Stressed-out Podocytes in Diabetes?

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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 proliferation10,11 except in disease states such as HIV nephropathy12 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 glomerular 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 vivo15 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-workers16 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-treated19 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.\textsuperscript{2,1} 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 \textit{et al.} advance our understanding of the pathogenesis of diabetic nephropathy, several caveats should be considered. First, MT is a multifunctional protein.\textsuperscript{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.\textsuperscript{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.\textsuperscript{22,23} Indeed, MT levels are altered in human cancer cell lines, and this may play a role in protecting against cellular apoptosis,\textsuperscript{22,23} in part by chelating zinc and decreasing the activity of the zinc-dependent tumor suppressor protein p53.\textsuperscript{23} The magnitude of ROS generation was not measured in the studies by Zheng \textit{et al.}; therefore, the relative contribution of antioxidant actions \textit{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 \textit{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 \textit{et al.}\textsuperscript{4} provide suggestive evidence that, similar to other target tissues such as the vasculature,\textsuperscript{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 \textit{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**

Kidney transplantation reduces mortality and cardiovascular deaths, more so than dialysis, although survival for both remains 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 LEscaloR 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 loss6 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 patients9 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.