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TREM-1 regulates macrophage polarization in ureteral obstruction

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Chronic kidney disease (CKD) is an emerging worldwide public health problem. Inflammatory cell infiltration and activation during the early stages in injured kidneys is a common pathologic feature of CKD. Here, we determined whether an important inflammatory regulator, triggering receptor expressed on myeloid cells (TREM)-1, is upregulated in renal tissues collected from mouse ureteral obstruction–induced nephritis. TREM-1 is crucial for modulating macrophage polarization, and has a pivotal role in mediating tubular injury and interstitial collagen deposition in obstructive nephritis. Lysates from nephritic kidneys triggered a TREM-1-dependent M1 polarization *ex vivo*, consistent with the observation that granulocyte-macrophage colony-stimulating factor (GM-CSF)-derived M1 macrophages express higher levels of TREM-1 in comparison with M-CSF-derived cells. Moreover, agonistic TREM-1 cross-link significantly strengthens the inductions of iNOS and GM-CSF in M1 cells. These observations are validated by a strong clinical correlation between infiltrating TREM-1-expressing/iNOS-positive macrophages and renal injury in human obstructive nephropathy. Thus, TREM-1 may be a potential diagnostic and therapeutic target in human kidney disease.

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Chronic kidney disease (CKD) is an emerging health problem that poses a growing socioeconomic burden for societies in North America and the Asia-Pacific regions.[1–5] A common pathologic feature of CKD is inflammatory cell infiltration occurring at early stages in the injured kidneys, followed by tubulointerstitial fibrosis at later stages of disease progression.[2–4] Among CKD, upper urinary tract obstruction resulting in renal dysfunction is a significant clinical problem in both adult and pediatric populations. Pathologic manifestations of renal obstructive injury include tubular dilation, tubular cell apoptosis,[5] as well as progressive interstitial fibrosis and massive macrophage infiltration in the obstructed kidney.[2]

Classically activated macrophages (M1), characterized by high major histocompatibility complex class II expression with strong interleukin-12 (IL-12) and IL-23 production, produce nitric oxide, reactive oxygen species, and proinflammatory cytokines, such as IL-1, IL-6, and tumor-necrosis factor (TNF),[2,6,7] leading to tubular cell apoptosis and renal tissue injury. In contrast, alternatively activated macrophages (M2) typically have immune-suppressive activity and express arginase (Arg)-1, promoting cell proliferation and collagen production. Besides, YM-1,[8,9] Mannose receptor,[10] galectin-3,[11] and transforming growth factor-β[12] also have been reported to be preferentially expressed on M2 cells. Microenvironmental cytokines, glucocorticoid hormones, and surrounding pathogens and apoptotic cells regulate M1/M2 differentiation.[10,13,14]

Triggering receptor expressed on myeloid cells (TREM) is an immunoglobulin-like family whose members have a critical role in modulating infection-induced inflammation.[15,16] A common feature of TREM downstream signaling is the link with adaptor DNAX activation protein (DAP)-12.[16,17] Activation of TREM leads to the production of M1 proinflammatory cytokines.[16,18] TREM-1 is the best-characterized member in the TREM family. Treatment with soluble TREM-1 decreases inflammation and mortality in high-dose lipopolysaccharide-mediated septic shock model.[15] Clinically, the levels of soluble TREM-1 in serum and bronchioalveolar lavage fluid have been suggested as a sensitive and specific predictor of bacterial pneumonia in humans.[19] Previously, we also reported that TREM-1-mediated bacterial clearance in the small intestine is an important immune response against *K. pneumoniae*.[20] A putative pathogenic role of TREM-1 was reported in rheumatoid arthritis[21] and inflammatory bowel disease.[22] However, the role of TREM-1 in the nephropathy of CKD has not yet been characterized.

A mouse model of experimental unilateral ureteral obstruction (UUO) is characterized by infiltrating macrophages and the different stages of obstructed nephropathy, which accelerates with disease progression.[23] In this study, we examine TREM-1 expression and function in UUO kidneys by using *Trem-1* knockout (KO) mice. Our results demonstrate a novel role of TREM-1 in regulating granulocyte-macrophage colony-stimulating factor (GM-CSF)/iNOS and M1 polarization. In addition, correlations of TREM-1 expression, macrophage profiling, and severity of renal injury in kidney specimens from patients of obstructive nephropathy further support that TREM-1 is a critical factor in obstructive nephritis.

RESULTS

Depleting TREM-1 ameliorates the UUO renal pathology

TREM-1 mRNA is not detectable in sham control kidneys but is significantly upregulated in the obstructed kidneys harvested from wild-type (WT) C57BL/6 mice at day 7 and day 14 after unilateral ureteral obstruction (UUO; Figure 1a), and TREM-1 protein is mainly detected in a subpopulation of F4/80$^+$ cells within the renal tubulointerstitium after UUO (Figure 1c and d).

To assess the *in vivo* role of TREM-1 in UUO, we compared the histological renal changes between WT and *Trem-1* KO mice (Supplementary Figure S1 online). TREM-1 deletion in KO kidneys is confirmed by quantitative reverse transcriptase-PCR (Figure 1a) and immunohistochemical analysis (Figure 1b). Histologically, UUO induces marked renal injury in WT mice (characterized by cortical tubular dilation with tubular epithelial cell necrosis, brush border loss, intratubular cast formation, and interstitial fibrosis), whereas *Trem-1* KO mice show significantly less renal damage after UUO (Supplementary Figure S2 online). Analyses by PAS and Masson's trichrome staining also show drastic tubular injuries at day 7 ($54 \pm 9\%$) and day 14 ($81 \pm 6\%$) in WT kidneys after UUO in comparison with the sham group (Figure 2a and b), accompanied by increased interstitial collagen deposition after UUO at day 7 (9.1 ± 2.6) and day 14 (34.9 ± 5.7) (Figure 2c and d). In contrast, TREM-1 deficiency results in significantly less tubular injuries ($30 \pm 7\%$; $50 \pm 13\%$) at day 7 and day 14 after UUO, and lower interstitial fibrosis (12.8 ± 5.4) at day 14 after UUO (Figure 2a–d).

Tubular cell injury was further examined by using terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling and Ki67 staining methods. In *Trem-1* KO kidneys, the numbers of apoptotic and proliferating cells are significantly lower than those in WT ones (Supplementary Figure S3a and b online). In addition, massive α-SMA$^+$ myofibroblasts accumulate in WT UUO kidneys but are relatively less in *Trem-1* KO kidneys isolated from day-14-UUO mice (Figure 2e and f).

TREM-1 is critical for regulating macrophage polarization but not recruitment

Renal inflammatory cell infiltration is a prominent feature associated with the pathogenesis of UUO. Renal macrophage recruitment was accessed by determining the distribution of F4/80$^+$ macrophages. Interestingly, similar levels of F4/80$^+$ cells are found in WT and *Trem-1* KO-obstructed kidneys (Supplementary Figure S4a–c online). A recent report suggests that TREM-1 has an important role in regulating neutrophil trafficking to amplify inflammation against bacterial infection,[24] leading us to examine the neutrophil infiltration after UUO. However, only very few Ly6G$^+$ neutrophils are observed in the obstructed kidneys, and no significant difference can be found in the numbers of infiltrating neutrophils between WT and *Trem-1* KO groups (Supplementary Figure S4d and e online). Thus, TREM-1 is dispensable for macrophage and neutrophil renal recruitment upon UUO.

M1 polarization has been suggested as a key step in UUO-mediated renal damage.[2] Whether TREM-1 regulates the balance of 'classical' and 'alternative' activation of macrophages in UUO remains to be investigated. M1 cells were evaluated by iNOS expression, which is presented in F4/80$^+$ infiltrating cells and tubular epithelial cells on WT renal sections, particularly at day 14 after UUO. By contrast, it is markedly attenuated in the cortex and medulla of UUO *Trem-1* KO kidneys (Figure 3a and b). Accordingly, the mRNAs of iNOS, TNF, IL-1β, and IL-6 (M1 markers) are highly expressed in WT UUO kidneys, but the expression is significantly lower in *Trem-1* KO UUO kidneys (Figure 3c). Furthermore, the expression of iNOS, TNF, and IL-1β mRNA is significantly lower in *Trem-1* KO renal macrophages freshly isolated from UUO kidneys compared with WT cells (Supplementary Figure S5a online). In parallel, M2 differentiation was evaluated by Arg-1 staining on renal sections. Unlike in WT samples, which only show a mild staining, Arg-1 is highly induced and significantly elevated in renal F4/80$^+$ infiltrating cells in *Trem-1* KO kidneys at day 7 and day 14 after UUO (Figure 3d and e). The mRNA expression levels of Arg-1, YM-1, IL-10, and Mannose receptor (M2 markers) are significantly higher in *Trem-1* KO UUO kidneys in