therapy). In this study, the actual incidence of post-transplant lymphoproliferative disease was similar in patients treated with IL-2 receptor antibodies versus patients who received no induction therapy but was significantly lower in these groups than in patients who received depleting antibodies as induction treatment. Whether mycophenolate mofetil (MMF) may reduce the risk of cancer is currently unclear. Although the analysis by Wimmer et al. suggests that MMF may even enhance the risk of cancer, when combined with calcineurin inhibitors, this conclusion could be biased by the retrospective design of their study and the preferential assignment of MMF to older recipients. Robson et al. prospectively examined the risk of lymphoma and other malignancies in 6751 de novo renal-transplant recipients treated with MMF and observed a similar risk at 3 years in comparison with an equal number of matched controls receiving non-MMF-based immunosuppression.

Obviously, malignancy is still a challenging complication of long-term immunosuppression. Immunosuppressive drugs, such as mTOR inhibitors and IL-2 receptor antibodies, probably reduce the risk for all or some specific malignancies and are currently tested in clinical trials designed to reduce the incidence of some specific cancers in kidney transplant recipients. Achieving tolerance, the Holy Grail for transplant physicians, would undoubtedly obviate the need for lifelong immunosuppression and its associated complications. Pilot clinical trials are currently running to explore the biology of tolerance in humans and will hopefully shed more light on the feasibility of this goal in the future.

**REFERENCES**


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**Diabetic nephropathy and proximal tubule ROS: Challenging our glomerulocentricity**

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Diabetic nephropathy is currently viewed as a predominantly glomerular process with glomerular injury driving secondary tubular loss. Brezniceanu and colleagues apply transgenic methods to support a prominent role for reactive oxygen species as mediators and for the proximal tubule as a major site of early disease activity in diabetes. Results support evidence for early tubular apoptosis and atrophy in human diabetic nephropathy.


Diabetic nephropathy has long been considered virtually synonymous with glomerulosclerosis, the latter viewed as the cardinal manifestation and primary lesion of this functionally devastating disease. Recent gentle challenges to our collective dogmatism on this topic have emerged, but the paper by Brezniceanu and colleagues (this issue) takes a giant step toward shifting the glomerulo-tubular balance in the field. These authors have made elegant use of transgenic technology to achieve selective overexpression of catalase in the renal proximal tubular epithelial cell (RPTC). Catalase, an enzyme that breaks down H$_2$O$_2$ to inactive components, is used as a tool to reduce generation of reactive oxygen species (ROS) and thereby probe their function. Using the RPTC-specific, androgen-regulated KAP promoter to drive transgenic catalase expression, Brezniceanu and colleagues found that adult males exhibited spontaneous overexpression of catalase protein and activity in RPTCs, without the addition of exogenous androgen. The investigators accordingly used the wild-type and transgenic adult male mice to examine the role of ROS in early streptozotocin (STZ)-induced diabetic nephropathy, specifically asking whether limiting ROS generation attenuates selected diabetes-induced abnormalities in the proximal tubule: increased angiotensin (Agt), plasminogen activator inhibitor-1, apoptosis, and histologic injury following 2 weeks of diabetes.
The emphasis by Brezniceanu and colleagues on the proximal tubule rather than the glomerulus is the first of several surprises. A handful of recent studies have in fact documented prominent tubular atrophy in both human and experimental diabetes. In eight type 1 diabetics with stage I–II chronic kidney disease, Najafi an et al. found by stereologic methods that atubular glomeruli and glomeruli attached to atrophic tubules made up 68% of all glomeruli, while fractional volumes of atrophic tubules were increased and those of normal tubules were reduced. Also in human diabetic kidneys, Kumar et al. demonstrated increased apoptosis localized to tubular and interstitial cells but, interestingly, not present in the glomerulus; a similar pattern was found in STZ-induced diabetic nephropathy in rats. Collectively, these reports point to a previously unrecognized atrophic process in the diabetic tubule and suggest that specifically tubular apoptosis may contribute.

The choice by Brezniceanu and colleagues to monitor expression of Agt in the proximal tubule reflects the recent recognition of an independently regulated intrarenal renin–angiotensin system (RAS) localized to the RPTC. The classic circulating RAS is based on renin secretion by the juxtaglomerular cells of the afferent arteriole; circulating renal renin in turn requires circulating Agt from liver and angiotensin-convert ing enzyme at the endothelial surface to effect angiotensin II (Ang II) production. The intrarenal RAS, on the other hand, appears to be self-contained, as all components required for Ang II generation can be synthesized within the RPTC. In contrast to the negative feedback exerted by Ang II on juxtaglomerular-cell renin secretion, the intrarenal RAS operates via a positive-feedback response to Ang II. In addition, Ang II in the tubular lumen is also actively taken up into RPTCs, in part by angiotensin receptor type 1 binding/internalization. Once inside, Ang II stimulates increased RPTC expression of both Agt and renin, and thus a feed-forward effect promoting more Ang II formation. By these processes, chronic exogenous Ang II infusion leads to increased Ang II in kidney cortex and specifically in the renal interstitial fluid. Renal interstitial Ang II levels have been found to be many-fold higher than plasma levels and to be regulated independently of plasma Ang II. Recent studies also show that transgenic overexpression of Agt confined to the RPTC is alone sufficient to induce systemic hypertension, lending support in principle to the concept that intrarenal RAS activation dominates extrarenal regulators of blood pressure. Of particular relevance to the article by Brezniceanu and colleagues, these Agt overexpression studies demonstrate that Agt excess alone appears capable of initiating intrarenal RAS activation.

The intrarenal RAS has generated intense interest in the setting of diabetes, where it was first appreciated that an activated intrarenal RAS can coexist with a suppressed circulating RAS. In experimental diabetes, investigators have subsequently confirmed an increase in intrarenal Ang II levels, together with increased renin and increased Agt in the early stages of diabetic nephropathy. There is, additionally, solid evidence that both high glucose and elevated Ang II considered the two most potent pathophysiologic agonists in diabetic nephropathy — induce increased ROS generation in proximal tubular and mesangial cells in vitro, and that ROS excess may in turn mediate tissue injury and apoptosis. However, we have not had evidence that can assign priority or establish a molecular sequence for how these signaling elements interact in vivo.

Brezniceanu and colleagues designed the transgenic model noted above to test the importance of ROS in mediating early diabetic nephropathy. They first clearly demonstrate that catalase overexpression is confined to the proximal tubule and is functional in situ: isolated renal proximal tubules from male transgenic mice exhibited virtually no increase in ROS and no increase in Agt mRNA or protein expression in response to either Ang II or high glucose, in contrast to the vigorous responses in wild-type renal proximal tubules. Turning next to the STZ-diabetic state, Brezniceanu and colleagues show similar outcomes in isolated renal proximal tubules from STZ-diabetic transgenic versus diabetic wild-type mice: catalase overexpression prevented the diabetes-induced increases in Agt expression, in the proinflammatory ROS-dependent plasminogen activator inhibitor-1, in apoptosis—inducing enzyme caspase-3 and p53, and in tubular-cell apoptosis. Finally, by standard renal histologic and immunohistochemical approaches, the authors confirmed in vivo by TdT-mediated dUTP nick end labeling that catalase overexpression prevented increases in RPTC Agt protein, apoptotic enzymes, and apoptosis in the STZ-diabetic transgenic kidneys. The molecular targets were chosen for their known sensitivity to ROS, underscoring the conclusion that constrained ROS generation in fact mediated the outcomes. Thus, we can reasonably predict that the processes represented by these molecules — specifically, intrarenal RAS activation, inflammation, and apoptosis — are downstream consequences of increased oxidative stress in early diabetic nephropathy.

The effects of transgenic catalase overexpression in diabetic RPTCs are equally interesting for what was not blocked. Proteinuria in diabetic transgenic mice persisted at levels comparable to those in diabetic wild-type mice. This is perhaps a reflection of the preserved hyperglycemia with its expected effects at glomerular sites. It is nonetheless a reminder that excess protein continued to bombard the catalase-overexpressing RPTCs in the diabetic transgenic mice. Hyperglycemia enhances tubular uptake of albumin and induces RPTC surface expression of CD36, mediating increased uptake of advanced glycosylation endproducts—modified albumin. It is interesting to speculate that the favorable results of limiting ROS in RPTCs might in part reflect prevention of the inflammatory and proapoptotic effects of excess RPTC albumin uptake. If true, this would predict an additional contribution of proteinuria-related injury in the early phase of diabetic nephropathy and raises the question of ROS—dependence of protein—induced tubulointerstitial injury. Figure 1 proposes one way of integrating the findings of Brezniceanu and colleagues and others in the field in a molecular scheme; major weight is given to intrarenal RAS activation and to the feed-forward, autoamplification features of this system in the RPTC.

commentary

The eff ects of transgenic catalase overexpression in diab
Another intriguing observation by Brezniceanu and colleagues\(^1\) is the apparent failure of catalase overexpression to block the RPTC hypertrophy in diabetic transgenic mice over the 2 weeks of STZ-diabetes. This also comes as a surprise. Ang II has been reported to induce RPTC hypertrophy via ROS-dependent pathways.\(^2\) Additionally, in prior work by Brezniceanu and colleagues in RPTC cell culture, hyperglycemia-induced hypertrophy was prevented by Ang II receptor blockade,\(^2\) suggesting that diabetic hypertrophy is Ang II dependent. On the basis of these observations, catalase overexpression would be expected to reduce diabetes-associated RPTC hypertrophy. The unexpected persistence of RPTC hypertrophy perhaps reflects the greater complexity of the \textit{in vivo} state, which cannot be fully modeled in the cell-culture setting. One is left to conclude that mechanisms of hyperglycemia-induced hypertrophy remain far from clear. Yet diabetic tubular hypertrophy may well have exceptionally important consequences. Recent studies by Thomson and colleagues have led to an equally tubulocentric proposal: that diabetic tubular hypertrophy is the primary event in diabetic nephropathy and in fact causes glomerular hyperfiltration.\(^2\) These investigators propose that proximal hypertrophy, via increased proximal sodium reabsorption and reduced sodium delivery to the macula densa, triggers a tubuloglomerular feed-back response that initiates and sustains diabetic hyperfiltration.\(^2\) The findings of Brezniceanu and colleagues\(^1\) appear to exclude a role for ROS in mediating diabetic RPTC hypertrophy. Studies by Thomson and colleagues provide evidence that ornithine decarboxylase is essential to hyperglycemia-induced RPTC hypertrophy.\(^2\) Taken together, these findings suggest it is likely that neither Ang II nor ROS are etiologic, whereas ornithine decarboxylase remains in the running as a mediator of diabetic tubular hypertrophy. It will be important to learn in the present model whether persistence of diabetic hypertrophy over a longer period leads to adverse consequences despite the catalase-induced protection from excess tubular ROS generation.

There are, as always, caveats to ponder. First, the diagnosis of hypertrophy is based primarily on kidney weight–body weight ratio; Brezniceanu and colleagues\(^1\) did not quantitate RPTC hypertrophy at a cellular level by either cell size or protein–DNA ratio. It thus remains possible that the apparent renal enlargement may reflect edema in diabetic transgenic mice, a histopathologic feature in fact noted by the investigators. If so, resolution of hypertrophy might be masked. Second, endogenous androgens are important, not only postpubertally, but also during fetal development (for example, in sex differentiation); catalase overexpression in fetal male RPTCs during development could induce programming events that permanently change proximal tubular function and confound results in unexpected ways. Thus, as usual, differing approaches to independently confirm these findings will be important. Because these observations cover a very early phase of disease, examination of ROS roles at later stages will also be crucial in developing rational therapeutic interventions.

Nonetheless, these remarkable findings provide compelling support for the \textit{in vivo} participation of ROS in the early phase of diabetic nephropathy and for the proximal tubule as a key site of early ROS-induced disease activity. That tubular processes leading to apoptosis and atrophy in the proximal tubule may drive loss of renal function independently of glomerular disease activity would be,
if confirmed, a paradigm shift in our currently glomerulocentric view of diabetic nephropathy.

REFERENCES