Stressed-out Podocytes in Diabetes?

Robert F. Spurney and Thomas M. Coffman

Division of Nephrology, Department of Medicine, Duke University and Durham VA Medical Centers, Durham, North Carolina

Diabetic nephropathy is the most common cause of ESRD in the United States.[1] Although current strategies can slow disease progression,[2,3] development of renal failure requiring renal replacement therapy is a distressingly common outcome in patients with diabetes.[1] As a result, much effort has been devoted to understanding the mechanisms that promote glomerular damage in diabetic kidney disease with the hope of identifying new therapeutic targets and strategies. In this issue of *JASN*, Zheng *et al.*[4] report that the specific expression of the antioxidant protein metallothionein (MT) in podocytes reduces albuminuria, attenuates the loss of glomerular podocytes, and ameliorates glomerular injury in a mouse model of diabetes. These findings suggest diabetes enhances oxidative stress in podocytes, causing podocyte depletion and promoting the development of nephropathy.

In times past, the mesangial cell was a major focus of research into the molecular mechanisms of diabetic nephropathy. This emphasis was driven by clinical observations that accumulation of extracellular matrix in the mesangium is a characteristic pathologic finding in patients with diabetes. In addition, mesangial cells are relatively easy to isolate and study in culture; however, accumulating evidence suggests that glomerular podocytes also play a pivotal role in the pathogenesis of diabetic kidney disease.[5] In this regard, foot process widening and loss of glomerular nephrin is observed early in the course of both experimental and human diabetic kidney disease.[5,6] More recently, reduced podocyte number has been reported in humans with either type 1 or type 2 diabetic nephropathy.[7–9] Podocytes are terminally differentiated cells with little potential for proliferation[10,11] except in disease states such as HIV nephropathy[12] and crescentic glomerulonephritis,[13] in which a maladaptive proliferation may occur. Thus, podocytes that are lost from the glomerulus cannot be effectively replaced, and sufficient loss of these glo-

merular epithelial cells may lead to instability of the tuft and glomerulosclerosis.[11] Several lines of evidence suggest podocyte loss contributes to progressive deterioration in kidney function in patients with diabetic nephropathy.[5,7–9] For example, reduction of podocyte number is a strong predictor of progressive renal disease in Pima Indians with type 2 diabetes and microalbuminuria.[7] Similarly, albumin excretion rates are negatively correlated with podocyte number in patients with proteinuria and type 1 diabetes.[8] Finally, the density of glomerular podocytes is reduced to a greater extent in patients with diabetes and overt proteinuria compared with patients with microalbuminuria.[9]

Although the precise mechanisms causing loss of podocytes from the glomerulus in diabetic nephropathy are not known with certainty, both apoptosis and detachment likely play a role.[5] Indeed, excretion of urinary podocytes is detected in both humans and rodents with diabetic nephropathy,[14,15] and the extent of podocyturia correlates with disease activity.[15] In rodent models, viable podocytes can be recovered from urine and cultured *ex vivo*[15] indicating that podocytes may detach without undergoing apoptosis. Other studies suggested a role for podocyte apoptosis as a mechanism of podocyte loss in diabetic nephropathy.[16,17] For example, Böttinger and co-workers[16] found that podocyte apoptosis coincided with the onset of albuminuria and preceded detectable loss of podocytes in two different mouse models of diabetes. Moreover, increased ambient glucose concentrations stimulated the generation of reactive oxygen species (ROS) in cultured podocytes, inducing apoptosis.[16] Podocyte apoptosis has also been documented early in the course of kidney disease in patients with type 2 diabetes.[17]

A role for exaggerated ROS generation has also been demonstrated in a number of diabetic complications,[18] and several reports suggested decreasing oxidative stress can protect against kidney injury in diabetic nephropathy.[16,19,20] Long-term treatment with an NADPH oxidase inhibitor prevented podocyte apoptosis and ameliorated glomerular injury in rodent models of type 1 and type 2 diabetes.[16] Moreover, a ubiquitously expressed superoxide dismutase 1 transgene reduced kidney injury in streptozotocin-treated[19] and *db/db* mice.[20] Although these studies support a role for ROS in diabetic nephropathy, they do not identify the key cellular sites where ROS must be neutralized to ameliorate renal disease. This is the issue addressed by Zheng *et al.*,[4] who tested whether providing antioxidant protection to the podocyte alone would confer protection from diabetic nephropathy. To this end, the authors generated transgenic mouse lines overexpressing the antioxidant protein MT in glomerular podocytes under control of the mouse nephrin promoter. Two founder lines were established with different levels of MT expression, six- or 18-fold higher than normal. These

MT-overexpressing mice were crossed with the OVE26 TG line, a mouse model of severe, early-onset type 1 diabetes.[21] Overexpression of MT in podocytes did not affect the basic metabolic characteristics of diabetes in OVE26 TG mice. Albuminuria, however, was reduced in both transgenic lines, although the reduction in albumin excretion was most marked in the founder line with lowest level of MT expression. Likewise, enhanced expression of MT in podocytes reduced apoptosis, preserved podocyte numbers, and prevented podocyte effacement. This was associated with attenuated glomerular pathology and reductions of glomerular and mesangial volumes. The authors, therefore, concluded that reducing oxidative stress in podocytes by overexpression of MT is sufficient to ameliorate diabetic kidney disease, providing further support for a central role of the podocyte in this disorder.

Although the studies by Zheng et al. advance our understanding of the pathogenesis of diabetic nephropathy, several caveats should be considered. First, MT is a multifunctional protein.[22,23] In addition to its antioxidant properties, cysteine-rich MT binds heavy metals and may act as a reservoir for essential metals such as zinc.[22,23] As a result, MT may modulate the activity of various transcription factors by either donating or chelating zinc that may be required for their biologic activity.[22,23] Indeed, MT levels are altered in human cancer cell lines, and this may play a role in protecting against cellular apoptosis,[22,23] in part by chelating zinc and decreasing the activity of the zinc-dependent tumor suppressor protein p53.[23] The magnitude of ROS generation was not measured in the studies by Zheng et al.; therefore, the relative contribution of antioxidant actions versus other properties of MT in the observed renal protection cannot be specifically distinguished. In the future, it would be important to document directly enhanced oxidative stress in diabetic podocytes in vivo and to determine whether the extent of kidney protection can be correlated with abrogation of ROS generation in podocytes.

Although the strategy of specific overexpression of transgene-encoded proteins in podocytes has been widely used in the field, this approach can also have unexpected consequences, confounding interpretation of such experiments. For example, diabetes-associated albuminuria was attenuated to a greater extent in the transgenic line with lower levels of MT transgene expression (six-fold above normal) compared with the higher expressing line (18-fold above normal). Moreover, preliminary studies reported by the authors suggested that further increasing podocyte MT levels may cause spontaneous albuminuria. One wonders whether the failure of even the low-expressing MT transgene to protect against albuminuria over the long term may relate to nonspecific, seemingly detrimental consequences of transgene overexpression. Alternatively, the observed differences in the phenotypes of the two transgenic mouse lines might be a consequence of off-target effects related to differences in integration sites. Conditional knockout of endogenous antioxidant proteins specifically in podocytes would be an alternative approach to explore this issue further that

potentially avoids some of the pitfalls inherent to overexpression studies.

In summary, the work by Zheng et al.[4] provide suggestive evidence that, similar to other target tissues such as the vasculature,[18] oxidative stress within the podocyte contributes to the pathogenesis of kidney damage in diabetes. A number of interesting questions remain. What is the extent of ROS generation in podocytes during diabetes? Are there biomarkers that can be used to monitor oxidative stress of the glomerulus in patients? What is the mechanism driving ROS generation in podocytes in diabetes? Can this be modulated in vivo? Answering these questions and developing therapeutic strategies that assuage podocyte stress may be useful in the future for preventing disease progression in diabetic nephropathy.

DISCLOSURES

None.

REFERENCES

1. USRDS: The United States Renal Data System. Am J Kidney Dis 42[Suppl 1]: 1–230, 2003
2. Lewis EJ, Hunsicker LG, Clarke WR, Bain RP, Rohde RD: The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. N Engl J Med 329: 1456–1462, 1993
3. Brenner BM, Cooper ME, Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med 345: 861–869, 2001
4. Zheng S, Carlson EC, Yang L, Kralik PM, Huang Y, Epstein PN: Podocyte-specific overexpression of the antioxidant metallothionein reduces diabetic nephropathy. J Am Soc Nephrol 19: 2077–2085, 2008
5. Wolf G, Chen S, Ziyadeh FN: From the periphery of the glomerular capillary wall toward the center of disease: Podocyte injury comes of age in diabetic nephropathy. Diabetes 54: 1626–1634, 2005
6. Jefferson JA, Shankland SU, Pichler RH: Proteinuria in diabetic kidney disease: A mechanistic viewpoint. Kidney Int 74: 22–36, 2008
7. Meyer TW, Bennett PH, Nelson RG: Podocyte number predicts long-term urinary albumin excretion in Pima Indians with type II diabetes and microalbuminuria. Diabetologia 42: 1341–1344, 1999
8. White KE, Bilous R, Marshall SM, Nahas ME, Remuzzi G, Pieras G, De Cosmo S, Viberti G: Podocyte number in normotensive type 1 diabetic patients with albuminuria. Diabetes 51: 3083–3089, 2002
9. Dalla Vestra M, Masiero A, Roiter AM, Saller A, Crepaldi G, Fioretto P: Is podocyte injury relevant in diabetic nephropathy? Studies in patients with type 2 diabetes. Diabetes 52: 1031–1035, 2003
10. Kritz W, Gretz N, Lemley KV: Progression of glomerular diseases: Is the podocyte the culprit? Kidney Int 54: 687–697, 1998
11. Wiggins RC: The spectrum of podocytopathies: A unifying view of glomerular diseases. Kidney Int 71: 1205–1214, 2007
12. He JC, Husain M, Sunamoto M, D'Agati VD, Klotman ME, Iyengar R, Klotman PE: Nef stimulates proliferation of glomerular podocytes through activation of Src-dependent Stat3 and MAPK1,2 pathways. J Clin Invest 114: 643–651, 2004
13. Ding M, Cui S, Li C, Jothy S, Haase V, Steer BM, Marsden PA, Pippin J, Shankland S, Rastaldi MP, Cohen CD, Kretzler M, Quaggin SE: Loss of the tumor suppressor Vhlh leads to upregulation of Cxcr4 and rapidly progressive glomerulonephritis in mice. Nat Med 12: 1081–1087, 2006

14. Nakamura T, Ushiyama C, Suzuki S, Hara M, Shimada N, Ebihara I, Koide H: Urinary excretion of podocytes in patients with diabetic nephropathy. *Nephrol Dial Transplant* 15: 1379–1383, 2000

15. Petermann AT, Pippin J, Krofft R, Bionski M, Griffin S, Durvasula R, Shankland SJ: Viable podocytes detach in experimental diabetic nephropathy: Potential mechanisms underlying glomerulosclerosis. *Nephron Exp Nephrol* 98: e114–e123, 2004

16. Susztak K, Raff AC, Schiffer M, Böttinger EP: Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. *Diabetes* 55: 225–233, 2006

17. Verzola D, Bandolfo MT, Ferrario F, Rastaldi MP, Villaggio B, Gianiorio F, Giannoni M, Rimoldi L, Lauria F, Miji M, Deferrari G, Garibotto G: Apoptosis in the kidneys of patients with type II diabetic nephropathy. *Kidney Int* 72: 1262–1272, 2007

18. Kuroki T, Isshiki K, King GL: Oxidative stress: The lead or supporting actor in the pathogenesis of diabetic complications. *J Am Soc Nephrol* 14[Suppl 3]: S216–S220, 2003

19. Craven P, Melhem M, Phillips SL, DeRubertis FR: Overexpression of Cu2+/Zn2+ superoxide dismutase protects against early diabetic glomerular injury in transgenic mice. *Diabetes* 50: 2114–2125, 2001

20. DeRubertis FR, Craven P, Melhem, Salah EM: Attenuation of renal injury in db/db mice overexpression superoxide dismutase: Evidence for reduced superoxide-nitric oxide interaction. *Diabetes* 53: 762–768, 2004

21. Zheng S, Noonan WT, Metreveli NS, Coventry S, Kralik PM, Carlson EC, Epstein PN: Development of late-stage diabetic nephropathy in OVE26 diabetic mice. *Diabetes* 53: 3248–3257, 2004

22. Cherian MG, Jayasurya A, Bay BH: Metallothioneins in human tumors and potential roles in carcinogenesis. *Mutation Res* 533: 201–209, 2003

23. Theocharis SE, Margeli AP, Klijanienko JT, Kouraklis GP: Metallothionen expression in human neoplasia. *Histopathology* 45: 103–118, 2004

See related article, "Podocyte-Specific Overexpression of the Antioxidant Metallothionein Reduces Diabetic Nephropathy," on pages 2077–2085.

Statin Use Prolongs Patient Survival after Renal Transplantation

Eberhard Ritz* and Christoph Wanner†

*Department of Internal Medicine, Division of Nephrology, Ruperto Carola University Heidelberg, Heidelberg, and †Department of Internal Medicine, Division of Nephrology, Julius-Maximilians University Würzburg, Würzburg, Germany

Kidney transplantation reduces mortality and cardiovascular deaths, more so than dialysis, although survival for both re-

Correspondence: Prof. Eberhard Ritz, Nierenzentrum, Im Neuenheimer Feld 162, 69120 Heidelberg, Germany. Phone: 00-49-6221-601-705; Fax: 00-49-6221-603-302; E-mail: prof.e.ritz@t-online.de

mains worse than in nonrenal disease populations. This may be for reasons of preexisting cardiovascular disease acquired during renal progression or dialysis; however, recent population data suggest even minor kidney dysfunction (which is almost universal in graft recipients) is associated with increased cardiovascular risk.[1]

The pathophysiology underlying increased cardiovascular risk is certainly complex, and it would be naive to assume that dyslipidemia is the only causal factor. Nevertheless, observational data suggest, even after renal transplantation, that cholesterol is a predictor of cardiovascular events.[2] This obviously raises the issue of whether statins should be administered to renal graft recipients with proven target organ damage for secondary prevention or even for primary prevention. Available evidence for statin treatment in renal patients is only relatively good for the early stages of chronic kidney disease because it is based only on *post hoc* analyses of subcohorts of patients who happened to have diminished estimated GFR and were included in the large statin trials.[3]

With respect to hemodialysis or transplant patients, matters are less clear. There is no large controlled prospective trial in a general hemodialysis population, and in the 4D study of patients who had type 2 diabetes and were on dialysis,[4] no significant effect of atorvastatin was seen on the composite cardiovascular end point. *Post hoc* analyses showed that adjudicated coronary death was reduced to the same extent as in nonrenal patients in the major statin trials (by 19% per 1-mmol lower LDL cholesterol); other cardiac causes—specifically sudden death—were much less affected, so the composite end point was not significantly reduced.

With respect to transplant patients, the only randomized, prospective, controlled trial, the Assessment of LEscol in Renal Transplantation (ALERT) study,[5] showed no significant difference in the primary composite end points (major adverse cardiac events defined as cardiac death, nonfatal myocardial infarction, and coronary intervention, despite 32% lowering of LDL cholesterol during a mean follow-up of 5.1 yr). Coronary intervention is usually regarded as a "soft" end point; it is of note, therefore, that fewer cardiac deaths or cases of nonfatal myocardial infarction from "harder" end points were observed (70 *versus* 104, a risk reduction of 35%; *P* = 0.005). The ALERT trial also found no significant effect of statin treatment on graft loss[6] or graft function,[7] in line with findings that fluvastatin fails to prevent renal transplant vasculopathy,[8] and in contrast to the positive finding of a past retrospective single-center study of protection by statins against acute graft rejection in sirolimus-treated patients[9] and despite animal experiments suggesting benefit on chronic allograft nephropathy.[10] Although a relation between lipid concentrations and loss of kidney function was observed in patients with primary kidney disease,[11] in the setting of kidney transplantation, lipid concentrations do not seem to play a major role in the genesis of renal function loss.