Twist1 in T Lymphocytes Augments Kidney Fibrosis after Ureteral Obstruction

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Key Points
- The transcription factor Twist1 acts in T lymphocytes to promote kidney fibrogenesis.
- T-cell Twist1 limits the accumulation of TNF-producing CD8+ T cells in the injured kidney.
- TNF produced by T lymphocytes limits kidney scar formation after ureteral obstruction.

Abstract
Background Twist1 is a basic helix-loop-helix domain–containing transcription factor that participates in diverse cellular functions, including epithelial-mesenchymal transition and the cellular immune response. Although Twist1 plays critical roles in the initiation and progression of kidney diseases, the effects of Twist1 in the T lymphocyte on the progression of renal fibrosis require elucidation.

Methods 129/SvEv mice with a floxed allele for the gene encoding Twist1 or TNFα were bred with CD4-Cre mice to yield CD4-Cre+ Twist1flox/lox (Twist1-TKO) or CD4-Cre+ TNFflox/lox (TNF-TKO) mice with robust, but selective, deletion of Twist1 or TNFα mRNA in T cells, respectively. Twist1 TKO, TNF TKO, and WT controls underwent UUO with assessment of kidney fibrosis and T-cell phenotype at 14 days.

Results Compared with WT controls, obstructed kidneys from Twist1 TKO mice had attenuated extracellular matrix deposition. Despite this diminished fibrosis, Twist1 TKO obstructed kidneys contained more CD8+ T cells than in WTs. These intrarenal CD8+ T cells exhibited greater activation and higher levels of TNFα expression than those from WT obstructed kidneys. Further, we found that selective deletion of TNFα from T cells exaggerated renal scar formation and injury after UUO, highlighting the capacity of T-cell TNF to constrain fibrosis in the kidney.

Conclusions Twist1 in T cells promotes kidney fibrogenesis, in part, by curtailing the renal accumulation of TNF-eliciting T cells.

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Introduction
Tubulointerstitial fibrosis is a common consequence of CKDs leading to end stage renal failure, irrespective of the cause (1–3). Interstitial infiltration of T lymphocytes into the kidney is an early and characteristic feature of progressive renal fibrosis in patients with CKD and in animal models. T-cell recruitment and activation after kidney injury can have profound effects on renal fibrogenesis (4). Under the influence of the local inflammatory microenvironment in injured tissues, T lymphocytes undergo polarization which culminates in their elaboration of divergent constellations of cytokines (5,6). Accordingly, T-lymphocyte heterogeneity plays an important role in regulating tissue injury and scar formation. For example, CD4+ type 1 T helper (Th1) cells can have opposing effects on organ fibrogenesis, depending on the specific clinical context (4,7–10). CD4+ Th2 cells have been shown to trigger renal fibrosis (7). Finally, γδ T cells and CD4+ Th17 cells that produce IL-17A can also drive collagen deposition in the kidney (11). In contrast, regulatory T cells suppress kidney injury and scar formation, despite their elaboration of TGF-β12 (12), and CD8+ effector T cells can similarly constrain fibrogenesis in kidneys or tumors (13–15). Although great efforts have been made to determine the molecular mechanisms governing T-cell phenotype and activation in kidney fibrosis, they remain largely unknown. Elucidating these mechanisms could lead to immunomodulatory interventions to mitigate kidney fibrosis.

Twist1 regulates the progression of several forms of renal disease, and operates through distinct mechanisms in different cell lineages (16–18). Twist1 also shapes cellular immune responses and contributes to heterogeneity in T-lymphocyte phenotype by modulating their elaboration of various inflammatory cytokines (19,20). Accordingly, Twist1 is upregulated in chronically activated Th1, Th17, and T follicular helper cells (21). Twist1 negatively regulates cytokine production in several T-cell subsets via diminishing NF-κB and Runx3 activation; limiting TNFα, IFNγ, or IL-2

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expression in Th1 cells (22); or repressing IL-6/STAT3 activation; leading to restricted IL-17 and IFNγ production (20). However, the role of its actions in T lymphocytes during fibrogenesis has not been explored. Given that T lymphocytes accumulate in the injured kidney and that their secreted cytokines modulate fibrosis, the contribution of Twist1 in lymphocytes to kidney fibrogenesis warrants scrutiny.

In this study, we find that Twist1 expression is induced in T lymphocytes that infiltrate fibrotic kidneys. Using a mouse model with selective deletion of Twist1 in T lymphocytes, we demonstrate that Twist1 in T lymphocytes augments kidney fibrosis accruing from unilateral ureteral obstruction (UUO). Furthermore, T-lymphocyte Twist1 curtails the renal accumulation of CD8+ T lymphocytes that elaborate TNFα. In turn, we find that TNFα from T lymphocytes constrains kidney fibrosis and injury. Thus, Twist1 promotes T cell–dependent fibrosis in the kidney, possibly by constraining TNFα production in lymphocytes.

Materials and Methods

Animals

All mice were housed and bred in the animal facilities at the Durham Veterans Affairs Medical Center, according to National Institutes of Health guidelines. All animal experiments were approved by the Durham Veterans Affairs Medical Center Institutional Animal Care and Use Committee. Homozygous Twist1-floxed mice (a kind gift from Richard Behringer) (23), backcrossed to the 129/SvEv back- ground, were bred with 129/SvEv CD4-Cre (24) mice to generate CD4-Cre+ Twist1floxed/floxed (Twist1 TKO) mice and wild-type (WT) Cre− control littermates. Similarly, 129/SvEv TNFfloxed/floxed mice (25) were bred with 129/SvEv CD4-Cre mice to yield CD4-Cre+ TNFfloxed/floxed (TNF TKO) mice and WT control subjects. To map the distribution pattern of Cre-recombinase expression, mT/mG mice from The Jackson Laboratory (Bar Harbor, ME) were crossed with the CD4–Cre recombinase transgenic lines; mT/mG mice normally express red fluorescent protein in all tissues. When Cre is present, the mT cassette is deleted, triggering expression of membrane-targeted enhanced green fluorescent protein (GFP). Male mice, aged 8–12 weeks, were used in our experiments.

UUO Model of Kidney Fibrosis

UUO was performed as previously described (17). In brief, mice were anesthetized with 4% isoflurane and maintained at 2% for the procedure, lasting approximately 10 minutes, and the left ureter was isolated and ligated 3–5 mm below its origin. At 14 days after ligation, mice were euthanized and the obstructed and contralateral nonobstructed kidneys were harvested for analysis.

Histologic Analysis and Immunohistochemistry Staining

Mouse kidney samples were fixed in 10% formalin (Sigma-Aldrich) overnight and embedded in paraffin. Sections (5 μm thick) were used for Periodic acid–Schiff staining and immunohistochemistry staining. Renal damage, including necrotic tubules, loss of brush borders, tubule dilation, cast formation, tubular epithelial swelling, and vacuolar degeneration, was semiquantitatively scored. A score of zero represents an injury area <5%, whereas one, two, three, and four connote damage involving 5%–25%, 26%–50%, 51%–75%, and >75% of the whole kidney area, respectively. The investigator, who was blinded to experimental conditions, assigned a score to each field on the basis of the degree of kidney injury. At least ten random fields were assessed under the microscope (original magnification, 200×), and an average score was calculated for each mouse. To visualize interstitial collagen deposition, sections were stained with anti-type I collagen, as previously described (18).

Western Blots

Kidney tissues (20 mg) were homogenized in radioimmunoprecipitation assay buffer (Sigma-Aldrich). Concentrations of protein were quantitated using the DC Protein Assay kit (Bio-Rad Laboratories, Hercules, CA). Equal amounts of sample were subjected to electrophoresis through 4%–12% Bis-Tris gels, and then transferred to polyvinylidene difluoride membranes. After blocking with 5% milk in Tris-buffered saline solution with Tween 20, the blots were incubated with anti-collagen type I (anti-Col-I) antibody (catalog number 1310-01; Southern Biotech, Birmingham, AL), fibronectin (FN; catalog number 2413; Abcam, Cambridge, United Kingdom), or anti-glyceraldehyde-3-phosphate dehydrogenase (catalog number 2118; Cell Signaling Technology, Danvers, MA) overnight in 4°C. The blots were then washed and incubated for 1 hour, at room temperature, with individual secondary antibodies accordingly. Bands were detected using an enhanced chemiluminescence detection system (Bio-Rad). The detected bands were quantified by densitometry through ImageJ 1.38 for Windows.

Kidney Flow Cytometry and T-Cell Isolation

Kidney single-cell suspensions were prepared by mechanical and enzymatic digestion, as described previously (18). To achieve homogenization, obstructed kidneys underwent mechanical dissociation and enzymatic digestion. Cell suspensions were then filtered through 70- and 40-μm cell strainers. The resulting single cells were incubated with Fc Block for 20 minutes at 4°C. For kidney flow cytometry, single cells were stained with CD45, CD8, CD4, CD44, CD62L, CD11b, Ly6G, CD64, TNFα, and near-infrared dead cell indicator (catalog number L34976; Life Technologies) for 30 minutes at 4°C. Cells were washed and fixed with Fix/Perm buffer (catalog number 554655; BD Biosciences, San Jose, CA). Then, 20 μl of CountBright Absolute Counting Beads (catalog number C36950; Invitrogen, Carlsbad, CA) were added to cells, and samples were analyzed on an LSR II Flow Cytometer (Becton Dickinson, Franklin Lakes, NJ).

For T-cell isolation, the single cells were stained with CD45, CD8, CD4, and 4′,6-diamidino-2-phenylindole, and then subjected to fluorescent cell sorting. The gating strategy was as follows: first gate on single live cells, second gate on CD45+ cells, and third gate on CD4 versus CD8 cells. The single CD4+ cells and single CD8+ cells were isolated and saved for real-time PCR.
**T-Lymphocyte Chemotaxis Assays**

Naive, splenic T lymphocytes were isolated using the Mouse T Cell Isolation Kit (negative selection; catalog number 19851A; STEMCELL). The purified T cells (1×10⁷/ml) were resuspended in 100 µl medium (RPMI 1640 supplemented with 1% BSA) and added to each insert (diameter, 6.5 mm) with 8-µm pore size (catalog number CLS3464; Corning) polyester membranes. Then, vehicle or 100 ng/ml CCL5-containing migration medium (500 µl) was placed in the lower compartment of the well. After 3 hours, the number of migrated T cells was determined by using a hemocytometer to count the cells present in the lower well media.

**Statistical Analyses**

The values of each parameter within a group are expressed as the mean±SEM. For comparisons between groups with normally distributed data, statistical significance was assessed using the two-tailed, unpaired *t* test. For comparisons between groups with non-normally distributed variables, we used the Wilcoxon test.

**Results**

**Generation of Mice with Genetic Deletion of Twist1 in T Lymphocytes**

To examine the functions of Twist1 in T cells during kidney interstitial fibrosis, we ablated Twist1 in T lymphocytes by using a Cre-Lox gene targeting approach. During the double-positive phase of maturation in the thymus, all thymocytes destined to become single CD4⁺ or CD8⁺ circulating T lymphocytes express the CD4 promoter.

Accordingly, by breeding a CD4-Cre mouse line with a double-fluorescence reporter mouse (mT/mG), we confirmed robust Cre expression which was marked by GFP within the thymus and spleen of CD4-Cre⁺ mT/mG mice. The absence of Cre expression was marked by red fluorescent protein. There were also sparse GFP-positive T cells around kidney tubular cells in naive CD4-Cre⁺ mT/mG kidneys (Figure 1A). Therefore, we bred the CD4-Cre mouse line with a Twist1-flox line harboring loxP sites on either side of the coding region for the transcription factor Twist1 (Figure 1B). For our experiments, we used Twist1-TKO mice and CD4-Cre⁻ Twist1flox/fox (WT) littermates (Figure 1C).

To confirm T cell–specific deletion of Twist1 in our Twist1-TKO animals, we labeled splenocytes for T- and B-lymphocyte markers and isolated CD4⁺ T cells (CD4⁺CD8⁻CD19⁻), CD8⁺ T cells (CD8⁺CD4⁻CD19⁻), and B lymphocytes (CD19⁺Thy1⁻) via fluorescent cell sorting, as in previous studies (26). Using mRNA from these immune cells and from kidney and heart, we then performed real-time PCR for the gene encoding Twist1 to confirm the degree and precision of T cell–specific deletion. Compared with their WT littermates, Twist1-TKO mice exhibited at least >85% deletion of Twist1 from both CD4⁺ and CD8⁺ T cells, but preserved Twist1 expression in B cells and all other tissues examined (Figure 1D).

**Twist1 Deficiency in T Cells Attenuates Kidney Interstitial Fibrosis Induced by UUO**

Twist1 in both renal tubular cells and myeloid cells plays an important role in renal fibrogenesis, but the actions of Twist1 in lymphocytes during renal interstitial fibrosis have not been established. Studies from several groups show...
prominent accumulation of T lymphocytes in fibrotic kidney after UUO (4, 5, 7). We detected enhanced expression of Twist1 mRNA in renal T cells after UUO compared with Twist1 levels from splenic T cells in naive animals (Supplemental Figure 1). WT and Twist1-TKO mice were, therefore, subjected to UUO. We then evaluated whether ablating Twist1 in T lymphocytes influences the progression of UUO-induced interstitial fibrosis. The weights of the UUO kidneys were similar between Twist1-TKO and WT groups (Supplemental Figure 2). We measured mRNA and protein levels for collagen, FN, and fibrosis mediators in UUO-induced fibrosis. First, immunohistochemistry staining for Col-I of kidney sections after UUO revealed diffuse interstitial collagen deposition in both groups. Twist1-TKO mice exhibited less severe kidney fibrosis at 2 weeks after UUO compared with WTs, as quantified by Col-I staining area (Figure 2, A and B). At day 14 after UUO, mRNA expressions for Col-I, FN, TGF-β1, and plasminogen activator inhibitor-1 were significantly less in Twist1-TKO kidneys compared with WT mice (Figure 2C). By Western blot analysis, UUO kidneys from Twist1-TKO animals showed markedly lower levels of Col-I and FN protein than WTs (Figure 2, D and E).

Compared with kidneys from WT controls, obstructed kidneys from Twist1-TKO animals also showed less kidney injury as determined by blinded pathology scores (Figure 3, A and B) and gene expression for the kidney injury marker, neutrophil gelatinase-associated lipocalin (Figure 3C). These data indicate that Twist1 in T cells exacerbates the severity of renal injury and fibrosis after ureteral obstruction.

Twist1 in T Lymphocytes Constrains Renal Accumulation of Activated CD8⁺ T Cells

Next, we examined leukocyte accumulation, including T lymphocytes, neutrophils, and monocytes/macrophages, in the kidney after ureteral obstruction. At day 14 UUO, we isolated infiltrating CD45⁺ cells from the injured kidney via flow cytometry (Supplemental Figure 3) and quantified the numbers of CD4⁺ and CD8⁺ T lymphocytes, CD11b⁺Ly6G⁺ neutrophils, and CD11b⁺CD64⁺

Figure 2. | Twist1 deficiency in T cells attenuates kidney fibrosis after ureteral obstruction. (A) Representative sections from obstructed WT and Twist1-TKO kidneys stained with collagen type I (Col-I) at day 14 after unilateral ureteral obstruction (UUO). Scale bar, 100 μm. (B) Blinded morphometric quantitation of Col-I (n=7). (C) mRNA levels for Col-I, fibronectin (FN), plasminogen activator inhibitor-1 (PAI-1), and TGF-β1 in contralateral (CTL) and obstructed (UUO) kidneys (n=7–11). (D) Western blot for FN and Col-I protein in CTL and UUO kidneys from WT and Twist1-TKO mice at day 14. (E) Semiquantitative determination of FN and Col-I protein levels (n=7). Data represent the mean±SEM. *P<0.05, #P<0.05. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
monocytes/macrophages, and—within these groups—naive and effector memory T cells marked by CD62LhiCD44lo and CD62LloCD44hi expression, respectively (27-30). As shown in Figure 4, A and B, the absolute numbers of CD8 T lymphocytes from Twist1-TKO kidneys after UUO were significantly higher than from WT obstructed kidneys. Although the numbers of CD4 T cells were increased in the Twist1-TKO kidneys, this difference did not achieve statistical significance (P<0.11). Numbers of effector memory T cells marked by CD62LloCD44hi expression were markedly increased within the CD8 T cell subset (Figure 4, C and D) from the Twist1-TKO kidneys, but were not significantly different from WTs in the CD4 T cell subset (Figure 4, E and F). Numbers of Ly6G+ neutrophils and CD64+ monocytes/macrophages were also similar in the kidneys from the two groups (Supplemental Figure 4). We then investigated the role of Twist1 in T-cell migration and splenic T-cell numbers after UUO using an in vitro transwell system and flow cytometry, respectively. No differences in migratory capacity of T cells in vitro or splenic T-cell numbers in vivo were found between the Twist1-TKO and WT groups (Supplemental Figures 5 and 6). Prior studies have demonstrated that Twist1 attenuates proinflammatory cytokine expression by suppressing NF-kB activation. TNFα, IL-1β, and IL-17A, all regulated by Twist1, have each been implicated in renal fibrogenesis (19,20,23). Therefore, we investigated the effects of Twist1 on the cytokine expression in CD4+ and CD8+ T lymphocytes infiltrating the obstructed kidney. To this end, we used fluorescent cell sorting to isolate CD4+ and CD8+ T cells from UUO kidney at day 14 and measured mRNA expression for these cytokines (Figure 5A). As shown in Figure 5, B-D, we were able to detect mRNA for TNFα, IL-1β, and IL-17A in these T cells. We found mRNA expression for TNFα, but not IL-1β or IL-17A, were significantly increased in the CD8+ T cells isolated from the Twist1-TKO kidneys compared to WT controls. In contrast, CD4+ T cell expression of TNFα, IL-1β, and IL-17A were all similar in the Twist1-TKO and WT kidneys after UUO (Figure 5, B-D). To further confirm the expression of TNFα at the protein level, we used flow cytometry to perform intracellular stains for TNFα in CD8+ T cells isolated from the obstructed kidney (Figure 5E). By this metric, the absolute numbers of CD8+TNFα+ T lymphocytes from Twist1-TKO kidneys after UUO were significantly higher than from WT obstructed kidneys (Figure 5F), and fluorescent stains of obstructed kidney corroborated this finding (Figure 5G). TNFα can promote renal fibrogenesis because global inhibition of TNF curtails renal scar formation (31). On the other hand, TNFα can inhibit fibroblast activation or promote matrix metallopeptidase production (32), and may act selectively within T cells to restrain renal damage (33). We therefore posited that TNFα produced by CD8+ T cells may play a protective role in kidney fibrogenesis.

Deletion of TNFα in T Cells Enhances UUO-Induced Kidney Fibrosis and Injury

To investigate the actions of TNFα in T cells in the pathogenesis of kidney fibrosis, we deleted TNFα in T cells, using a Cre-Lox approach (Supplemental Figure 7),
and subjected TNF-TKO and WT control mice to UUO (Figure 6A). The extent of fibrosis was examined after 14 days by scoring of Col-I stains and Western blot of kidney fibrosis markers. Blinded scoring of Col-I stains revealed the obstructed kidneys from TNF-TKO mice exhibited significantly more deposition of collagen than WT littermates (Figure 6, B and C). mRNA expression for TGF-β1 was significantly higher in obstructed TNF-TKO kidneys compared with WT mice (Figure 6D). Western blot analysis confirmed that the TNF-TKO obstructed kidneys expressed higher levels of FN and Col-I (Figure 6, E and F). Moreover, blinded injury scores indicated that tubular injury in TNF-TKO kidneys after UUO was more severe than that in WTs (Figure 7, A and B). These findings were confirmed at the mRNA level for neutrophil gelatinase-associated lipocalin (Figure 7C). Collectively, these data suggest that TNFα from T cells in Twist1-TKO kidneys attenuates UUO-induced kidney fibrosis and injury.

**Discussion**

Regardless of etiology, renal fibrosis typically features a robust inflammatory response, leading to the accumulation of large numbers of T cells and myeloid cells in the injured kidney. T lymphocyte-elicited immune responses exert critical roles in the initiation and development of kidney disease. CD8+ T cells, in particular, play a complex part in regulating kidney scar formation (14,15,28). In this study, we find that Twist1 in lymphocytes promotes kidney fibrogenesis by limiting CD8+ T-cell accumulation, activation, and TNFα production after UUO.

CD4+ and CD8+ T lymphocytes play divergent roles in kidney fibrogenesis. Transfer of CD4+ but not CD8+ T cells...
into lymphocyte-deficient recipients exacerbates kidney scar formation (7), likely due to the profibrotic effects of multiple CD4+ T-cell subpopulations. For example, the transcription factor T-bet, which drives differentiation of proinflammatory CD4+ Th1 cells, is required to mediate the full fibrotic response in the injured kidney (4). Despite elaborating a completely different cytokine profile, anti-inflammatory CD8+ Th2 cells also worsen the progression of renal fibrosis (34). In contrast, CD8+ T cells may constrain kidney fibrosis. For example, recruiting CD8+ T cells into the kidney inhibits activation of the myofibroblasts that produce collagen and, in turn, protects against renal fibrosis (13). Inversely, depleting CD8+ T cells augments renal fibrosis after ureteral obstruction by facilitating CD4+ T-cell differentiation toward a Th2-cell phenotype (14). As another mechanism for CD8+ T-cell protection from renal fibrosis, Wang et al. (15) reported that CD8+ T cells coexpressing CD11c could induce fibroblast death via the secretion of several cytotoxic mediators in the setting of kidney fibrosis. Analogously, cytotoxic CD8+ T cells can limit cancer growth by killing tumor-associated fibroblasts (35,36). In our current studies, to determine the effect of Twist1 on the accumulation and

Figure 5. | CD8+ T cells from UUO kidneys of Twist1-TKO animals have augmented TNFα expression. (A) Representative flow plot of CD4 versus CD8 staining on single-cell suspensions from obstructed kidney of a WT mouse at day 14. (B–D) mRNA levels for (B) TNFα, (C) IL-17A, and (D) IL-1β in CD4+ and CD8+ T cells from WT and Twist1-TKO UUO kidneys (n=7–9). (E) Representative plots for TNFα staining in CD8+ T cells from WT and Twist1-TKO UUO kidneys. (F) Absolute numbers of CD8+ TNFα+ T cells per milligram of WT and Twist1-TKO kidney at day 14 after UUO. Scale bar, 40 μm. Data represent the mean±SEM. Arrows mark TNF-expressing cells. *P<0.05. DAPI, 4',6-diamidino-2-phenylindole; SSC, side-scattered light.
activation of T lymphocytes after UUO injury, we measured the numbers of CD62LloCD44hi effector memory T cells in the UUO kidneys by flow cytometry. These data indicate that Twist1 in T cells limits the accumulation and activation of CD8+ T cells in the kidney and aggravates renal fibrosis. However, in our studies, the augmented renal infiltration of protective CD8+ T cells was not associated with clear alterations in the phenotype of accompanying renal CD4+ T cells. Rather, Twist1 blunted the generation of TNFα in the infiltrating CD8+ T cells, consistent with its capacity to restrain cytokine production by mononuclear cells during chronic disease (19,37). Because we detected no difference in migratory capacity between WT and Twist1-knockout T cells or T-cell numbers in WT and Twist1-TKO spleens after UUO, we speculate that the enhanced CD8+ T-cell accumulation in Twist1-TKO obstructed kidneys may relate to effects of Twist 1 on T-cell survival in the obstructed kidney, or rates of T-cell egress from the injured kidney. In this regard, TNFα has been shown to promote T-cell survival (38,39).

In our studies, Twist1 restrained expression of TNFα, but not IL-17A or IL-1β, in CD8+ T cells infiltrating the obstructed kidney. In other systems, Twist1 can also regulate IL-17A and IL-1β expression, so this isolated effect of Twist1 on T-cell TNFα may be specific to the UUO microenvironment (19,20). TNFα is a pleiotropic cytokine exhibiting a dual effect on fibrogenesis that may depend on its cellular source or site of injury, so we posited that TNF in T cells could be mediating renal protection in our model. Consistent with this notion, we found that selective deletion of TNFα from T lymphocytes augmented extracellular matrix deposition and kidney injury after UUO. Our studies cannot distinguish whether the primary effect of TNF in this context was to limit tubular injury, interstitial fibrosis, or both.
Regardless, this finding contrasts with a multitude of studies implicating TNF in tissue fibrogenesis. For example, treatment with TNF antagonists attenuates silica- and bleomycin-induced pulmonary fibrosis (40,41), whereas continuous infusion of recombinant TNF enhances scar formation in the lung (40), and overexpression of TNF in the lungs promotes local fibroblast accumulation and collagen deposition (42). On the other hand, inhibiting TNF worsened lung fibrosis in a patient with systemic sclerosis and led to progression of fibrosis (43). Moreover, TNF inhibits the synthesis of Col-I, Col-III, and FN in cultured dermal fibroblasts (44,45), and reduces the numbers of fibrocytes and deposition of Col-I in the obstructed kidney (8). On a molecular level, TNF may blunt collagen accumulation through interactions with the classical NF-κB and TGF-β1/Smad signaling pathways or the matrix-degrading enzyme system (32). These studies corroborate a protective function for TNF in T lymphocytes during renal fibrogenesis after UUO and are in agreement with our finding that TNF in T cells can restrain inflammatory injury to the glomerulus (33).

Collectively, these data indicate that Twist1 in T lymphocytes promotes kidney injury and fibrosis, potentially via Twist1’s effects to suppress TNF generation in infiltrating T cells. Our studies suggest that blocking TNF directly in T lymphocytes could exacerbate kidney scar formation, warranting further scrutiny to identify targets that act downstream of Twist1 and TNF in T cells to regulate the pathogenesis of kidney fibrosis.

Disclosures
J.R. Privratsky reports serving on the scientific advisory board for the Association of University Anesthesiologists, and being a scientific advisor for, or member of, Life Sciences. All remaining authors have nothing to disclose.

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Figure 7. | TNF deletion in T cells enhances UUO-induced kidney injury. (A) Representative images of sections of obstructed kidneys from WT and TNF-TKO mice, stained with Periodic acid–Schiff (PAS). Scale bar, 100 μm. (B) WT and TNF-TKO obstructed kidney pathology scores (n=8). (C) Renal mRNA levels for neutrophil gelatinase-associated lipocalin (NGAL) from contralateral (CTL) and obstructed (UUO) kidneys (n=8). Data represent the mean±SEM. *P<0.05.

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Author Contributions
S.D. Crowley was responsible for funding acquisition and resources, and provided supervision; S.D. Crowley, R. Griffiths, J.R. Privratsky, and J. Ren were responsible for methodology; S.D. Crowley, R. Griffiths, and J. Ren were responsible for investigation;
S.D. Crowley, J.R. Privratsky, and J. Ren were responsible for formal analysis and reviewed and edited the manuscript; S.D. Crowley and J. Ren conceptualized the study; R. Griffiths, X. Lu, and J. Ren were responsible for project administration; J.R. Privratsky, X. Lu, and J. Ren were responsible for data curation; and J. Ren wrote the original draft.

Supplemental Material

This article contains the following supplemental material online at http://kidney360.asnjournals.org/lookup/suppl/doi:10.34067/KID.00071820/~/DCSupplemental.

Supplemental Figure 1. mRNA levels of Twist1 in CD3+ T lymphocytes from naïve spleens and obstructed kidneys (“UUO”).

Supplemental Figure 2. The weights of UUO kidneys from WT and Twist1 TKO mice.

Supplemental Figure 3. The gating strategy for excluding cellular debris and analyzing viable CD4 and CD8+ T cells from Twist1 TKO mice.

References


41. Piguet PF, Vesin C: Treatment by human recombinant soluble TNF receptor of pulmonary fibrosis induced by bleomycin or silica in mice. *Eur Respir J* 7: 515–518, 1994 https://doi.org/10.1183/09031936.94.07030515


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