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IL-18 neutralization ameliorates obstruction-induced epithelial–mesenchymal transition and renal fibrosis

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Ureteral obstruction results in renal fibrosis in part due to inflammatory injury. The role of interleukin-18 (IL-18), an important mediator of inflammation, in the genesis of renal fibrosis was studied using transgenic mice overexpressing human IL-18-binding protein. In addition, HK-2 cells were analyzed following direct exposure to IL-18 compared to control media. Two weeks after ureteral obstruction, the kidneys of wild-type mice had a significant increase in IL-18 production, collagen deposition, α-smooth muscle actin and RhoA expression, fibroblast and macrophage accumulation, chemokine expression, and transforming growth factor-β1 (TGF-β1) and tumor necrosis factor-α (TNF-α) production, whereas E-cadherin expression was simultaneously decreased. The transgenic mice with neutralized IL-18 activity exhibited significant reductions in these indicators of obstruction-induced renal fibrosis and epithelial–mesenchymal transition, without demonstrating alterations in TGF-β1 or TNF-α activity. Similarly, the HK-2 cells exhibited increased α-smooth muscle actin expression and collagen production, and decreased E-cadherin expression in response to IL-18 stimulation without alterations in TNF-α or TGF-β1 activity. Our study demonstrates that IL-18 is a significant mediator of obstruction-induced renal fibrosis and epithelial–mesenchymal transition independent of downstream TGF-β1 or TNF-α production.

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Obstructive nephropathy is a major cause of renal dysfunction[1] characterized by progressive tubulointerstitial fibrosis.[2] Interstitial fibrosis is a complex pathophysiological process involving inflammatory cell infiltration, fibroblast proliferation, and an imbalance in extracellular matrix (ECM) synthesis, deposition, and degradation.[3–5] In the kidney, interstitial fibrosis is characterized by *de novo* activation of α-smooth muscle actin (SMA)-positive myofibroblasts, the principal effector cells responsible for excess ECM deposition. Growing evidence suggests that renal tubular epithelial cells (TECs) are capable of undergoing a phenotypic transformation into matrix-producing fibroblasts in pathologic states.[6–9] This transformation in the phenotype of the cell epithelial—mesenchymal transition (EMT) is thought to contribute greatly to renal fibrosis, with a large proportion of interstitial fibroblasts originating from TECs.[10,11] Epithelial cells that have undergone EMT are characterized by the loss of epithelial cell markers, such as E-cadherin, and *de novo* α-SMA expression.[12] The transformed epithelial cells also begin to express fibroblast (FSP-1), a marker specific for EMT.[13] TGF-β1 can independently initiate and complete the entire course of EMT, suggesting that induction of EMT may be a major pathway by which TGF-β1 stimulates interstitial fibrosis.[14–19] EMT appears to be integral to progressive TIF in diseased kidneys,[8,9,20] as selective blockade of EMT in animal models significantly reduces obstruction-induced fibrosis.[21,22]

IL-18 is a pro-inflammatory cytokine implicated in the pathogenesis of many inflammatory renal diseases, including renal ischemia–reperfusion injury, allograft rejection, and autoimmune disease.[23–28] In humans, IL-18 is a sensitive and early marker of renal tubular damage [26,29] and in animal models of renal ischemia–reperfusion injury, IL-18 exacerbates acute tubular necrosis.[30–32] A recent study suggests that IL-18 can also directly stimulate fibrotic changes in renal TECs *in vitro*,[33] and we therefore hypothesized that IL-18 is an important mediator of obstruction-induced renal fibrosis and EMT. To study this we examined renal cortical IL-18 production, collagen expression and deposition, α-SMA and RhoA expression, E-cadherin expression, FSP-1 and macrophage accumulation, chemokine expression, and TGF-β1 and

TNF-α production in male C57B16 wild type (WT) mice and mice transgenic for IL-18BP using a well-established model of unilateral urethral obstruction (UUO). C57BL6 mice transgenic for IL-18-binding protein transgenic (IL-18BP Tg) have been previously demonstrated to overexpress human IL-18-binding protein isoform *a* and reliably inhibit IL-18 activity.[34] In addition, human proximal tubular cells (HK-2) were directly stimulated with IL-18 and the cells were subsequently examined for α-SMA and E-cadherin expression, collagen production, and TGF-β1 and TNF-α activity.

RESULTS

IL-18 and IL-18BP production

Before surgical intervention, serum levels of human IL-18BP were measured and found to be dramatically elevated in the IL-18BP Tg mice when compared with WT mice (Figure 1a). Renal cortical tissue obtained from sham-operated animals revealed low levels of IL-18 production; however, IL-18 levels increased significantly in response to 1 or 2 weeks of obstruction (Figure 1b). IL-18 gene expression was similarly found to be significantly increased in WT animals exposed to 1 or 2 weeks of obstruction when compared with sham-treated animals (Figure 1c), whereas no significant change in IL-18 gene expression was detected in IL-18BP Tg animals exposed to the same degree obstruction.

Renal tissue sections were subsequently stained for IL-18. Minimal IL-18 was detected in sham-treated animals; however, a significant increase in renal cortical IL-18 staining was evident following 1 or 2 weeks of UUO (Figure 1d). IL-18 production predominantly localized to renal TECs, with minimal staining occurring in the glomeruli and interstitium.

Collagen expression and deposition

Renal tissue cross sections were analyzed for collagen I and III mRNA production and total soluble collagen concentration. Low levels of collagen I and III mRNA were present in sham-treated samples; however, collagen I and III mRNA expression significantly increased in WT mice exposed to 1 or 2 weeks of UUO (Figure 2a and b). In the obstructed IL-18BP Tg animals, collagen I and III mRNA levels were increased over sham levels, but significantly reduced when compared with WT-obstructed mice. Similarly, total renal collagen concentrations were significantly elevated in WT mice exposed to 1 or 2 weeks of UUO (Figure 2c). Although collagen concentrations were increased over sham levels in

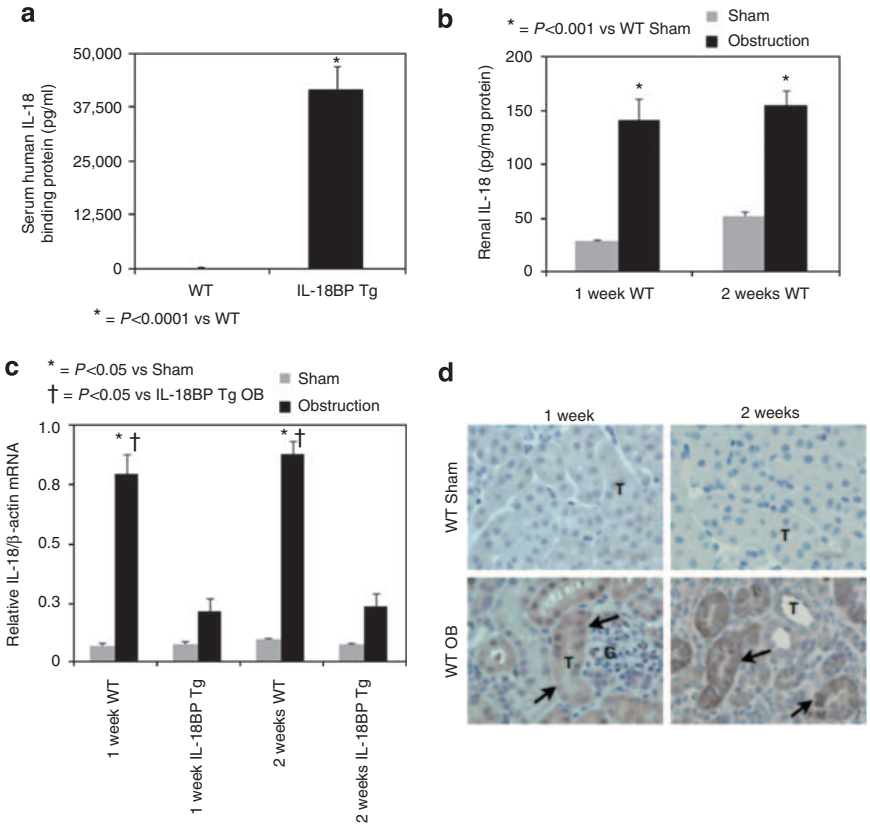

Figure 1 | Serum human IL-18BP levels and renal IL-18 production, quantitative mRNA expression, and immunolocalization following UUO. (**a**) Serum human IL-18BP levels (pg/ml) in WT or IL-18BP transgenic (IL-18BP Tg) animals. (**b**) Renal cortical IL-18 protein levels in WT sham and 1 or 2 week obstructed kidneys. (**c**) Quantitative IL-18 mRNA expression represented as a percentage of β-actin in WT and IL-18BP Tg animals exposed to sham operation or 1 or 2 weeks of UUO. (**d**) Renal cortical immunolocalization of IL-18 production (brown stain; arrows) in WT sham and 1- or 2-week obstructed kidneys (WT OB). G = glomerulus; T = tubule; OB, obstruction; UUO, unilateral urethral obstruction; WT, wild type. Original magnification ×400.