Toward a Mouse Model of Diabetic Nephropathy: Is Endothelial Nitric Oxide Synthase the Missing Link?

Susan E. Quaggin* and Thomas M. Coffman†

*The Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, and Division of Nephrology, St. Michael’s Hospital, University of Toronto, Toronto, Ontario, Canada; and †Division of Nephrology, Duke University School of Medicine, Durham, North Carolina


Two articles in the October and February issues of JASN reported that diabetic mice that lack the eNOS gene developed accelerated kidney disease with features that resemble human diabetic nephropathy (DN). These articles are significant given the lack of good mouse models to study this important clinical issue.

Why Is the Mouse Such a Poor Model for DN?

Over the years, a number of mouse models of diabetes have been developed and studied. Although in-depth investigation of renal function and pathology has been somewhat limited, no mouse model to date exhibits the classic pathologic lesions of DN that are observed in humans: Glomerular nodulosclerosis, Kimmelstiel-Wilson nodules, fibrin drop lesions, or capsular drop lesions. Similarly, they also fail to develop characteristic functional features of overt DN in humans, including robust proteinuria and progressive loss of renal function.

Many potential explanations have been proposed for the apparent resistance to the renal complications of diabetes in mice. One possibility is that the lifespan of a mouse is simply too short to permit complications that typically take a decade or more to develop in patients. Alternatively, there are a number of differences in dietary patterns and metabolism, including lipid and cholesterol pathways, that may affect susceptibility to nephropathy. Whereas hypertension is a common accompaniment and a major risk factor for progressive kidney injury in patients with diabetic nephropathy, normal or low BP is typical in hyperglycemic mice. Finally, the complex genetic and environmental overlay in patients with diabetes is much different from that in the current favorite strains of laboratory mice that are housed in “barrier” facilities devoid of pathogens. Within this array of potential factors, the articles from Zhao et al. (1) at Vanderbilt and Nakagawa et al. (2) at Florida suggest that endothelial function may be a key determinant for susceptibility to nephropathy in diabetic mice.

Loss of Endothelial Nitric Oxide Synthase Produces Nodular Glomerular Disease in Diabetic Mice

To examine the role of endothelial nitric oxide (eNO) in renal responses to diabetes, both groups induced diabetes in mice that carry a genetic deletion of the eNOS gene (2). Zhao et al. examined eNO synthase (eNOS) deficiency in the db/db mouse strain, a widely used model of type 2 diabetes. These mice carry a mutation in the leptin gene. On the typical C57BLKS/J background, renal disease in db/db mice is limited to the early features of DN with mild proteinuria, mesangial expansion, and mild thickening of the glomerular basement membrane (2).

Nakagawa et al. carried out chemical induction of hyperglycemia using streptozotocin, which is toxic to islet cells. On wild-type backgrounds, streptozotocin-treated mice also typically develop only mild renal disease.

The eNOS knockout mice lack the NOS-3 isoform that is responsible for a major portion of NO generation by endothelium. The most notable phenotype in nondiabetic eNOS mice is an increase in systolic BP and mild glomerular defects. Dysregulation of NO has been described in patients with DN, including increased NO expression in early DN, followed by a marked downregulation. Furthermore, polymorphisms in the eNOS gene that lead to decreased eNOS expression have been associated with advanced DN in patients (3–6).

Strikingly, in both studies, diabetic mice with deletions of eNOS developed profound glomerular changes with increased proteinuria, marked thickening of the glomerular basement membrane, mesangial expansion, and prominent nodular sclerosis. The eNOS-deficient db/db mice also developed an impressive reduction in GFR. Furthermore, these dramatic changes were largely rescued in the streptozotocin mice by insulin therapy. Taken together, these findings suggest an important facilitating role for endothelial dysfunction in the pathogenesis of diabetic kidney disease. Moreover, this critical role of eNOS seems to transcend the cause of diabetes because similar acceleration of renal injury was seen in models of type 1 and type 2 diabetes.

eNOS modulates a number of endothelial functions, including vascular tone, and contributes to vasodilation and hyperfiltration, features of early DN. The articles suggest that loss of these functions promotes the development of kidney pathol-
ogy. This notion is consistent with clinical studies suggesting that microalbuminuria, the earliest clinically detectable sign of renal involvement in diabetes, is a direct reflection of endothelial dysfunction. Recently, several reviews have focused on the role of the podocyte and/or mesangial cell in the development and progression of DN (14). The proposed contribution of endothelial dysfunction to the pathogenesis of DN does not preclude a key role for podocytes and/or mesangial cells in this process. Given the cross-talk between glomerular cell compartments, the initiation and/or propagation of the defect may occur in any or all of these cell lineages and may involve or require interactions between them.

**Vascular Endothelial Growth Factor, eNOS, and Diabetes**

Although both articles reported similar major findings, some differences were observed. In the article by Nakagawa et al., elevated vascular endothelial growth factor (VEGF) levels and endothelial proliferation were observed. Dysregulation of VEGF production was reported previously in glomeruli of patients with DN. However, determining a role for VEGF in DN on the basis of the current literature is somewhat confusing with publications reporting both increased and decreased levels of VEGF in diabetes (7–13,15). One potential explanation for this discrepancy is that these measurements reflect different stages of disease. Podocytes are a major source of VEGF, and podocyte dropout is a characteristic feature of DN. Therefore, one possible scenario is that diabetes may cause an early stimulation of VEGF followed by a loss of VEGF production coincident with podocyte dropout.

Nakagawa et al. suggested that there may also be an uncoupling of VEGF-NO signaling. Normally, VEGF signals through its major tyrosine kinase receptor VEGFR-2, causing phosphorylation of eNOS via the phosphatidylinositol-3 kinase/Akt pathway (16). Nakagawa and colleagues argued that primary loss of eNOS function in diabetes causes upregulation of VEGF, resulting in endothelial proliferation with abnormal glomerular angiogenesis (2). An alternative or complementary interpretation of the data is that primary injury or dysfunction of the endothelium in eNOS diabetic mice has an impact on adjacent vasculature support cells, such as podocytes in the glomerulus and pericytes in the retina, resulting in a “hypoxic” response or signal that triggers upregulation of VEGF.

In diabetes, it is not yet clear whether the reported alterations in VEGF production are primary or secondary or represent “rebound” phenomena to hypoxia or another stimulus. We suggest that the consistent decrease in VEGF that has been observed in late stages of DN is likely the result of podocyte dropout, leading to vascular rarefaction and glomerulosclerosis. However, it is not clear whether the alterations in VEGF production and signaling have a direct causal role in the pathogenesis of nephropathy or are simply a marker for renal pathology. Available models for genetically manipulating components of the VEGF system may be useful for addressing these questions.

**Conclusion**

The articles by Zao et al. (1) and Nakagawa et al. (2) provide clear evidence that loss of eNOS enhances the susceptibility to glomerular disease in the metabolic environment of diabetes. The observation of a similar pathologic outcome, nodular glomerulosclerosis, in two independent mouse models of diabetes suggests that this pathway may be broadly relevant to the renal complications of type 1 and type 2 diabetes. In future studies, it will be interesting to determine whether eNOS deficiency predisposes to additional microvascular complications, such as diabetic retinopathy.

Given the dearth of preclinical models in this area, these articles provide an interesting and important step toward better models for diabetic research. Furthermore, they suggest that therapies that are targeted toward preservation of endothelial function may be useful in preventing or attenuating DN in humans.

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**Disclosures**

None.

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