models of endothelial dysfunction have resulted in diabetic glomerulosclerosis (23–27), suggesting substantial crosstalk between endothelial and mesangial cells. Finally, there is reason to believe that extraglomerular cells, such as bone marrow–derived mesangial cell progenitors (28) and macrophages (29,30), may significantly contribute to glomerulosclerosis in diabetic nephropathy.

Thus, with evidence for all three glomerular cell types, as well as extraglomerular cells, contributing to the progressive accumulation of extracellular matrix proteins in diabetic nephropathy, the pathogenesis of this lesion is necessarily complex and multifactorial. In the review of newer observations on contributors to diabetic glomerulosclerosis, some reductionism is inevitable, but the interaction of multiple cell types and systemic responses must be kept in mind and will be emphasized throughout this review.

NEWER OBSERVATIONS ON MECHANISMS OF DIABETIC GLOMERULOSCLEROSIS

While the classic view of the pathogenesis of diabetic nephropathy presented above remains largely valid, there

have been a number of interesting insights into mechanisms of diabetic glomerulosclerosis since 2000 that have increased our understanding of the complexities of this process. Some of these mechanisms are briefly described in this section and are organized into whether they are systemic or originate in one or more of the glomerular cell compartments. It must be stressed that many of these observations have been derived from animal models and cell signaling experiments and have yet to be confirmed in humans. Whenever data are available for human glomerulosclerosis we will highlight it, but otherwise these newer mechanisms must be regarded as only potentially involved in diabetic injury until validated in human disease. This caveat is especially important because conventional rodent models of diabetic nephrosclerosis do not completely recapitulate the disease in humans and have not reliably developed glomerulosclerosis to the same extent as seen in human diabetic glomerulopathy (31). Finally, these represent only a portion of the newly identified mechanisms, and the following discussion should not be assumed to be comprehensive.

1. Systemic and paracrine: inflammation. A number of clinical and animal model studies have implicated inflammatory mechanisms as important pathogenic factors in diabetic microvascular complications, including diabetic glomerulosclerosis. Whereas the best data for the role of inflammation are in progressive tubulointerstitial injury in diabetic nephropathy (32,33), several reports also implicate inflammatory mechanisms in the pathogenesis of glomerulosclerosis (34–36). Circulating markers of inflammation are increased in both type 1 and type 2 diabetic patients, and a number of these markers (C-reactive protein, fibrinogen, serum amyloid A protein, and interleukin-6) correlate with both albuminuria and glomerular ECM protein deposition, as well as increased risk for progression toward end-stage kidney disease (34). Inflammatory cytokines, such as tumor necrosis factor-α (33,37), and other mediators may stimulate glomerular cells to enhance production or reduce degradation of ECM proteins. The participation of macrophages in the development of glomerulosclerosis has been recently highlighted (29,35,36,38,39). For example, targeted deletion of the macrophage scavenger receptor-A ameliorated many of the glomerular changes of experimental diabetic nephropathy in mice, including albuminuria, glomerular hypertrophy, mesangial matrix expansion, and overexpression of TGF-β, at 6 months after induction of diabetes. Moreover, in this model, macrophage infiltration was decreased, proinflammatory genes were suppressed, and attachment of monocytes to type IV collagen was reduced (35). Evidence over the last few years also implicates inflammatory mechanisms in podocyte injury in diabetic models and suggests that interventions that block inflammation-induced injury can ameliorate diabetic glomerulosclerosis (39). The importance of inflammatory mechanisms in diabetic glomerulosclerosis is also underscored by the evidence that many therapeutic agents that prevent or retard progression of human diabetic glomerular disease are potent anti-inflammatory agents (36,40,41). In addition, advanced glycation end products and oxidant stress, critical factors in the progression of diabetic nephropathy, augment and are augmented by inflammatory mechanisms of injury in the kidney (35,42). Finally, it should be stressed that glomerular cells produce a multitude of inflammatory mediators in a diabetic milieu, especially as glomerular injury proceeds, that can augment inflammatory damage and even lead to systemic effects.

2. Systemic and mesangial cell: bradykinin 2 receptor blockade. An association between the onset and progression of type 1 diabetic nephropathy in humans and the D-allele of the ACE gene has been reported by several groups (43,44) In addition, ACE inhibitors and angiotenisin receptor blockers are mainstays of renal protection in human diabetic nephropathy. However, using knockout and knockin mice and computer modeling, Smithies and coworkers have found that substantial changes in ACE gene dose lead to only modest increases in ACE with minimal effects on blood pressure and angiotensin II levels but interestingly led to substantial decreases in bradykinin, suggesting that perhaps bradykinin rather than angiotenisin II was more important in renal responses in diabetes (45,46). Therefore, this group studied the contribution of targeted deletion of the bradykinin 2 receptor on the evolution of diabetic nephropathy in Akita mice on a C57BL/6 background (47). In this model, diabetic homozygote bradykinin 2 receptor knockout mice developed profound mesangial sclerosis that resembled glomerular changes of human diabetic glomerulosclerosis. There were no changes in the glomerular endothelial cells or podocytes. Although the mechanism of this increased glomerulosclerosis phenotype remains unclear, there is normally a high level of bradykinin 2 receptor expression in mesangial cells, and knockout of these receptors was associated with enhanced renal expression of several genes involved in progressive glomerulosclerosis, including TGF-β1, connective tissue growth factor, which is a TGF-β effector, and p53 (48). Although these mice have substantial increases in bradykinin 1 receptors (47), this provided no protection from the effects of bradykinin 2 receptor gene deletion. Similar effects to bradykinin 2 receptor knockout were found in diabetic rats treated with a specific nonpeptidic bradykinin 2 receptor antagonist (49). This agent reversed almost all of the salutary effects of ACE inhibitors on albuminuria, glomerular ERK, and TGF-β signaling pathways and glomerular gene expression changes. It also enhanced oxidative stress in glomeruli from treated diabetic rats. Hence, activation of bradykinin 2 receptors in the kidney, presumably in mesangial cells, is protective and, through some yet-to-be-determined mechanism(s), must dampen activation of ERK– and TGF-β–dependent prosclerotic pathways.

3. Paracrine and mesangial cell: lipids and lipid mediators. Increased circulating lipids and enhanced glomerular lipid synthesis have been clearly implicated in diabetic glomerulosclerosis, although the mechanisms by which elevated circulating lipids might contribute to this process remain unclear (50). In addition, several recent studies by Levi and coworkers (51–53), as well as others, have documented enhanced kidney synthesis of triglycerides and cholesterol. This increased local lipid synthesis appears to be stimulated in diabetes due to a number of factors, including increased renal expression of the transcription factor, sterol regulatory element–binding protein-1, which, when overexpressed in mice, causes lipid accumulation and induces expression of TGF-β, plasminogen activator inhibitor-1, and vascular endothelial growth factor (VEGF). This endogenous kidney lipid synthesis pathway appears to directly result in enhanced accumulation of ECM proteins, mesangial expansion, and glomerulosclerosis (51–53), suggesting that diabetes induces renal glomerular synthesis of triglycerides and cholesterol, which

then promotes glomerulosclerosis. In effect, local production of triglycerides and cholesterol is analogous to that of local synthesis of other lipid mediators, which are generated in glomerular and renal vascular cells and have profound effects on the development of glomerulosclerosis.

These latter mediators, known collectively as eicosanoids (prostanoids, leukotrienes, hydroxyeicosatetrenoic acids, and epoxyeicosatrienoic acids) exert diverse and complex effects on renal glomeruli, and the specific influence of each eicosanoid varies from cell to cell and depends on specific gene transcription and other machinery (54). However, many of these effects appear to promote diabetic glomerulosclerosis (54). For example, selective COX2 inhibition inhibits the development of glomerular sclerosis in rats with streptozotocin diabetes and hypertension (55,56). However, the role of COX-derived prostanoids in the pathogenesis of diabetic glomerulosclerosis has not yet been clarified and will require further exploration. Finally, since many of these lipid mediators participate crucially in inflammatory responses, separating out lipid mediators from inflammation as a glomerulosclerosis factor is artificial at best.

4. Mesangial cell: microRNA regulation of TGF-β signaling. MicroRNAs (miRNAs) are short noncoding RNAs of 22 nucleotides that have been shown to play important roles in mammalian gene expression. They induce posttranscriptional gene regulation by blocking protein translation (by binding to the 3′ untranslated region of their target genes) or by inducing miRNA degradation and therefore have the potential to play central roles in gene regulation in both physiologic and pathophysiologic conditions in a number of disease states (57). More than 500 human miRNAs have been identified, and it is predicted that up to 30% of human protein coding genes may be regulated by miRNAs (57). Recently, Natarajan and coworkers (58) have found that expression of miRNA-192 is enhanced in glomeruli from mice with both type 1 and type 2 diabetes, as well as by TGF-β treatment of cultured mesangial cells. These investigators found that TGF-β–induced miRNA-192 mediates an increase in collagen-1α2 expression by reducing expression of two E-box repressors of collagen-1α2 gene activation. Because miRNA-192 was increased in tissues from both type 1 and type 2 diabetic mice, the authors felt that hyperglycemia may be a common factor in inducing miRNA-192 expression, but the mechanisms of this regulation remain to be elucidated. This appears to be the first demonstration of a functional role for a miRNA in kidney disease of any type. Since miRNA-192 is downstream of TGF-β, its stimulation of extracellular matrix synthesis should be a better target for therapy, since such interventions could have fewer nonspecific effects than interrupting TGF-β signaling in general. These findings should open up this important regulatory field for further study.

5. Mesangial cell: Janus kinase/signal transducer and activation of transcription pathway activation. Many growth factors and agonists, including angiotenisin II, act via Janus kinase (JAK)/signal transducers and activation of transcription (STAT) signaling pathways; these pathways may therefore be important in the glomerular response to diabetes. Marerro and coworkers (59,60) have found that high glucose augments angiotensin II activation of the JAK/STAT pathway in rat kidney glomeruli and that activation of JAK/STAT signaling in mesangial cells enhances TGF-β, collagen IV, and fibronectin production. These effects appear to be directly due to hyperglycemia, perhaps mediated by enhanced production of reactive oxygen species, as incubation of cultured mesangial cells in high glucose results in enhanced phosphorylation and hence activation of JAK2 and the downstream substrates of JAK2, STAT1, STAT3, and STAT5A/B (60,61). Moreover, inhibition of JAK2, with AG-490, prevented diabetic proteinuria (60,61), but there has been no report yet on the effects of this inhibitor on diabetic glomerulosclerosis per se.

We have recently obtained independent confirmation of the potential role of JAK/STAT pathways in diabetic kidney disease. Using a transcriptomic approach with human cDNA samples derived from glomerular and tubulointerstitial regions from humans with both early and more progressive diabetic nephropathy, we found that a host of JAK/STAT genes were expressed at higher levels in both these regions (62). These results were obtained in screenings designed to identify pathways in which gene expression was altered in humans with diabetic nephropathy but not in conventional mouse models of diabetic nephropathy, all of which have failed to recapitulate the progressive glomerulosclerosis and tubulointerstitial fibrosis seen in the human disease. JAK1-3 and STAT1 were each expressed at significantly higher levels in glomeruli of patients with diabetic nephropathy. Immunohistochemistry showed a strong JAK2 staining in the glomeruli, as well as in proximal tubules, from patients with diabetic nephropathy compared with those from healthy control subjects. In contrast, there was no increase in JAK2 expression in several common mouse models of diabetic nephropathy, suggesting one reason for lack of progressive glomerulosclerosis in these models. We also found that JAK2 expression in mesangial cells, without direct agonist stimulation, leads to activation of JAK/STAT signaling as evidenced by enhanced STAT3 phosphorylation (C. Berthier, H. Zhang, F.C.B., M.K., unpublished data). We also found that JAK2 overexpression alone led to enhanced oxidative stress (C. Berthier, H. Zhang, F.C.B., M.K., unpublished data). Thus, enhanced JAK2 expression and JAK2-mediated signaling, triggered by high glucose and possibly angiotensin II, appears to occur in progressive diabetic glomerulosclerosis in humans and may result in enhanced glomerulosclerosis. JAK2 signaling has been identified in podocytes (63), but nothing is known about the effects of diabetes on JAK/STAT signaling in this cell type.

6. Endothelial cell/podocyte: reduction in endothelial nitric oxide synthase and vascular endothelial growth factor. As noted above, the role of endothelial cells in the pathogenesis of diabetic nephrosclerosis has received increased attention. One endothelial abnormality in diabetes is altered regulation of endothelial nitric oxide (NO) production (64,65). Although there is some controversy (66), most reports suggest higher levels of NO production early in diabetes but reduced levels in progressive diabetic nephropathy (65). *eNOS* gene polymorphisms that result in decreased endothelial NO synthase (eNOS) expression are associated with advanced diabetic nephropathy (67,68). Three reports that analyzed the effects of diabetes in mice with targeted deletion of the *eNOS* gene have underlined the critical participation of endothelial responses in diabetic glomerular pathology in animal models (25,26,64). Diabetic eNOS knockout mice developed substantial mesangial expansion and glomerulosclerosis, including nodular sclerosis, as well as other signs of advanced diabetic nephropathy including reduction in

glomerular filtration rate. The mechanisms by which reduced NO production results in glomerular pathology are not clear. Resultant hemodynamic effects may play a role, but it seems most likely that paracrine effects between endothelial cells, podocytes, and mesangial cells account for the bulk of the impressive pathology resulting from altered endothelial NO production.

Regulation of eNOS in diabetic nephropathy may come via VEGF. VEGF has been implicated in podocyte and endothelial alterations in diabetic nephropathy (69). VEGF stimulates eNOS activity in the glomerulus (69) and may exert some of its protective effects through this mechanism. In many chronic kidney diseases, VEGF levels are low and are associated with impaired angiogenesis with capillary loss. Conversely, in most animal models of diabetic nephropathy, VEGF levels are elevated, suggesting an uncoupling of the VEGF-NO axis as postulated by Nakagawa et al. (26,69). While such an uncoupling may play a role in rodent models, the recent observation that VEGF gene expression is actually decreased in biopsy samples from humans with progressive diabetic nephropathy (70) would suggest that diminished VEGF levels could reduce eNOS activity, leading to glomerular pathology similar to that seen in the eNOS knockout mice in human diabetic glomerulosclerosis. Both low and high levels of VEGF can have deleterious effects on glomerular endothelium via mechanisms that may be either NO-dependent or -independent (71). Hence, better characterization of the VEGF and eNOS pathways will be essential for understanding podocyte-endothelial crosstalk in diabetic glomerulosclerosis.

7. Endothelial cell/podocyte and paracrine: reduction of activated protein C. As another piece of evidence underlining the importance of endothelial cell responses in the evolution of diabetic glomerulosclerosis, a quite recent study nicely elucidated the effects of the thrombomodulin/activated protein C (APC) pathway on glomerular endothelial cells in diabetes (24). In diabetic patients, function of the endothelial thrombomodulin–protein C system is impaired (72), and in diabetic mice, glomerular endothelial thrombomodulin A expression and protein C activation are substantially reduced (24). APC prevented high glucose–induced apoptosis of glomerular endothelial cells and podocytes, but not mesangial cells, in vitro. The role of APC in glomerular injury in diabetes was further explored using two mouse models, one with impaired protein C activation and one expressing a hyperactivatable protein C mutation. Mesangial ECM expansion was enhanced in the diabetic mice with impaired protein C activation and was completely prevented in those with the hyperactivatable protein C mutation. Moreover, mice with impaired protein C activation demonstrated enhanced endothelial and podocyte apoptosis, whereas the hyperactivatable protein C mice had fewer apoptotic cells. In an interesting experiment, the investigators showed that they could inhibit glomerular mesangial expansion by a generalized apoptosis inhibitor, minocycline. All of these effects were shown to be independent of any changes in coagulation due to altered protein C activation. In summary, this single comprehensive study established that a reduction in protein C activation promotes glomerular capillary dysfunction and apoptosis of endothelial cells and podocytes in experimental diabetes. Moreover, APC seems to be a remarkable mediator of crosstalk between the endothelial cells, podocytes, and the mesangium to regulate nephropathy. If these interesting results are confirmed by further analyses and validated in humans with diabetic nephropathy, it will likely open new pathways for treatment.

CONCLUSIONS

A simple model of the pathogenesis of diabetic glomerulosclerosis has been confounded by a series of recent reports, each of which underscores the complexity and interrelatedness of the mechanisms of this critical aspect of type 1 and type 2 diabetic nephropathy. Many of the new pathogenic pathways that been revealed by genetic manipulation of animal models in the last few years are likely to individually contribute to our understanding and treatment of glomerulosclerosis. Undoubtedly, other pathways not yet elucidated or described in this review will be found to contribute importantly to this complication. In addition, the features and pathways involved in tubulointerstitial fibrosis, which appears to be a uniform feature of progressive diabetic nephropathy and which best predicts renal failure (73), have not been considered in this review. It is conceivable that some of the mechanisms involved in glomerulosclerosis may participate in tubulointerstitial fibrosis, but this remains to be proven. Finally, some of the mechanisms that induce diabetic glomerulosclerosis may be the same as and some may differ from those involved in other chronic renal diseases. Identifying pathways that are specific for diabetic nephropathy may allow for development of specific treatments for this complication that may add materially to our current armamentarium of rather nonspecific therapies. A comprehensive understanding of the pathogenesis of diabetic glomerulosclerosis and how it is similar to and differs from other causes of nephrosclerosis may require a systems biology and computational approach to knit the new and exciting observations into a coherent if multidimensional paradigm. This complexity, though problematic on one hand, opens up a number of new possibilities for intervention to prevent or forestall diabetic nephropathy in patients with diabetes.

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