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Increased susceptibility to acute kidney injury due to endoplasmic reticulum stress in mice lacking tumor necrosis factor-α and its receptor 1

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Endoplasmic reticulum (ER) stress is actively involved in acute organ injury. Since tumor necrosis factor α (TNFα) plays a role in acute kidney injury, and induces ER stress and cell death *in vitro*, we examined the contribution of TNFα to acute kidney ER stress induced by tunicamycin. Contrary to expectation, tunicamycin caused much more severe kidney injury in TNFα−/− than in wild-type mice. The major site of kidney injury in TNFα−/− mice was proximal tubules, which showed extensive cell vacuolation, lipid accumulation, and apoptosis. Reconstitution of TNFα−/− mice with TNFα 24 h before tunicamycin injection reversed the susceptibility. When TNFα-receptor-deficient mice were treated with tunicamycin, severe renal injury developed in TNFR1−/− but not TNFR2−/− mice, suggesting this aspect of TNFα action was through TNF receptor-1 (TNFR1). In response to tunicamycin-induced acute ER stress, kidneys from neither TNFα−/− nor TNFR1−/− mice showed a significant increase in phosphorylated eukaryotic translation initiation factor 2α (eIF2α), a key step in ER stress regulation. Moreover, proximal tubular cells from TNFR1−/− mice did not show increased eIF2α phosphorylation in response to tunicamycin and were susceptible to ER stress-induced cell death. Finally, treatment of proximal tubule cells isolated from TNFR1−/− mice with an inhibitor of eIF2α phosphatase increased the levels of phosphorylated eIF2α and substantially reduced tunicamycin-induced cell death. Thus, disruption of TNFR1 signaling leads to dysregulation of eIF2α and increased susceptibility to acute ER stress injury in the kidney.

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Acute kidney injury caused by ischemia, drug toxicity, or infection is an important health problem. Although the mechanism of acute kidney injury likely varies among different causes, common factors such as oxidative stress, decreased energy supply, as well as inflammation are implicated in its pathogenesis.[1–5] Endoplasmic reticulum (ER) stress is an important form of cellular stress that occurs because of the accumulation of excessive amounts of unfolded proteins in the ER.[6,7] Three major ER stress response pathways have been identified in mammals: (1) pancreatic ER kinase (PERK)-eukaryotic initiation factor 2α (eIF2α),[8,9] (2) inositol-requiring enzyme 1 (IRE1)–X-box binding protein 1 (XBP-1),[10–12] and (3) activating transcription factor (ATF6) pathways.[13–15] Activation of eIF2α decreases protein synthesis. XBP-1 increases cellular degradation of unfolded proteins. ATF6 enhances the protein folding machinery. Thus, all three pathways are critical for handling ER stress and for return to normal homeostasis.[16,17] The presence of unresolved ER stress can cause cell death, inflammation, and excessive oxidant production.[18–21] Therefore, chronic ER stress is thought to be involved in atherosclerosis, diabetes, and neurodegenerative diseases.[22–24] As multiple factors that are present in acute kidney injury such as hypoxia, free radicals, and a reduced supply of glucose and amino acids are known to cause ER stress,[25–28] this stress may be involved in the pathogenesis of acute kidney injury. This hypothesis is consistent with the observation that the administration of a single ER stress-inducing agent (tunicamycin) is sufficient to cause acute kidney injury in animals.[17,18] Tunicamycin is an inhibitor of *N*-acetylglucosamine phosphotransferase, an enzyme that catalyzes the first step of protein *N*-glycosylation. Tunicamycin induces the accumulation of unfolded proteins and extensive ER stress *in vivo* and *in vitro*.[16,17] In this study, we examined

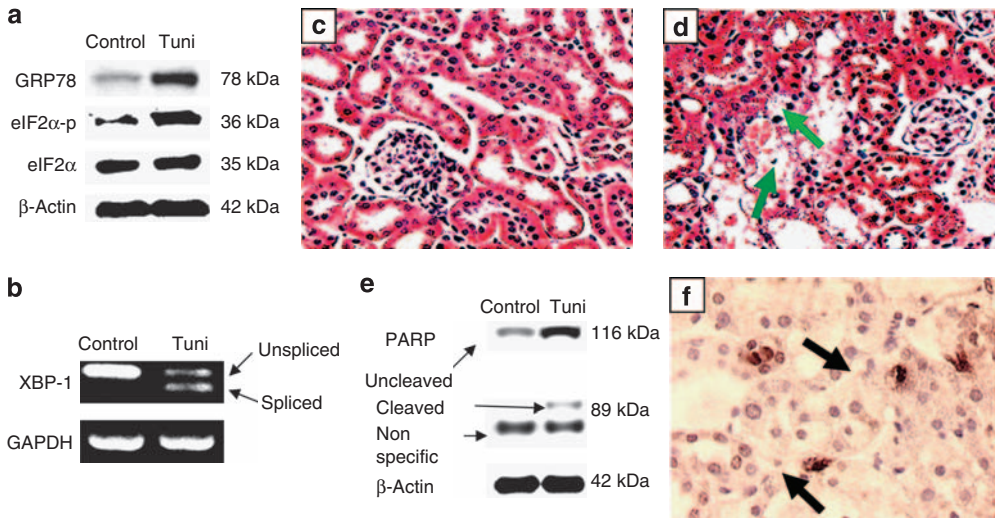

Figure 1 | Tunicamycin-induced ER stress kidney injury. (**a**) Representative gel of western blot shows that the levels of GRP78 and phosphorylated eIF2α are increased in mouse kidney 24 h after tunicamycin (tuni) injection. (**b**) RT-PCR. Spliced XBP-1 is present in tunicamycin-treated mouse kidney. (**c**) Normal renal histology of control mouse. (**d**) Renal histology of mouse killed 72 h after tunicamycin injection. Tubular vacuolation (light green arrow) and nuclear changes (dark green arrow) are prominent. (**e**) Cleaved PARP is present only in tunicamycin-treated mouse kidney. (**f**) Appearance of apoptotic cell death in the kidney from tunicamycin-treated mouse. TUNEL staining was performed. Arrow points to apoptotic cell. eIF2α, eukaryotic translation initiation factor 2α; ER, endoplasmic reticulum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GRP78, glucose regulatory protein 78; PARP, poly ADP ribose polymerase; RT-PCR, reverse transcriptase-PCR; TUNEL, TdT-mediated dUTP nick end labeling; XBP-1, X-box binding protein 1.

ER stress in the kidneys of tunicamycin-treated mice. Tumor necrosis factor-α (TNFα) induces ER stress in cultured cells, is a pro-death and proinflammatory cytokine, and has been shown to actively participate in acute kidney injury.[29–32] Therefore, we examined the contribution of TNFα to acute ER stress kidney injury.

RESULTS

Tunicamycin induced acute ER stress in kidney and caused pronounced damage in proximal tubules

The levels of glucose regulatory protein 78 (GRP78), a marker for ER stress, were significantly increased in kidneys 24 h after tunicamycin injection (Figure 1a). The levels of phosphorylated eIF2α and XBP-1 splicing were elevated, an evidence that ER stress pathways were activated in the kidneys of tunicamycin-treated mice (Figure 1b). Histological evidence of renal tubular damage was seen 48–72 h after tunicamycin injection, and was characterized by vacuolation of tubular cells and nuclear changes including chromatin condensation, pyknosis, and fragmentation. Cleaved poly ADP ribose polymerase and cell apoptosis were observed in the kidneys of tunicamycin-treated mice (Figure 1). As the morphological injury seemed to predominate in proximal tubules, sections were stained for alkaline phosphatase, a proximal tubular cell marker. The number of alkaline phosphatase-positive proximal tubules was visibly decreased, and urinary alkaline phosphatase activity was significantly increased in tunicamycin-treated mice (Figure 2). Double staining showed that apoptotic cells were localized in alkaline phosphatase-positive proximal tubules, whereas tubules positive for aquaporin 2, a marker for collecting ducts, were essentially negative for apoptosis (Figure 2). Proximal tubular cells *in vitro* were also found to be more susceptible to tunicamycin-induced cell death compared with distal tubular cells.

More severe acute ER stress kidney injury in TNFα-deficient mice

As TNFα is actively involved in acute kidney injury,[30,31] we examined serum and kidney TNFα levels in tunicamycin-treated wild-type mice. Serum TNFα levels were not significantly increased at 24–72 h after tunicamycin injection. Kidney TNFα mRNA and protein levels and TNF receptor 1 and 2 (TNFR1 and TNFR2) mRNA levels remained largely unchanged (data not shown). However, tunicamycin-treated TNFα−/− mice had a much more extensive and severe ER stress injury in kidney compared with wild-type mice (Figure 3a and b). Histologically, >80% of tubules in the cortex and at the corticomedullary junction in TNFα−/− mice (Figure 3c) were affected, whereas the injury in wild-type mice occurred predominantly in ~20% of tubules at the corticomedullary junction. There was a substantial reduction in the number of alkaline phosphatase-positive tubules in tunicamycin-treated TNFα−/− mice when compared with wild-type mice (Figure 3d and e). When alkaline phosphatase staining was present in TNFα−/− mice, it was irregular, suggesting that the brush border was less well preserved (Figure 3e). Additionally, tunicamycin-treated TNFα−/− mice had increased alkaline phosphatase in the urine (Figure 3f). Apoptosis was prominent in the kidneys of TNFα−/− mice. Double staining for apoptosis and aquaporin 2 showed that apoptosis occurred mostly in tubular cells other than collecting ducts in both wild-type (Figure 3g) and TNFα−/− mice (Figure 3h). The number of apoptotic cells was 7.5-fold higher

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