Tubulointerstitial fibrosis can sensitize the kidney to subsequent glomerular injury

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Chronic glomerular injury is associated with eventual development of tubulointerstitial fibrosis. Here we aimed to assess whether, and how, mild chronic tubulointerstitial injury affects glomeruli. For this, we generated mice expressing different toxin receptors, one on their proximal tubular epithelial cells (diphtheria toxin receptor [DTR]) and the other only on podocytes (human CD25 [IL-2R] driven by the nephrin promoter [Nep25]), allowing serial induction of tubule-specific and glomerular (podocyte)-specific injury, respectively. Six weeks after diphtheria toxin injection, mild interstitial fibrosis was found in Nep25$^+$/DTR$^+$, but not in Nep25$^+$/DTR$^-$ mice. However, atubular glomeruli and neuronal nitric oxide synthase, a mediator of tubuloglomerular feedback, were higher in Nep25$^+$/DTR$^+$ than in DTR$^-$ mice and these atubular glomeruli had less podocyte density as assessed by WT-1 biomarker expression. Peritubular capillary density, hypoxia-inducible factor-1 and -2, and cyclooxygenase 2 expression were similar at week six in the two groups. At week seven, all mice were given the immunotoxin LMB-2, which binds to CD25 to induce podocyte injury. Ten days later, proteinuria, podocyte injury, and glomerulosclerosis were more severe in Nep25$^+$/DTR$^+$ than Nep25$^+$/DTR$^-$ mice with more severe sclerosis in the tubule-connected glomeruli. This supports the concept that even mild preexisting tubulointerstitial injury sensitizes glomeruli to subsequent podocyte-specific injury. Thus, increased atubular glomeruli and abnormal tubuloglomerular feedback significantly contribute to the crosstalk between the tubulointerstitium and glomeruli.

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Chronic glomerular injury is associated with eventual development of tubulointerstitial fibrosis, resulting in progressive loss of entire nephrons and chronic kidney disease. This glomerulotubular spread of injury has been observed in animal models, and several potential mechanisms have been proposed.[1] Although chronic progressive tubulointerstitial disease plays a critical role in the outcome of patients with primary glomerular lesions, the basic mechanisms that generate the tubulointerstitial damage remain unclear.[2] Proposed mechanisms can be broadly classified as "tubular" and "glomerular" hypotheses.[3] The tubular hypothesis states that excessive tubular protein load due to glomerular protein leakage induces inflammation and fibrosis, which are harmful to tubular epithelial cells. The glomerular hypothesis postulates that obstruction of the glomerulotubular junction by local fibrosis causes tubules to be disconnected from glomeruli, resulting in tubular degeneration.[4] Recently, altered hypoxia inducible factors (HIFs) 1 and 2 have been suggested as another possible mechanism by which glomerular injury affects the tubules.[5] These studies all have focused on the glomerular lesion as an initial injury, and assessed subsequent effects on the tubulointerstitium. In these schemas, tubulointerstitial lesions are secondary and do not have an active role in the progression of glomerulosclerosis in chronic kidney disease.

Tubulointerstitial injury could also play an active, primary role in progressive nephron loss. Previously, patients who survived and had short-term return of normal renal function after acute kidney injury (AKI) were considered to have experienced complete recovery. However, more recent epidemiologic studies show that AKI is a major risk factor for long-term chronic kidney disease (CKD).[6] Previous experimental studies using Six2-Cre-LoxP technology selectively activated diphtheria toxin receptor (DTR) expression in renal epithelia derived from the metanephric mesenchyme.[7] These studies showed that mild tubular injury induced adaptive repair processes and few long-term consequences, while severe injury induced maladaptive repair, severe interstitial fibrosis, and glomerulosclerosis. This study suggests the transition of AKI to CKD due to peritubular capillary rarefaction and other mechanisms.[8] In a diabetic nephropathy

experimental model, tubulointerstitial injury was not merely a result of glomerular injury, but was also a primary target of diabetic injury and even appeared to affect the progression of glomerular lesions.[9] These findings raise the question of whether mild tubulointerstitial injury could sensitize to subsequent glomerular injury.

Previous studies have either used models with tubular injury, such as ischemia-reperfusion, with long-term follow-up, or the injury model targeted both tubules and glomeruli, such as diabetic nephropathy. In contrast, we have now developed an animal model with distinct sequential tubular and glomerular injuries to specifically investigate novel mechanisms whereby tubular injury sensitizes to subsequent glomerular injury. We achieved proximal tubular cell-specific injury using diphtheria toxin (DT)-mediated conditional cell ablation in transgenic mice, where the DT receptor is expressed on proximal tubules. After DT injection, mice have increased blood urea nitrogen and creatinine, and initial polyuria with subsequent reversible oliguria. Injury is present in the proximal tubule without glomerular change, and injured tubular cells recover rapidly.[10,11] Glomerular-specific injury is possible in the so-called nephrin promoter 25 (Nep25) mouse model, through selective podocyte injury by injection of a modified toxin, LMB-2, in mice that express human CD25 only on podocytes, driven by the nephrin promoter. These mice show progressive nonselective proteinuria, with resulting edema, and focal segmental glomerulosclerosis due to podocyte injury.[12] We now have generated combined double-transgenic mice to investigate the effects of mild, functionally recovered tubulointerstitial injury on the development of subsequent glomerular injury, and mechanisms for this crosstalk.

RESULTS

Diphtheria toxin-induced acute tubular injury and chronic interstitial fibrosis

At baseline, urinary neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (Kim-1) excretion levels were similar in Nep25$^+$/DTR$^-$ and Nep25$^+$/DTR$^+$ mice. Two weeks after DT injection, urinary NGAL and Kim-1 were markedly increased in Nep25$^+$/DTR$^+$ mice (NGAL 810.7 $\pm$ 148.0 ng/mg, Kim-1 503.3 $\pm$ 128.8 ng/mg), but not in Nep25$^+$/DTR$^-$ mice (NGAL 46.1 $\pm$ 9.5 ng/mg, Kim-1 2.7 $\pm$ 0.6 ng/mg, $P < 0.05$ vs. Nep25$^+$/DTR$^+$). At week 6, urinary NGAL and Kim-1 returned toward normal in Nep25$^+$/DTR$^+$ mice (NGAL 233.4 $\pm$ 8.5 ng/mg, Kim-1 49.6 $\pm$ 5.0 ng/mg), but were still higher than Nep25$^+$/DTR$^-$ mice (NGAL 149.8 $\pm$ 13.7 ng/mg, Kim-1 2.5 $\pm$ 0.4 ng/mg, $P < 0.05$ vs. Nep25$^+$/DTR$^+$) (Figure 1a).

All mice underwent uninephrectomy at week 6 after DT injection. These kidneys showed focal tubular epithelial cell regeneration and mild tubulointerstitial fibrosis only in Nep25$^+$/DTR$^+$ mice (Figure 1b). Interstitial fibrosis, quantified by sirius red staining, was higher in Nep25$^+$/DTR$^+$ (0.235 $\pm$ 0.031% area stained) than Nep25$^+$/DTR$^-$ kidney (0.060 $\pm$ 0.009%, $P < 0.05$) (Figure 1c). Neither group showed glomerular abnormalities by light microscopy at this time point.

Thus, after recovering from acute tubular injury induced by DT injection, Nep25$^+$/DTR$^+$ mice developed tubulointerstitial fibrosis, while Nep25$^+$/DTR$^-$ had no tubular injury, as expected.

Preexisting tubulointerstitial injury amplified subsequent glomerular injury

Urinary albumin increased at week 2 due to acute tubular injury, and returned to baseline at week 6 in Nep25$^+$/DTR$^+$ mice. Total proteinuria was also similar in Nep25$^+$/DTR$^+$ and Nep25$^+$/DTR$^-$ mice at week 6 (33.85 $\pm$ 0.69 vs. 38.73 $\pm$ 2.37 mg/mg). LMB-2 injection at week 7 injured podocytes and resulted in maximal massive albuminuria in both Nep25$^+$/DTR$^+$ and Nep25$^+$/DTR$^-$ mice (10363.44 $\pm$ 1762.80 vs. 9495.00 $\pm$ 546.12 μg/mg) (Figure 2a). At 10 days after LMB-2 injection total proteinuria was more severe in Nep25$^+$/DTR$^+$ vs Nep25$^+$/DTR$^-$ mice (228.02 $\pm$ 19.09 vs. 161.21 $\pm$ 19.56 mg/mg, $P < 0.05$) (Figure 2a). Renal function, measured by blood urea nitrogen, was similar between Nep25$^+$/DTR$^+$ and Nep25$^+$/DTR$^-$ mice at killing (94.30 $\pm$ 4.95 vs. 92.40 $\pm$ 8.82 mg/dl).

Although podocyte foot process effacement was less in Nep25$^+$/DTR$^+$ versus Nep25$^+$/DTR$^-$ mice (74.0 $\pm$ 2.2 vs. 87.0 $\pm$ 4.4%, $P < 0.05$), segments of denuded glomerular basement membrane with stripped podocytes were more extensive in Nep25$^+$/DTR$^+$ than Nep25$^+$/DTR$^-$ mice (26.0 $\pm$ 2.2 vs. 3.0 $\pm$ 1.8%, $P < 0.05$) (Figure 2b). Glomerular Wilms' tumor-1 antigen (WT-1)$^+$ cell density, a marker of differentiated podocytes, was decreased in Nep25$^+$/DTR$^+$ versus Nep25$^+$/DTR$^-$ mice (26.94 $\pm$ 1.48 vs. 33.52 $\pm$ 2.32 $\times$ 10^{-4}/μm^2, $P < 0.05$) (Figure 2b).

Nep25$^+$/DTR$^+$ mice also had more glomerulosclerosis than Nep25$^+$/DTR$^-$ mice at day 10 after LMB-2 (Nep25$^+$/DTR$^+$ 1.48 $\pm$ 0.15 vs. Nep25$^+$/DTR$^-$ 0.78 $\pm$ 0.15, $P < 0.05$) (Figure 2c). Tubular injury markers, urine NGAL and KIM-1, increased similarly in both groups after LMB-2 injection (Figure 1a). Kidney fibrosis, measured as total collagen amount, was increased in the Nep25$^+$/DTR$^+$ versus Nep25$^+$/DTR$^-$ group at killing (11.6 $\pm$ 2.1 vs. 6.9 $\pm$ 0.7, $P < 0.05$) (Figure 2d).

Preexisting tubulointerstitial injury induced more atubular glomeruli and abnormal tubuloglomerular feedback

Atubular glomeruli were more frequent in Nep25$^+$/DTR$^+$ versus Nep25$^+$/DTR$^-$ mice at week 6 before LMB-2 injection (20.6 $\pm$ 2.6 vs. 12.1 $\pm$ 2.4, $P < 0.05$) (Figure 3b). WT-1$^+$ cell density was decreased in atubular glomeruli versus tubule-connected glomeruli in both groups (Figure 3c). LMB-2 injection increased the extent of atubular glomeruli in both groups (Figure 3b). Glomerulosclerosis was similar in atubular glomeruli in the 2 groups, while tubule-connected glomeruli showed more severe glomerulosclerosis in Nep25$^+$/DTR$^+$ than in Nep25$^+$/DTR$^-$ mice (Figure 3d).