Cathepsin S–Dependent Protease–Activated Receptor-2 Activation: A New Mechanism of Endothelial Dysfunction

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Endothelial dysfunction plays an important role in the development of diabetic nephropathy.1 Although the loss of endothelial nitric oxide synthase is well recognized as a cause of hypertension and endothelial dysfunction, other mechanisms are likely to contribute to endothelial damage. One such possible mechanism is endothelial damage caused by infiltrating macrophages. Although macrophages play a role in the induction and progression of human and experimental diabetic nephropathy;2–4 there is no evidence that macrophage-derived factors are a cause of endothelial damage. A study by Kumar et al.5 in this issue of the Journal of the American Society of Nephrology has established a new pathologic mechanism in diabetic nephropathy in which macrophage–derived cathepsin S activates protease–activated receptor-2 (PAR-2) on endothelial cells to cause substantial endothelial damage, giving rise to vascular albumin leakage, inflammation, and glomerulosclerosis.

Cathepsins are a family of cysteine proteases. One member of this family, cathepsin S, has been implicated in the pathogenesis of a range of disease states.6 Cathepsin S contributes to protein degradation in the endosomal/lysosomal pathway, including proteolytic degradation of the lig chaperone protein, which allows peptide antigens to bind to MHC class 2 molecules and be presented to T lymphocytes. In addition to its well defined role in the adaptive immune response, cathepsin S can also be secreted. Cathepsin S enzymatic activity operates across a relatively wide range of pH values, which enables activity in acidic endosomes as well as the more neutral conditions of the extracellular space, in which it can cleave substrates, such as elastin, E-cadherin, secretory leukoprotease inhibitor, junctional adhesion molecule-B, and PAR-2.6 One distinct feature of cathepsin S is that its expression is largely restricted to leukocyte subsets, particularly macrophages, although cathepsin S expression can be induced in a variety of nonleukocyte cell types. Experimental studies using cathepsin S gene–deficient mice or cathepsin S inhibitors have defined a role for this enzyme in autoimmune diseases, atherosclerosis, airway hyper-responsiveness, cancer metastasis, neovascularization, and neuropathic pain.6 In addition, elevated serum levels of cathepsin S have been described in a number of diseases and correlate with insulin resistance, diabetes, atherosclerosis, and heart disease.7–8

The study by Kumar et al.5 proposes that macrophage–derived cathepsin S induces endothelial cell damage by activation of PAR-2 on the endothelial cell surface. This is on the basis of several key findings. Injection of recombinant cathepsin S into normal mice induced marked endothelial damage, resulting in vascular leakage and albuminuria; however, Par-2 gene–deficient mice were protected from these injurious effects, and studies of cultured endothelial cells showed that cathepsin S acts through PAR-2 and not through other members of the PAR family. A key finding was that treatment with a selective cathepsin S inhibitor, RO5461111, in established type 2 diabetic nephropathy in uninephrectomized db/db mice reduced endothelial damage, albuminuria, and glomerulosclerosis as well as albumin leakage in the retina.5 These beneficial effects were associated with protection from endothelial cell damage and loss and protection from podocyte damage and loss as well as a reduction in macrophage infiltration and markers of inflammation without any apparent effect on body weight or blood glucose levels. Importantly, administration of a PAR-2 inhibitor, GB83, gave parallel findings with reduced endothelial cell loss and glomerulosclerosis, providing the first direct evidence that PAR-2 plays a pathogenic role in diabetic nephropathy. Interestingly, combined drug treatment showed no added benefit, implying that cathepsin S and PAR-2 activations operate through the same mechanism to cause diabetic kidney injury. Finally, CD68+ macrophages were identified as the main source of cathepsin S synthesis in both human and experimental diabetic nephropathy.5

Studies that make a substantial contribution to a field inevitably raise additional questions. For example, does cathepsin S promote diabetic renal injury through actions on other cell types? This is a difficult issue to address, because studies of PAR-2 expression in the kidney have been plagued by problems of a lack of specificity of antibodies using for immunohistochemistry. Tubular epithelial cells, mesangial cells, and podocytes all express PAR-2 in culture, and activation of PAR-2 in these cell types induces a variety of proinflammatory and profibrotic responses, including CCL2 and TGF-β1 production.9 It is also known that the kidney expresses relatively high levels of PAR-2 mRNA, suggesting that PAR-2 may be widely expressed in the kidney; however, defining which renal cell types do, in fact, express PAR-2 in situ remains unresolved. It may require conditional Par-2 gene deletion in endothelial cells to formally address this question.

Another question raised by the study by Kumar et al.5 relates to the source of cathepsin S that causes PAR-2 activation

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and consequent endothelial cell damage in the diabetic kidney. Although macrophages were identified as the main source of local cathepsin S production within the diabetic kidney, serum cathepsin S levels are increased in many diseases, including diabetes. Thus, damage to the endothelium could be mediated through circulating cathepsin S, the source of which is unclear. Although serum levels of cathepsin S can be elevated in a range of diseases, there is lack of data on the actual level of cathepsin S enzymatic activity in these conditions. This is a particularly important issue in CKD, in which there are increased blood levels of the cathepsin inhibitor, cystatin C. These data provide impetus for measuring circulating levels of cathepsin S activity as both a biomarker and a pathogenic mechanism in progressive kidney disease.

Is this mechanism of cathepsin S/PAR-2–induced endothelial damage specific to diabetic renal injury, or is it of general importance in progressive kidney disease? We lack data on cathepsin S expression in other forms of kidney disease. The only well characterized inducer of cathepsin S synthesis in macrophages is IFN-γ, suggesting production by M1–type polarized proinflammatory macrophages. This is consistent with the presence of macrophages expressing the archetypal M1–type marker, inducible nitric oxide synthase, within the diabetic kidney. Indeed, M1–type proinflammatory macrophages are a common feature in biopsies from patients with kidney disease, especially rapidly progressive forms of disease, suggesting that macrophage–derived cathepsin S may be a common mechanism of renal injury.

One implication of the study by Kumar et al. is that we have two new therapeutic targets for diabetic nephropathy: cathepsin S and PAR-2. However, which should we target given the lack of added benefit with combined blockade over that of individual blockade? There are several pharmaceutical companies that have cathepsin S inhibitors in clinical trials in areas such as neuropathic pain, rheumatoid arthritis, psoriasis, and osteoporosis. In addition, PAR-2 inhibitors are in preclinical studies. In the case of diabetic nephropathy, cathepsin S may be the more attractive target, because cathepsin S has also been implicated in related comorbidities, such as insulin resistance, atherosclerosis, and aortic stiffening. However, it may also be the case that combined cathepsin S and PAR-2 inhibition will provide added benefit in other types of kidney diseases. For example, experimental models of rapidly progressive GN have identified a role for cathepsin S in the adaptive immune response leading to lupus nephritis, whereas PAR-2 promotes glomerular thrombosis in angiotensin II–treated basal membrane disease. In addition, PAR-2–gene–deficient mice exhibit reduced interstitial fibrosis in the unilateral ureteric obstruction model.

In conclusion, this exciting study has identified a specific approach to targeting endothelial dysfunction in diabetic nephropathy. These findings may also be highly relevant in nondiabetic kidney disease.

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DISCLOSURES

None.

REFERENCES

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The Ever–Expanding Kidney Repair Shop

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When entering the kidney repair shop, a sign on the first floor directs you to the replacement parts warehouse. There, you can find unlimited and ready to use cell supplies: podocytes, parietal epithelia, proximal and distal tubules, and collecting duct cells. More complex renal structures are on the second floor. There, the most recent and exciting display was constructed by Sharmin et al.,1 showing glomeruli that were generated from induced human pluripotent stem cells (iPSCs).

The potential of human pluripotent stem cells was shown previously.2 When human embryonic stem cells were injected into immunodeficient mice, they generated teratoma. Inspection of the teratoma disclosed areas of embryonic glomeruli and renal tubules adjacent to nonrenal tissues.2 It took some time for this uncontrolled in vivo potential to be translated into highly directed and controlled in vitro differentiation of pluripotent stem cells toward renal lineages.3,4 The understanding of how the mammalian kidney develops in vivo laid the foundation for deciphering the inductive signals required for the in vitro directed renal differentiation protocols from pluripotent stem cells. For instance, Six2+Cited1+ nephron progenitors residing in the nephrogenic cortex were shown to give rise to all types of nephron epithelia.5 Therefore, derivation of nephron epithelial progenitors from pluripotent stem cells represents an attractive intermediate step in the differentiation protocol from which renal tissue and nephron-like structures can be further induced in culture or mature after in vivo grafting into mice. Nishinakamura and colleagues6 further translated knowledge of early kidney development into an efficient differentiation protocol; they were able to separate anterior from posterior intermediate mesoderm in the early stages of renal differentiation.7 Although a transcription factor T (also known as Brachyury) is mainly expressed in the immature mesoderm of the primitive streak at E7.5, Nishinakamura and colleagues7 found that the precursors of nephron progenitors remained as T+ immature cells at the posterior end of E8.5 embryos. These cells convert to Osrl+ posterior intermediate mesoderm at E9.5, whereas the ureteric bud precursors convert from T+ to Osrl+ anterior intermediate mesoderm 1 day earlier (E8.5). On the basis of these findings, Nishinakamura and colleagues7 were able to establish a protocol to induce nephron progenitors from T+ cells of E8.5 embryos and subsequently, succeeded in inducing nephron progenitors from mouse embryonic stem cells and human induced pluripotent stem cells (iPSCs). The induced nephron progenitor aggregates readily formed three-dimensional primordial glomeruli and renal tubules on Wnt stimulation in vitro, similar to those spontaneously observed in teratomas.2 Importantly, like other recent in vitro renal differentiation protocols,6 Nishinakamura and colleagues7 use iPSCs generated according to the Yamanaka method as the starting material, potentially affording patient–derived autologous cells rather than allogeneic cells for transplant and the possibility of personalized renal medicine.

In this study, Sharmin et al.1 used their elegant differentiation protocol to perform in-depth inspection of the human iPSC–derived three–dimensional primordial glomeruli that they had generated.3 Sharmin et al.1 did so by inserting the GFP gene into the nephrin (NPHS1) locus of human iPSCs and visualizing the GFP–tagged podocyte formation in vitro. Sharmin et al.1 went on to show that the podocytes in vitro have the typical transcriptional and morphologic features of those in vivo. On grafting of the iPSC–derived human glomeruli under the renal capsule of immunodeficient mice, Sharmin et al.1 showed that these induced glomeruli could be vascularized with the mouse vasculature, forming chimeric glomeruli with human epithelia and mouse endothelium. Even more remarkable, the iPSC–derived podocytes adjacent to the host–derived vasculature formed complex cellular processes between which slit diaphragm–like structures could be observed.1 Thus, iPSC–derived avascularized fetal glomeruli grown in vitro became fully vascularized after in vivo transplantation in mice, showing for the first time a connection of host vasculature to iPSC–derived kidney tissues and pushing the field

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