Pathogenesis of Diabetic Nephropathy

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Our understanding of the natural history of diabetic nephropathy has emerged largely from patients with type 1 diabetes. However, histological manifestations among those with type 2 diabetes are similar (10). Both the clinical manifestations and the histological appearances of kidney disease associated with diabetes have been well characterized. The pathogenesis, however, is less well understood, and there are gaps in our understanding of how various causal factors relate to the histological manifestations of diabetes; in part, this is because of a paucity of kidney biopsies and longitudinal data. Here, we will focus on the pathogenesis, summarizing our current understanding of the histological and clinical correlates and pointing out remaining controversies in the context of pathogenesis.

The pathogenesis of diabetic nephropathy is initiated and maintained by four causal factors, which can be classified broadly into metabolic, hemodynamic, growth, and proinflammatory or profibrotic factors (**Figure 1**). Although there is both a substantial overlap among these factors and variability in their relative contribution among individuals and over time, for ease of discussion, we will describe the pathogenesis as if each factor played an isolated role. These pathogenetic factors produce lesions in various kidney compartments: glomeruli, tubuli, interstitium, and vasculature. A complex series of molecules, receptors, enzymes, and transcription factors participate in the process that drives the earliest stages of kidney disease to an enlarged kidney with hypertrophy, expanded extracellular matrix (ECM), glomerulosclerosis, vascular hyalinosis, interstitial fibrosis and tubular atrophy, and loss of function culminating in end-stage renal disease (ESRD).

Metabolic Factors

The earliest changes are triggered by metabolic factors, namely hyperglycemia. Damage resulting from hyperglycemia can occur by alteration of tissues or can be induced by products of glucose metabolism (11). An overview of the deranged metabolic pathways that mediate the pathogenesis of nephropathy in people with diabetes is shown in **Figure 2**.

Glycation of Tissues

Hyperglycemia through a nonenzymatic mechanism can lead to production of advanced glycation end products (AGEs), which by glycation of various tissue constituents such as proteins, collagen, lipids, and ECM can provoke organ dysfunction. This process is likened to that of accelerated aging through browning of tissues or the Maillard reaction (11).

Glycation of molecules provokes downstream injury by several mechanisms that can be broadly classified into receptor-mediated and non–receptor-mediated categories (12).

Glycation leads to activation of receptors on cells—the best characterized of which is the receptor of advanced glycation end products (RAGE)—that trigger the synthesis and release of nuclear

FIGURE 1 Overview of pathogenic factors in diabetic nephropathy. The key drivers of diabetic nephropathy can be broadly classified as metabolic, hemodynamic, growth, and proinflammatory or profibrotic factors.

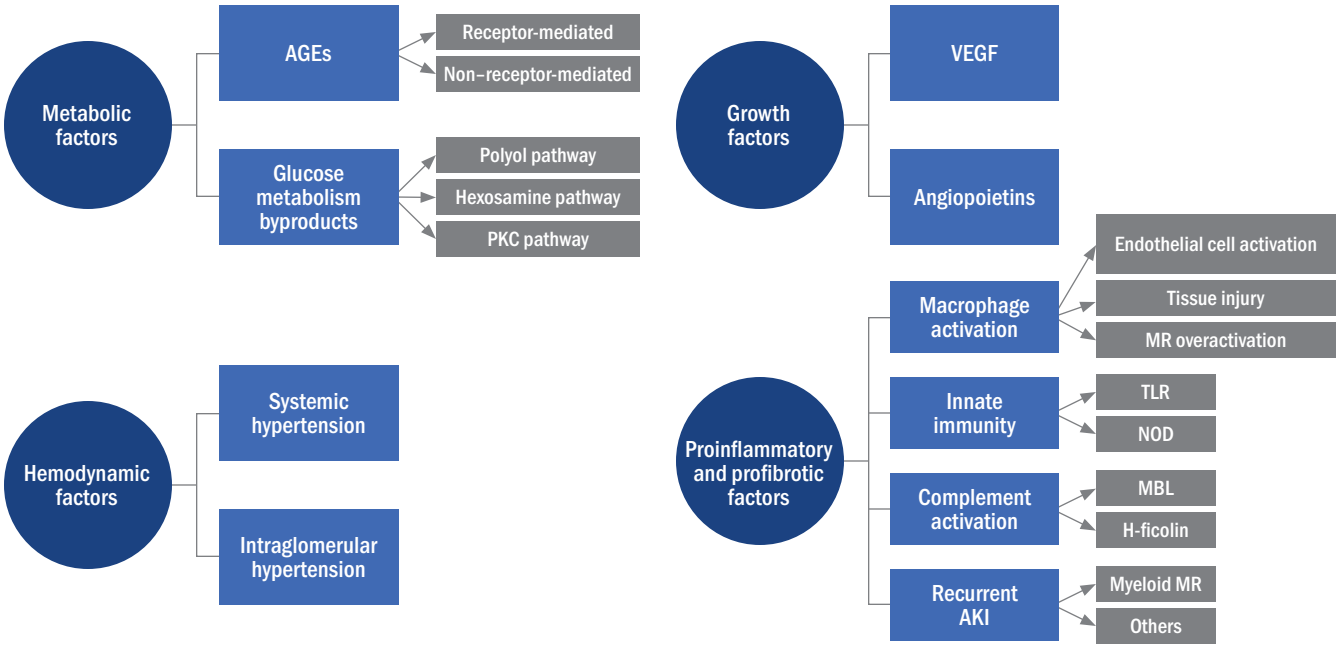
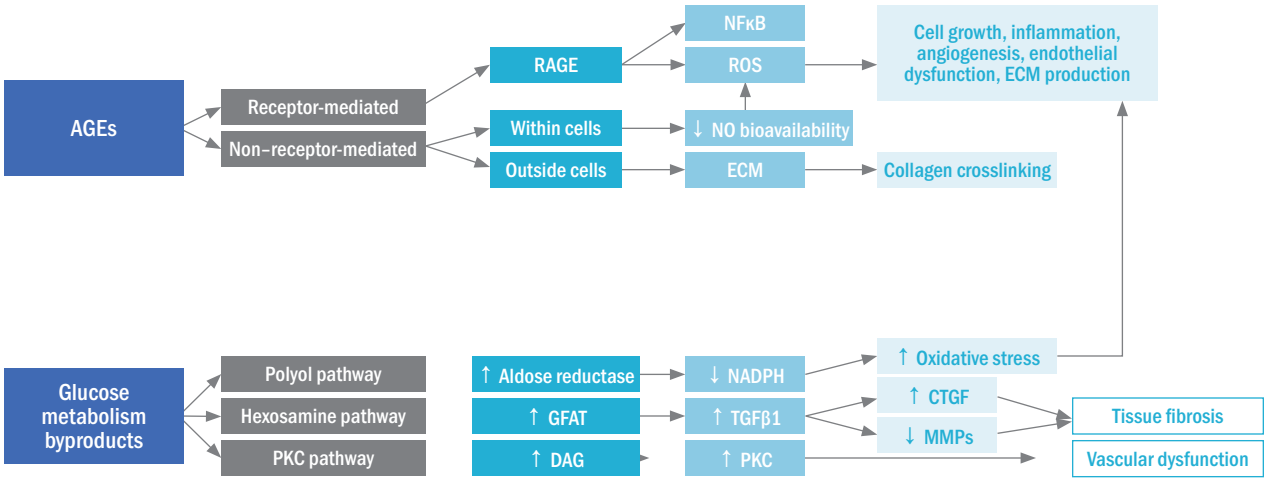


FIGURE 2 Metabolic pathways of diabetic nephropathy. Hyperglycemia provokes the accumulation of AGEs and other products of glucose metabolism. Activation of each of these pathways can injure the kidney. AGEs can produce cell injury by receptor and non-receptor pathways. Outside the cells, they can cause tissue damage by glycating molecules such as collagen that can reduce tissue compliance through crosslinking. Increased glucose flux can result in activation of pathways such as polyol, hexosamine, and PKC that can result in cellular injury and organ dysfunction.



factor κB (NFκB) and the generation of reactive oxygen species (ROS). These molecules, although transcription factors, initiate and maintain kidney damage by several processes (12), including cell growth and hypertrophy, inflammation, angiogenesis, endothelial dysfunction, and ECM production.

Within the cells, AGEs can produce cellular dysfunction without binding to a receptor. For example, glycation of cytosolic proteins can reduce nitric oxide (NO) bioavailability and provoke oxidative stress (12). Similarly, outside the cells, AGEs can provoke tissue dysfunction without binding to a receptor. For example, glycation of connective tissue constituents such as collagen can crosslink molecules in the ECM and cause dysfunction (12).

Histological manifestations of AGE accumulation include basement membrane thickening, reduced protein degradation that results in an increase in mesangial matrix, and an increase in interstitial extracellular volume.

Damage Induced by Products of Glucose Metabolism

Glucose can induce damage in cells independent of glycation such as by the activation of the polyol pathway, hexosamine pathway, or protein kinase C (PKC) pathway or through the generation of ROS.

Polyol Pathway

The polyol pathway involves the activation of the enzyme aldose reductase within cells when intracellular concentrations of glucose rise to hyperglycemic levels (11). This depletes the cellular nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) concentration and alters the redox ratio, which can reduce NO bioavailability and alter enzyme function. Although aldose reductase inhibitors were found to be effective in rodent models of diabetes, human trials have failed to reveal protection from an important microvascular complication of diabetes—eye disease—in a randomized trial (13).

Hexosamine Pathway

The hexosamine pathway is important for the synthesis of proteoglycans, glycolipids, and glycoproteins (14). The synthesis of these molecules requires an amino sugar substrate called UDP-N-acetylglucosamine, which is the final product of the hexosamine pathway. The rate-limiting enzyme of the hexosamine pathway is glutamine:fructose-6-phosphate-amidotransferase (GFAT), which catalyzes the reaction between fructose-6-phosphate and the amine-donor glutamine to produce glucosamine-6-phosphate (14). In cultured mesangial cells, high glucose levels provoke production of transforming growth factor β1 (TGF-β1); this effect is eliminated by inhibition of GFAT. In contrast, stable overexpression of GFAT increases TGF-β1 production. Furthermore, the effects appear to be transduced by PKC. In humans, GFAT is absent in glomerular cells. However, in patients with diabetic nephropathy, GFAT is expressed in the glomerulus, suggesting that it may play a pathophysiological role (14).

PKC Pathway

PKC is a family of enzymes that are critical intracellular signaling molecules and are important for vascular function. In the physiological state, receptor-mediated activation of PKC releases intracellular calcium ions and diacylglycerol (DAG) and activates these enzymes. In pathological states such as in diabetes, DAG production can be abnormally increased and can lead to activation of PKC. In diabetes, DAG production is increased by increased glycolysis and an elevated level of intracellular glyceraldehyde-3-phosphate and glycerol-3-phosphate. PKC

can also be activated by ROS and AGEs. An inhibitor of PKC-β—ruboxistaurin—has been tested in a phase 2 randomized clinical trial in patients with type 2 diabetes and persistent albuminuria (albumin-to-creatinine ratio [ACR] 200–2,000 mg/g creatinine) despite therapy with renin-angiotensin system inhibitors (15). Compared to placebo, the reduction in ACR at 1 year—the primary endpoint of the study—was not significant.

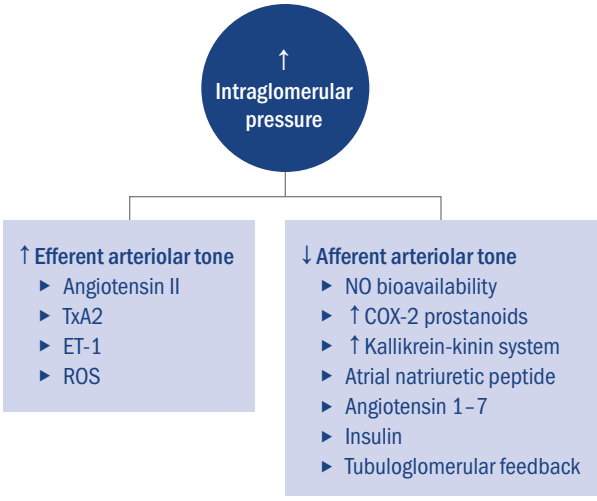
Hemodynamic Factors

The increases in glomerular capillary pressure increase the single nephron glomerular filtration rate—hyperfiltration—and this occurs early in the course of diabetes. An increase in intraglomerular pressure is the result of an increase in efferent arteriolar tone and a reduction in afferent arteriolar tone (**Figure 3**) (16). How this process occurs is not settled, but two theories have emerged.

One group believes that hyperfiltration is mediated by circulating molecules that primarily operate within the glomerulus (17). Several mediators have been proposed to increase intraglomerular pressure via increasing efferent arteriolar tone and reducing afferent arteriolar tone. Increase in efferent arteriolar resistance can result from an increase in the concentration of angiotensin II, thromboxane A2 (TxA2), endothelin 1 (ET-1), and ROS (16). Reduction in afferent arteriolar resistance can be provoked by reduction in NO oxide bioavailability; increased cyclooxygenase-2 (COX-2) prostanoids; activation of the kallikrein-kinin system, atrial natriuretic peptide, and angiotensin 1-7; and an increase in insulin (16).

However, another group proposes that tubular mechanisms remain the primary driver of the intraglomerular hypertension (12). The activation of glucose transporting pathways in the proximal tubule early in the course of diabetes stimulates the reabsorption of both glucose and sodium in the proximal nephron (12). Sodium delivery to the distal nephron is reduced. This triggers tubuloglomerular feedback; the afferent arteriole dilates, and the efferent arteriole constricts (12). An increase in insulin by itself can increase sodium and glucose transport in the proximal tubule and provoke tubuloglomerular feedback. Insulin, as noted above, can also reduce afferent arteriolar tone directly. Thus, insulin can both directly and indirectly cause hyperfiltration.

FIGURE 3 Mechanisms of intraglomerular hypertension. Intraglomerular pressure can increase as a result of either an increase in efferent arteriolar tone or a reduction in afferent arteriolar tone. The mediators of these alterations are shown.



Growth Factors

It has long been recognized that microangiopathy such as that occurs in the eye also associates with kidney disease. Therefore, investigators have explored the relation between vascular proliferation and endothelial permeability—factors known to be important in the pathogenesis of diabetic eye disease—with the occurrence of diabetic nephropathy. Vascular endothelial growth factor (VEGF) is activated early and leads to vascular expansion, which can provoke hyaline arteriosclerosis and hypertensive changes in the kidney (18). Similarly, angiopoietins can cause vascular proliferation and have been implicated in the pathogenesis of diabetic nephropathy (19).

Proinflammatory and Profibrotic Factors

Inflammation and fibrosis are important causes of diabetic nephropathy (20). Whether this is causal or in response to injury remains a matter of debate. However, there is a strong relation between the degree of infiltration of macrophages and subsequent occurrence of tubular interstitial fibrosis and progression of diabetic kidney disease (21,22).

Macrophages are attracted to the kidney by a variety of mechanisms (23). Endothelial cell dysfunction, activation, and injury all stimulate the production of adhesion molecules on the endothelial surface that facilitate transendothelial migration of macrophages. Injury and activation of resident kidney cells such as podocytes, mesangial cells, and tubular cells result in secretion of chemokines that facilitate intrarenal macrophage infiltration. Macrophages are activated to the proinflammatory (M1) phenotype by ROS, angiotensin II, and the activation of mineralocorticoid receptors (MRs). That by itself can damage podocytes, endothelial cells, mesangial cells, and tubular cells. Activated macrophages, by releasing profibrotic cytokines, can increase cell proliferation and matrix volume expansion and provoke fibrosis. Fibrosis at a molecular level is mediated in part because of activation of TGFβ1, which has two synergistic effects: activation of connective tissue growth factor (CTGF) and a reduction in matrix metalloproteinases (MMPs). In contrast, MR antagonists can coax macrophages to the antiinflammatory (M2) phenotype and be protective (24). Thus, macrophages play an important role in the pathogenesis of diabetic nephropathy (23).

Acute Kidney Injury, Inflammation, Chronic Kidney Disease, and the Role of MRs

Inflammation and fibrosis may also be important promoters of progression of chronic kidney disease (CKD) in patients with

diabetes, and this may be the result of acute kidney injury (AKI). It is increasingly being recognized that single or repeated bouts of AKI on a background of CKD in diabetes may play a vital role in the progression of CKD to ESRD (25). Macrophage infiltration is commonly seen in AKI, and depletion of macrophages in preclinical models can protect from AKI (26). In two different rodent models of AKI, bilateral ischemia reperfusion (IR) pretreatment with the nonsteroidal MR antagonist finerenone prevented the development of AKI (27). In a separate set of experiments, unilateral IR injury was also associated with reduced fibrosis when animals were pretreated with finerenone (27). Furthermore, in a pig model of IR AKI, the administration of the MR antagonist potassium canrenoate prevented the progression of AKI to CKD at 90 days (27).

The relative contributions of the knockout of MRs in smooth muscle cells versus their knockout in myeloid cells have been investigated in mouse models (**Figure 4**) (27). With MR knockout in smooth muscle cells, IR models demonstrated that the short-term elevation of serum creatinine and blood urea nitrogen was prevented. However, at 30 days, there was no difference between wild-type and smooth muscle cell MR knockouts. In contrast to MR knockout in smooth muscle cells, among myeloid MR knockout mice, there was no immediate protection from AKI. However, at 30 days, there was a marked improvement in renal function and markers of inflammation. Furthermore, there was a shift in the polarization of macrophages infiltrating the kidney. Although the total number of macrophages in wild-type and myeloid MR knockouts were similar, there was a shift in the nature of macrophages such that the M2 macrophages associated with an antiinflammatory response were increased in relation to the M1 macrophages, which are proinflammatory (27).

Although these studies were done in animals without diabetes, the experiments demonstrate the importance of inflammation and MRs in mediating CKD after AKI; similar mechanisms likely operate in patients with CKD resulting from diabetes (**Table 1**) (28,29).

FIGURE 4 Short- and long-term effects of MRs are location dependent. In smooth muscle cells, MRs protect from short-term AKI. In contrast, MRs in myeloid cells have no short-term effects but prevent long-term inflammation and fibrosis. These experiments are helpful in understanding the long-term consequences of repeated AKI in the progression of kidney disease in diabetes.

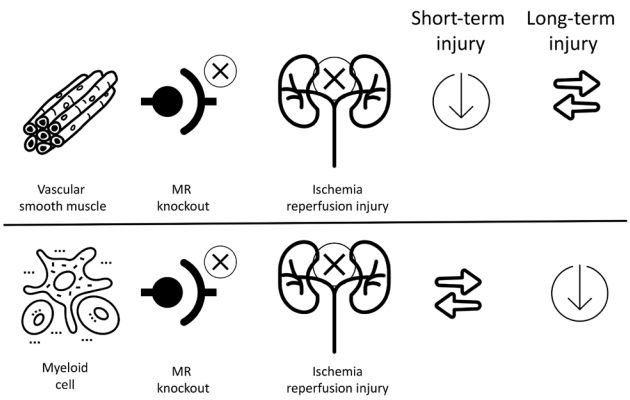


TABLE 1 MR Blockade and Kidney Protection in Diabetes

- Reduced maladaptive response
- Reduced ROS
- Improved endothelial function
- Shift in macrophage phenotype from proinflammatory (M1) to antiinflammatory (M2)
- Better blood pressure control

Innate Immunity, Complement Activation, and Diabetic Nephropathy

Activation of the innate immune system through pattern recognition receptors such as membrane-bound toll-like receptors (TLR) and nucleotide-binding oligomerization domain (NOD)-like receptors may play an important role in the pathogenesis of diabetic nephropathy (30). The complement system, in addition to fighting infections, facilitates the removal of damaged cells by antibodies and phagocytic cells. The activation of the complement component C3 generates the membrane attack complex (MAC) that lyses, damages, or activates target cells. Mannose-binding lectin (MBL) activates the lectin pathway; pattern recognition molecules called ficolins can also activate the lectin pathway. The lectin pathway is activated after binding of ficolins to glycated proteins. Glycation of complement regulatory proteins such as CD59 might by itself activate complement; this is so because CD59 normally inhibits MAC (30).

A causal relation between MBL activation and diabetic nephropathy is firmly established in animals. For example, compared to wild-type mice with streptozotocin-induced diabetes, MBL knockout mice have less kidney damage, less kidney hypertrophy, lower urine albumin excretion, and less type IV collagen expression (31).

Several lines of evidence in humans suggest the important role of complement activation in CKD progression. As examples, *1*) in patients with type 1 diabetes, concentrations of MBL associate with progression of kidney disease from macroalbuminuria to ESRD (32); *2*) in a prospective cohort study of 270 patients with newly diagnosed type 1 diabetes, H-ficolin was associated with an increased risk of worsening of albuminuria (33); and *3*) MAC detected by antibodies directed against the C9 component of MAC localize it to the glomerular basement membrane (GBM), tubules, and Bowman capsule in patients with type 1 diabetes (34–36).

Taken together, these data point out the important role of the complement system and its components in the pathogenesis of diabetic nephropathy.

Interrelations Among Pathogenic Factors in Diabetic Nephropathy

The interplay of metabolic, hemodynamic, growth, and profibrotic factors is illustrated by consideration of the following preclinical experiments (37). Cultured mesangial cells exposed to CTGF

increase production of profibrotic molecules such as fibronectin and collagen type I (37). Although the baseline production of CTGF by mesangial cells is low, exposure of mesangial cells to increased glucose concentration (a metabolic factor) or cyclic metabolic strain (a hemodynamic factor) increases the production of CTGF (a growth factor). The induction of CTGF protein by a high glucose concentration is blocked by TGFβ1-neutralizing antibody. This suggests that another growth factor—TGFβ1—mediates the effect of high glucose concentration to provoke CTGF production. In vivo studies in obese db/db diabetic mice demonstrate that CTGF transcription was increased 28-fold after ~3.5 months of diabetes (37). At 3.5 months of diabetes, mesangial expansion was mild, and interstitial disease and proteinuria were absent. Furthermore, rather than being diffusely increased throughout the kidney, the CTGF production was limited to the glomerular compartment. These experiments demonstrate the interplay of all the pathogenic factors discussed above and underscore the complex interrelations of these factors, over time and at different locations in the kidney, in producing the histological manifestations of diabetic nephropathy.

Pathological Classification of Diabetic Nephropathy

According to an international consensus conference, the histological manifestations of diabetic nephropathy follow four progressive classes (**Table 2**) (38). The classification acknowledges lesions in the glomeruli, tubuli, and vessels, but the root of the classification system is based on the appearance of the glomerulus. According to this classification system, diabetic nephropathy progresses from thickening of the GBM, to mesangial expansion, Kimmelstiel–Wilson lesions, and global glomerulosclerosis, which is reflected in the four classes, as discussed further below. Although this system has not been validated with clinical outcomes, it serves as an important clinical and research tool to classify the severity of diabetic nephropathy lesions.

Class I Diabetic Nephropathy

On ultrastructural evaluation of the kidney histology, among the earliest change that occurs in the kidney is thickening of the GBM; light microscopy shows minimal, non-specific, or no changes. Thickening of the GBM does not directly correlate with clinical injury. Patients may have such thickening but have no increase in urine albumin excretion rate or impairment of glomerular filtration rate (39,40). Although an increase in diastolic blood pressure (40) or nocturnal blood pressure (39) is correlated with GBM thickening, the causal relation is not established because of a lack of longitudinal data and interventional studies. GBM thickening occurs as a result of either an increased rate of deposition or a reduced rate of removal of connective tissue. Target molecules include collagen IV and VI, fibronectin, and laminin (35,41).

TABLE 2 Pathological Classification of Diabetic Nephropathy

Class I	▸ GMB thickening on electron microscopy; minimal, non-specific, or no changes on light microscopy
Class II	▸ Increase in mesangial matrix
Class IIa	▸ Mesangial expansion ≤25%
Class IIb	▸ Mesangial expansion >25%
Class III	▸ Nodular glomerulosclerosis: Kimmelstiel-Wilson lesion
Class IV	▸ Advanced glomerulosclerosis; >50% glomeruli sclerotic

Class II Diabetic Nephropathy

Among the earliest manifestations on kidney histology that correlate with kidney damage is an increase in mesangial matrix, as seen in class II diabetic nephropathy. Class II is further subclassified based on the degree of mesangial expansion; class IIa is characterized by ≤25% mesangial expansion, and class IIb involves >25% of the mesangial expansion. An increase in mesangial matrix, glomeruli, and kidney volume is clinically manifested as kidney enlargement; kidneys are often 11 cm or larger on kidney ultrasound. Urine albumin excretion is often increased in these patients.

Class III Diabetic Nephropathy

An increase in mesangial matrix is followed by mesangial sclerosis. The hallmark lesion on a kidney biopsy is nodular glomerulosclerosis, or Kimmelstiel-Wilson nodules. The presence of Kimmelstiel-Wilson nodules on kidney biopsy correlates with the occurrence of diabetic retinopathy, suggesting activation of common pathogenetic pathways such as VEGF.

Class IV Diabetic Nephropathy

Advanced, or class IV, diabetic nephropathy is characterized by sclerosis in >50% of the glomeruli. These patients often have a loss of kidney function at the time of biopsy.

An enlargement of glomeruli is often seen along with thickening of the walls of the glomerular capillaries. Arteriolar hyalinosis of both the afferent and efferent arteriole should alert health care professionals to the possibility of diabetic nephropathy. The proximal tubules can contain protein resorption droplets. In the setting of severe persistent hyperglycemia, glycogen deposits may be seen rarely in the proximal tubules (i.e., Armanni Ebstein lesion). Interstitial fibrosis and tubular atrophy (IFTA) and interstitial inflammation are often seen. Despite tubular atrophy, the basement membranes are often thickened in patients with diabetes.

The Heterogeneity of Kidney Injury in Type 2 Diabetes: A Pathogenetic Explanation

Although kidney disease is histologically similar in type 1 and type 2 diabetes, the relative contributions of causes of kidney damage differ in these two conditions. Compared to patients

with type 1 diabetes, those with type 2 diabetes are older, have a greater BMI, and are more likely to have dyslipidemia, hypertension, and other cardiovascular risk factors and, consequently, atherosclerosis and arteriosclerosis. Thus, the nature of kidney injury in patients with type 2 diabetes may be modified by environmental factors and genetic background. This heterogeneity in environmental and genetic factors in patients with type 2 diabetes may explain the distinct kidney injury phenotypes.

As an example, consideration of an animal experiment provides evidence for interplay between genetics and environment with regard to kidney injury phenotype (42). Progeny of rats with one parent with heart failure and another with obesity were fed a diet either high in carbohydrate or high in fat; all progeny had diabetes (42). Compared to animals fed a high-carbohydrate diet, animals fed a high-fat diet demonstrated a greater preponderance of tubulointerstitial injury and non-nodular glomerulosclerosis. There was evidence of lipid peroxidation and increased kidney TGFβ1 that correlated with kidney injury. Furthermore, injury in animals fed a high-fat diet was seen in the arterial wall and renal microcirculation. In contrast, animals fed a high-carbohydrate diet had increased glycoxidation stress biomarkers, but these did not correlate with kidney injury (42).

Conclusion

The pathogenesis of diabetic nephropathy is similar in type 1 and type 2 diabetes. Diabetic nephropathy is classified histologically by the appearance of the glomerulus on kidney biopsy. It progresses from GBM thickening, to mesangial expansion, nodular glomerulosclerosis, and global glomerulosclerosis. Glomerulomegaly, vascular lesions, IFTA, and tubular resorption droplets are all commonly seen. The pathogenesis of diabetic nephropathy involves metabolic, hemodynamic, growth, and inflammatory and fibrotic factors. The relative contributions of these factors vary among patients, over time, and even in different compartments of the kidney, and genetic and environmental factors can modify the appearance of the kidney lesions. AKI plays an important role in the progression of kidney disease in patients with diabetes. MR activation, particularly in the myeloid cells, may be important in mediating inflammation and fibrosis in CKD and after AKI in individuals with type 2 diabetes, and MR antagonist therapy may be protective.

See references starting on p. 34.

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