Nephrol Dial Transplant (2007) 22: 2775–2777
doi:10.1093/ndt/gfm380
Advance Access publication 25 June 2007

Translational Nephrology

NDT
Nephrology Dialysis Transplantation

A breakthrough in diabetic nephropathy: the role of endothelial dysfunction*

Takahiko Nakagawa[1], Mark Segal[1], Byron Croker[2] and Richard J. Johnson[1]

[1]The Division of Nephrology, University of Florida, Gainesville FL 32610 and [2]Pathology and Laboratory Medicine Service, North Florida/South Georgia Veterans Health System, and Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL 32608, USA

Keywords: mesangiolysis; glomerular microaneurysm; KimmelSteil-Wilson nodule; VEGF; eNOS; animal model

Summary of key findings

Kanetsuna *et al*. [1] have published an important paper in the *American Journal of Pathology*, reporting that renal lesions resembling human diabetic nephropathy can be induced in mice made diabetic (with streptozotocin) which genetically lack endothelial nitric oxide synthase (eNOS). eNOS is a key enzyme in endothelial cells that produces nitric oxide (NO). In turn, NO has multiple functions in the vasculature, including acting as a vasodilator, anti-inflammatory, anti-thrombotic and anti-proliferative activities. In this study, diabetic eNOS knockout mice developed both renal functional (proteinuria, reduced glomerular filtration rate) and structural changes consistent with human diabetic nephropathy. Up to now, it has been difficult to develop in mice models of diabetic nephropathy that resemble human disease, so this article represents a breakthrough in the pathogenesis of diabetic nephropathy.

Review of the field

Diabetic nephropathy is currently one of the most serious complications of longstanding diabetes and has emerged as the most common cause of end-stage renal disease worldwide [2]. Despite our best efforts, 20 to 40% of subjects with type I diabetes and as many as 40% of subjects with type 2 diabetes [3] will develop this complication, typically 10 to 15 years after the onset. Numerous studies have been performed to identify why diabetic nephropathy develops in certain individuals but not in others. In this regard, the development of microalbuminuria [4] has been considered of most significance. Others have proposed that there may be a specific 'nephropathy' gene that could account for why some individuals develop diabetic nephropathy, whereas others remain free of this complication [5]. Unfortunately, the lack of a good murine model of human diabetic nephropathy has hampered progress towards an understanding of the pathophysiological changes that are required to induce the fully developed diabetic renal lesion.

Classically human diabetic nephropathy is characterized early, by thickening of the basement membrane and by mesangial expansion [6]. In more severe disease, frank mesangiolysis, microaneurysm and nodule formation can occur, often with local fibrin deposition [7]. These late changes are less commonly observed, with better blood sugar and blood pressure control, as compared with studies performed decades ago [7–9]. It has been well described that these lesions can be largely prevented when control of blood sugar, blood pressure and inhibition of the renin angiotensin system can be achieved [10]. However, if the mechanisms leading to these changes were better understood, it is possible that more targeted therapies could be developed.

Most mouse models of diabetic nephropathy can reproduce some of the changes of human diabetic nephropathy, especially the basement membrane thickening and mesangial matrix expansion. However, there are few models that develop full blown diabetic nephropathy with hypertension, proteinuria, chronic kidney disease (CKD), vascular lesions and mesangial nodule formation. Most of these latter models involve inducing diabetes in a hypertensive mouse model, and the lesions generated

Correspondence and offprint requests to: Takahiko Nakagawa, MD, PhD, Division of Nephrology, Hypertension and Transplantation, University of Florida, PO Box 100224, Gainesville FL 32610-0224. Email: nakagt@medicine.ufl.edu

*Comment on Kanetsuna Y, Takahashi K, Nagata M *et al*. Deficiency of endothelial nitric-oxide synthase confers susceptibility to diabetic nephropathy in nephropathy-resistant inbred mice. *Am J Pathol* 2007; 170: 1473–1484.

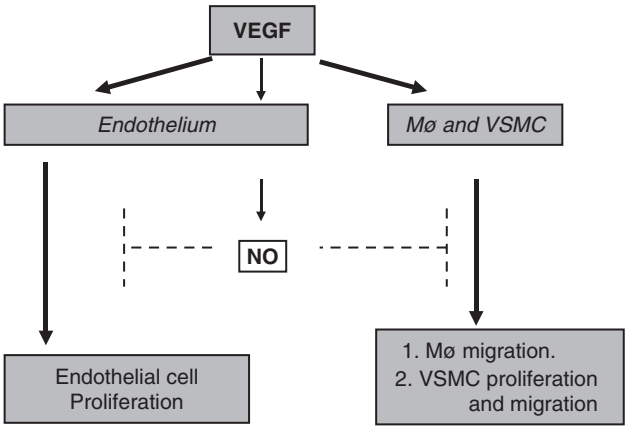

Fig. 1. Hypothesis: Uncoupling of VEGF with endothelial NO causes diabetic nephropathy. We have hypothesized that endothelial dysfunction induced by hyperglycaemia or other factors may underlie the pathogenic mechanisms of a high VEGF state. VEGF normally stimulates endothelial nitric oxide (NO) release and acts in concert with elevated NO levels as a trophic factor for vascular endothelium. The increased NO derived from the endothelial cell acts as an inhibitory factor that prevents excess endothelial cell proliferation, vascular smooth muscle cell proliferation, and macrophage infiltration. In the setting where NO bioavailability is reduced in diabetes, high level of VEGF leads to excessive endothelial cell proliferation, stimulation of macrophage chemotaxis and vascular smooth muscle cell activation, which could lead to development of diabetic nephropathy.

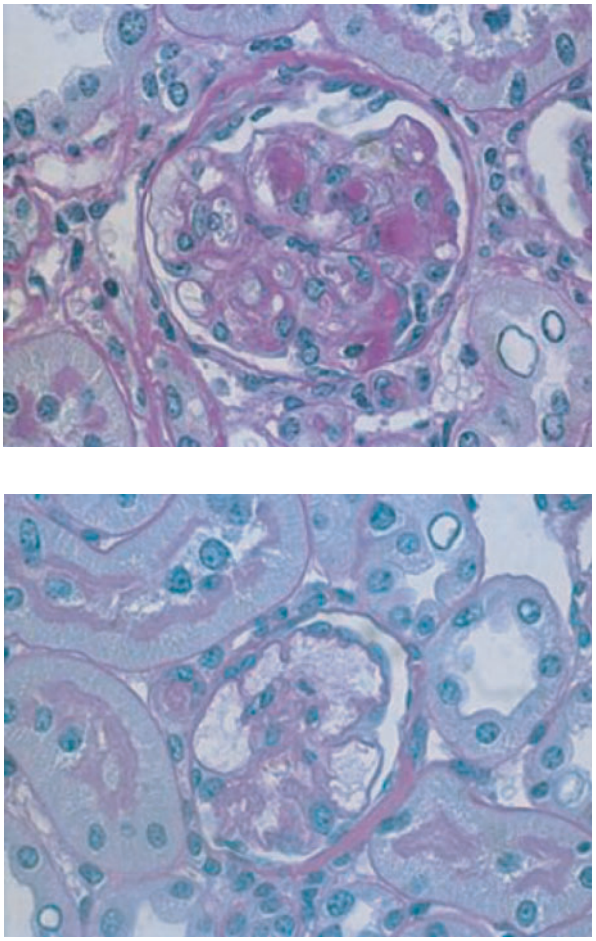

Fig. 2. Glomeurlar lesion in diabetic eNOS knock-out mice (PAS staining). (**A**) Glomerular nodular lesion in diabetic eNOS knock-out mice (original magnification 1000×): Diabetes was induced by streptozotocin. At 5 months, the nodular lesion developed in the glomerulus. Some nodules composed of insudative lesions, which can be seen in human diabetic glomerular injury [7]. (**B**) Glomerular mesangiolysis and microaneurysm in diabetic eNOS knock-out mice (original magnification 1000×): diabetic eNOS knock-out mice developed mesangiolysis as well as glomerular microaneurysms, which are also observed in human diabetic nephropathy [7].

rarely resemble the full blown human diabetic nephropathy. As a consequence, the National Institutes of Health developed a multicentre consortium, to try to develop a murine model that better resembles the human disease.

Recently it has been appreciated that endothelial dysfunction is common in subjects with diabetic nephropathy [11]. Specifically, it has been shown that diabetic subjects with renal disease often have an impaired release of NO, which is a key vasodilator involved in keeping the endothelium healthy. In animal models of CKD and arteriosclerosis, blocking endothelial NO leads to an increase in microvascular disease [12,13], known to impair renal autoregulation [14]. In addition, endothelial dysfunction has also been shown to lead to an uncoupling of the vascular endothelial growth factor (VEGF)-nitric oxide axis resulting in enhanced proinflammatory and proliferative effects of VEGF (Figure 1) [15–17].

The possibility that this 'endothelial dysfunction' is the missing second factor in human diabetic nephropathy is now supported by the recent publication of three papers, which all report that eNOS knockout mice with diabetes (either type 1 or 2) develop lesions similar to that observed in human diabetic renal disease [1,16,18]. Mice develop not only basement membrane thickening and mesangial expansion, but also vascular lesions, mesangiolysis, microaneurysms, nodules, proteinuria and CKD (Figure 2) [1,16,18]. Interestingly, simple blood sugar control with insulin will block both the hypertension and renal injury [1,16]. Historically, scientists had not considered that blood sugar control might also prevent the development of hypertension, but there is increasing evidence that hypertension is largely mediated by subtle renal injury and inflammation [19].

What is in it for the practicing nephrologist?

These studies should ignite the field to determine what mechanisms account for the endothelial dysfunction in human diabetic renal disease, and whether this might represent a new target for preventing diabetic complications. For example, it has recently been shown that endothelial progenitor cells from

diabetic subjects also have a migratory defect due to a relative absence of NO [20]. Various potential mediators need to be investigated, including the role of oxidative stress, the presence of asymmetric dimethyl arginine (an eNOS inhibitor) and uric acid. Investigation of the mechanisms of endothelial dysfunction and for means to reverse these changes might lead to new breakthroughs in this important medical condition.

Take home message

New studies in animal models suggest that endothelial dysfunction may represent a key risk factor for the development of nephropathy in diabetic patients. Future studies investigating the role of endothelial dysfunction in human diabetic nephropathy are needed.

Acknowledgement. Supported by NIH grants DK-52121 and HL-68607 and generous funds from Gatorade.

Conflict of interest statement. Dr. Richard J Johnson is a consultant on the Scientific Board of Nephromice. The other authors have no conflict of interest to declare.

References

1. Kanetsuna Y, Takahashi K, Nagata M *et al*. Deficiency of endothelial nitric-oxide synthase confers susceptibility to diabetic nephropathy in nephropathy-resistant inbred mice. *Am J Pathol* 2007; 170: 1473–1484
2. US RDS: USRDS 2005 Annual Data Report: Atlas of ENd-Stage Renal Disease in the United States. Bethesda, MD *National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Disea*: http://www.usrds.org
3. Warram JH, Gearin G, Laffel L, Krolewski AS. Effect of duration of type I diabetes on the prevalence of stages of diabetic nephropathy defined by urinary albumin/creatinine ratio. *J Am Soc Nephrol* 1996; 7: 930–937
4. Parving HH, Chaturvedi N, Viberti G, Mogensen CE. Does microalbuminuria predict diabetic nephropathy? *Diabetes Care* 2002; 25: 406–407
5. Seaquist ER, Goetz FC, Rich S, Barbosa J. Familial clustering of diabetic kidney disease. Evidence for genetic susceptibility to diabetic nephropathy. *N Engl J Med* 1989; 320: 1161–1165
6. Chavers BM, Bilous RW, Ellis EN, Steffes MW, Mauer SM. Glomerular lesions and urinary albumin excretion in type I diabetes without overt proteinuria. *N Engl J Med* 1989; 320: 966–970
7. Stout LC, Kumar S, Whorton EB. Focal mesangiolysis and the pathogenesis of the Kimmelstiel-Wilson nodule. *Hum Pathol* 1993; 24: 77–89
8. Mazzucco G, Bertani T, Fortunato M *et al*. Different patterns of renal damage in type 2 diabetes mellitus: a multicentric study on 393 biopsies. *Am J Kidney Dis* 2002; 39: 713–720
9. Stout LC, Kumar S, Whorton EB. Insudative lesions–their pathogenesis and association with glomerular obsolescence in diabetes: a dynamic hypothesis based on single views of advancing human diabetic nephropathy. *Hum Pathol* 1994; 25: 1213–1227
10. Fioretto P, Steffes MW, Sutherland DE, Goetz FC, Mauer M. Reversal of lesions of diabetic nephropathy after pancreas transplantation. *N Engl J Med* 1998; 339: 69–75
11. Chan WB, Chan NN, Lai CW *et al*. Vascular defect beyond the endothelium in type II diabetic patients with overt nephropathy and moderate renal insufficiency. *Kidney Int* 2006; 70: 711–716
12. Kang D, Nakagawa T, Feng L, Johnson RJ. Nitric oxide modulates vascular disease in the remnant kidney model. *Am J Pathol* 2002; 161: 239–248
13. Zhao Q, Egashira K, Inoue S *et al*. Vascular endothelial growth factor is necessary in the development of arteriosclerosis by recruiting/activating monocytes in a rat model of long-term inhibition of nitric oxide synthesis. *Circulation* 2002; 105: 1110–1115
14. Beierwaltes WH, Sigmon DH, Carretero OA. Endothelium modulates renal blood flow but not autoregulation. *Am J Physiol* 1992; 262: F943–F949
15. Nakagawa T: Uncoupling of the VEGF-Endothelial Nitric Oxide Axis in Diabetic Nephropathy: An explanation for the paradoxical effects of VEGF in renal disease. *Am J Physiol Renal Physiol* 2007; 292: F1665–F1672
16. Nakagawa T, Sato W, Glushakova O. *et al*. :Diabetic eNOS knockout mice develop advanced diabetic nephropathy. *J Am Soc Nephrol* 2007; 18: 539–50
17. Nakagawa T, Sato W, Sautin YY *et al*. Uncoupling of vascular endothelial growth factor with nitric oxide as a mechanism for diabetic vasculopathy. *J Am Soc Nephrol* 2006; 17: 736–745
18. Zhao HJ, Wang S, Cheng H *et al*. Endothelial nitric oxide synthase deficiency produces accelerated nephropathy in diabetic mice. *J Am Soc Nephrol* 2006; 17: 2664–2669
19. Johnson RJ, Rodriguez-Iturbe B, Nakagawa T *et al*. Subtle renal injury is likely a common mechanism for salt-sensitive essential hypertension. *Hypertension* 2005; 45: 326–330
20. Segal MS, Shah R, Afzal A *et al*. Nitric oxide cytoskeletal-induced alterations reverse the endothelial progenitor cell migratory defect associated with diabetes. *Diabetes* 2006; 55: 102–109

Received for publication: 7.5.07
Accepted in revised form: 22.5.07