IL-1RI deficiency ameliorates early experimental renal interstitial fibrosis

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Abstract

Background. IL-1β has the potential to promote progressive renal disease by effects on macrophage recruitment and activation or by effects mediated through tubular cell transforming growth factor (TGF)-β production, previously demonstrated in vitro.

Methods. The in vivo roles of endogenous IL-1β and its type I receptor (IL-1RI) in renal fibrosis were studied using wild-type C57BL/6 mice, IL-1β−/− and IL-1RI−/− mice with unilateral ureteric obstruction.

Results. After 7 days, IL-1RI−/− mice (IL-1α and IL-1β deficient) were protected from injury and collagen accumulation. IL-1β−/− mice demonstrated some histological protection, but no reduction in α1(1) procollagen mRNA or biochemically measured collagen accumulation. Compared with obstructed kidneys from wild-type mice, TGF-β1 mRNA was reduced in IL-1RI−/− mice (with trends to reduced TGF-β2 and TGF-β3). Expression of a downstream TGF-β effector, connective tissue growth factor, was decreased in IL-1RI−/− mice. IL-1RI−/− mice exhibited less tubulointerstitial apoptosis compared with wild-type mice. Macrophage infiltration and adhesion molecule mRNA expression was unchanged in IL-1β−/− or IL-1RI−/− mice. While TNF expression was similar to wild-type mice, IFN-γ expression was reduced in both IL-1β−/− and IL-1RI−/− mice. IL-1RI−/− mice at 14 days showed a catch-up in fibrosis compared with wild-type mice.

Conclusion. IL-1/IL-1RI interactions are profibrotic in renal fibrosis. IL-1RI−/− mice were more protected at an early stage, associated with changes in TGF-β and downstream mediators of fibrosis, but independent of the presence of infiltrating macrophages.

Keywords: IL-1; interstitial fibrosis; macrophages; obstructive uropathy; TGF-β

Introduction

Tubulointerstitial fibrosis is a common pathway to end-stage renal failure occurring after a variety of immune and inflammatory, metabolic or haemodynamic renal insults. The initiating injury, timing and persistence of the initiating insult are all important in tubulointerstitial fibrosis. However, observations from human disease and in vitro and in vivo models have defined several processes common to tubulointerstitial fibrosis induced by a variety of different insults. These include tubular injury, the infiltration of innate immune effectors, epithelial myofibroblast
transformation and the laying down of matrix proteins, including collagen and fibronectin, with resulting interstitial fibrosis, tubular atrophy and loss of renal function [1–3].

Potential pro-fibrogenic mediators present in the tubulointerstitium include cytokines, secreted by immune/inflammatory cells and/or intrinsic renal cells. IL-1β possesses a number of proinflammatory effects relevant to a range of diseases [4,5]. IL-1β binds to the IL-1 receptor (IL-1RI), constitutively expressed at low levels on most cells, including ‘immune’ cells, endothelial cells, epithelial cells and fibroblasts [4]. Another member of the IL-1 family, IL-1α, can bind to and signal via this receptor. IL-1α plays an autocrine role in cell activation, being expressed on the cell surface, intracellularly and also released from cells [4,5]. The IL-1 receptor antagonist (IL-1Ra) antagonizes the activity of IL-1 in vivo by competitively binding to IL-1RI [6]. Another membrane receptor, IL-1RII, acts as a decoy receptor and does not signal on binding to IL-1 [7]. Both IL-1RI and IL-1RII can exist in soluble forms [4]. IL-1β has a number of biological actions that might promote fibrosis, including promoting leukocyte infiltration, inducing proinflammatory mediators and inducing the production of transforming growth factor (TGF-β), a key profibrotic growth factor. In vitro, both IL-1α and IL-1β promote TGF-β production [8–10] as well as fibronectin production and loss of E-cadherin expression (by IL-1α) or induction of α-smooth muscle actin (α-SMA), a myofibroblast marker expression (by IL-1β). These changes in primary cultures of human proximal tubular cells are TGF-β dependent [8,10]. Both IL-1α and IL-1β are produced by fibroblasts [11,12]. IL-1 has similar effects on TGF-β and matrix production in cultured cortical fibroblasts and also promotes fibroblast proliferation [11,13], and fibroblasts derived from diseased kidneys demonstrate greater IL-1 responsiveness than those from normal kidneys [11,12].

Obstructive uropathy is an important cause of renal fibrosis, with the involvement of macrophages, tubular cells and apoptosis of tubular cells, interstitial myofibroblasts, soluble cytokines/growth factors (the best known being TGF-β), proteolytic enzymes and matrix proteins [5,14–16]. It is commonly modelled by ligating one ureter (unilateral ureteric ligation, UUO) in mice. In this system, bone marrow-derived CD11b+ and CD18+ cells modified to overexpress IL-1Ra limit injury in murine UUO after 6 days [17]. The hypothesis tested by the current studies was that mice genetically deficient in IL-1β or the IL-1RI would be protected from experimental interstitial fibrosis induced by UUO.

**Methods**

**Experimental mice**

Mice with a deletion of the IL-1β (IL-1β−/−, from Y. Iwakura, University of Tokyo, Tokyo, Japan [18]) or the IL-1RI gene (IL-1RI−/−, from A. Satsokar, Ohio State University, OH, USA [19]) on a C57BL/6 background were bred in an SPF facility (Monash Medical Centre, Clayton, Victoria, Australia). Male mice (6–8 weeks old) were used. The left ureter was ligated under general anaesthesia [20] and renal injury was studied after 7 or 14 days. Experiments were approved by the Monash University, Monash Medical Centre Animal Ethics Committee. Histological assessment was performed on coded slides, results expressed as mean ± SD.

For analysis of two groups, the significance of differences between groups was determined by an unpaired t-test, and for more than two groups by ANOVA, with Tukey’s post-test (Prism, GraphPad Software, San Diego, CA, USA). Seven days after UUO, C57BL/6 wild-type (WT) mice (n = 7), IL-1β−/− mice (n = 13) and IL-1RI−/− mice (n = 7) were studied. For 14-day analyses, C57BL/6 WT mice (n = 7) and IL-1RI−/− mice (n = 9) were studied.

**Detection of IL-1RI in mice subjected to UUO**

Renal IL-1RI protein was demonstrated using anti-mouse CD121a (IL-1 receptor type I p80, 35F5, BD Biosciences, San Jose, CA, USA) conjugated with R-phycocerythrin. Cryostat-cut snap frozen sections (6 μm) were incubated with 15% rat serum in 5% BSA/PBS (10 min, room temperature) and then with anti-IL-1RI-PE (1:50 in 1% BSA/PBS, 1 h, room temperature). Sections from IL-1RI−/− mice were negative controls.

**Assessment of morphological changes and collagen accumulation**

Kidney tissues were fixed in Bouin’s fixative and embedded in paraffin, and 3-μm tissue sections from all mice were stained with picrosiris red (Sigma, St Louis, MO, USA) identifying collagen fibres (red) [20,21]. Histological assessment of matrix deposition was determined by point counting using a 10 × 10 grid. A minimum of 10 high power fields (×400, hpf) were assessed per animal, and results expressed as % interstitial cortical area, excluding glomeruli, blood vessels, periglomerular and perivascular areas [20,22]. Renal collagen content was measured by determining total hydroxyproline [23] as previously described [20], and results expressed as μg/mg kidney wet weight.

**Intersitial macrophages, neutrophils, cleaved caspase-3 and myofibroblasts**

For neutrophils and macrophages, paraformaldehyde-fixed frozen tissue sections (6 μm) were stained with RB6-8C5 (anti-Gr-1) or F4/80 using a three-layer immunoperoxidase technique [20,24]. Neutrophil numbers were counted in ≥10 hpf per animal. As individual macrophages could not be reliably counted [20], ≥10 hpf were assessed per animal and cortical interstitial infiltrate scored as 0–3+ (0-equivalent to normal animals and the contralateral kidney of experimental animals, 1: 10–40% interstitium, 2: 40–70% interstitium, 3: >70% interstitium). For cleaved caspase-3, 4 μm paraffin-embedded sections were de-paraffinized (1 h, 60°C), followed by hydration and antigen retrieval. After blocking biotin and endogenous peroxidases, the sections were incubated with 10% swine serum (1 h, room temperature), then with a rabbit anti-human caspase-3 antibody (1:400, overnight, 4°C; Cell Signaling Technology, Danvers, MA, USA) that cross-reacts with mouse caspase-3 [25] and then with swine anti-rabbit biotin (1:100, 1 h, room temperature; Dako), followed by avidin/biotin (Dako) with 3,3′-diaminobenzidine. Ten consecutive hpf were assessed per animal, excluding glomeruli and large vessels and results expressed as cells/hpf (c/hpf). Immunohistochemistry using Bovin’s fixed tissue sections (4 μm) was used for α-SMA. The sections were stained with a peroxidase-conjugated mouse anti-human α-SMA antibody (1A4) using the Enhanced Polymer One-Step Staining reagent (Dako) [20] and then incubated overnight at 4°C; binding was detected using 3,3′-diaminobenzidine and the sections were counterstained with nuclear fast red (BDH Chemicals, Poole, UK). Dako negative control EPOS immunoglobulins-HRP were used as a negative control. Intestinal α-SMA accumulation was assessed by point counting.

**Assessment of intrarenal chemokine, cytokine and collagen mRNA expression**

The ribonuclease (RNAse) protection assay was performed as previously described [26]. Kidney RNA was extracted with a TRizol reagent (Invitrogen, CA, USA) from randomly selected mice of a single experiment (n = 5–9 each group with UUO). Multiprobes incorporating [α-32P]UTP were transcribed from two custom templates (RiboQuant System, Pharmingen), the first containing probes for ICAM-1 IL-1RI, E-selectin, TNF, VCAM-1, IL-1β, IL-1Ra, IL-18, F4/80, IFN-γ and macrophage migration inhibitory factor (MIF), the second containing probes for lymphotixin-α, lymphotixin-β, TNF, IL-13, IFN-γ, type 1 procollagen α1 chain [α1(I) procollagen], TGF-β1, TGF-β2 and TGF-β3. Gene expression was normalized to the housekeeping gene L32.
Table 1. Expression of IL-1 and IL-1 receptor family mRNA in kidneys 1 week after unilateral ureteric ligation

<table>
<thead>
<tr>
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<th>WT contralateral</th>
<th>Fold induction (× contralateral kidney)</th>
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<tbody>
<tr>
<td>IL-1α mRNA</td>
<td>0.13 ± 0.07</td>
<td>0.76 ± 0.55a 5.84</td>
</tr>
<tr>
<td>IL-1β mRNA</td>
<td>0.74 ± 0.08</td>
<td>2.79 ± 1.28 3.77</td>
</tr>
<tr>
<td>IL-1RI mRNA</td>
<td>1.33 ± 0.21</td>
<td>3.76 ± 1.20 2.82</td>
</tr>
<tr>
<td>IL-1Ra mRNA</td>
<td>0.54 ± 0.10</td>
<td>8.28 ± 1.54 15.33</td>
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All values are expressed as mean ± SD, arbitrary units. 

For IL-1α mRNA, 1 µg RNA (n = 6–7 for each group with UUO) was treated with 1 unit of amplification grade DNase I (Invitrogen), then primed with 500 ng Oligo(dT)12-18 (Roche, Mannheim, Germany) and reverse-transcribed (Super Script II, Invitrogen). For IL-1α (113 bp product) and β-actin (388 bp product) primers (Vector NTI software, Invitrogen), see the Supplementary data table. Real-time PCR was performed on a Rotor Gene RG-3000 (Corbett Research, NSW, Australia) using FastStart DNA Master, SYBR Green I (Roche). IL-1α and β-actin mRNA expression was quantified using serial dilutions of an exogenous standard, and IL-1α levels normalized to β-actin and expressed as arbitrary units. For connective tissue growth factor (CTGF) and α-SMA mRNA (n = 7–10 each group with UUO), TaqMan minor groove binder probes (Applied Biosystems 7500, Foster City, CA, USA) were linked to 6-carboxyfluorescein, and gene expression was analysed by real-time quantitative RT-PCR (TaqMan, Applied Biosystems) with 18S ribosomal RNA expression assessed (18S rRNA TaqMan Control Reagent kit) as a control. Results were expressed proportional to values obtained from WT mice obstructed kidneys.

Results

IL-1β and IL-1RI expression in obstructed kidneys of mice with UUO

IL-1 and IL-1R family gene expression was assessed in mice with obstructed kidneys 7 days after UUO. Both IL-1α and IL-1β mRNA were induced in kidneys of mice with disease (Table 1). In addition to induction of IL-1α and IL-1β expression, induction of the mRNA for IL-1RI and IL-1Ra was observed in obstructed kidneys. Immunofluorescent staining for IL-1RI revealed staining in areas of the interstitium and in tubules in obstructed kidneys from WT mice at Day 7 after UUO (Figure 1A), with minimal fluorescence in obstructed kidneys from IL-1RI−/− mice (Figure 1B) as a negative control.

Renal interstitial fibrosis is reduced in the absence of the IL-1RI

Compared with the unobstructed contralateral kidney (Figure 2A and B), obstructed kidneys from 7-day UUO mice demonstrated increased picrosirius red staining and increased matrix production (Figure 2C and D). Histologically, mice deficient in either IL-1β or IL-1RI exhibited reduced injury with reduced picrosirius red staining (Figures 2E–H and 3A). Analyses of the biochemical accumulation of collagen (Figure 3B) and α1(I) procollagen mRNA expression (Figure 3C) confirmed that IL-1RI−/− mice were relatively protected from collagen accumulation.

Fig. 1. Expression of IL-1RI in obstructed kidneys 7 days after undergoing unilateral ureteric ligation. In obstructed kidneys from C57BL/6 wild-type (WT) mice (A), IL-1RI expression was observed in the interstitium and at varying intensity within tubules. Signal was absent in obstructed kidneys from IL-1RI−/− mice (B). Original magnification ×200.

Fig. 2. Renal histopathology of C57BL/6 wild-type (WT) mice, IL-1β−/− mice and IL-1RI−/− mice 7 days after undergoing unilateral ureteric obstruction. Contralateral kidneys from WT mice demonstrated no abnormalities (A and B). Seven days after ureteric ligation, WT mice demonstrated tubular dilatation, interstitial expansion and increased matrix expression (red, C and D). In IL-1β−/− mice, these changes were relatively less severe (E and F). Mice deficient in the IL-1RI demonstrated substantial reduction of inflammation and tubular matrix expression (red). (G and H). Picrosirius red staining of paraffin-fixed sections. All photomicrographs are taken of the outer cortex. Original magnifications ×100 (A, C, E and G) and ×200 (B, D, F and H).
in interstitial fibrosis, although values for IL-1β−/− mice were not different from those for WT mice.

Endogenous IL-1/IL-1RI interactions enhance TGF-β and CTGF mRNA expression

To understand why IL-1/IL-1RI interactions play a profibrotic role in vivo in UUO, the expression of the three isoforms of TGF-β and of CTGF, a downstream effector of TGF-β, was measured. Compared with WT, IL-1RI−/− obstructed kidneys expressed less TGF-β1 mRNA and less CTGF mRNA (Figure 4). Apparent reductions in TGF-β2 and TGF-β3 did not reach statistical significance. In IL-1β−/− mice, TGF-β1 mRNA and CTGF mRNA were similar to WT values (Figure 5D), IL-1β−/− mice had similar α-SMA mRNA expression and a similar proportion of the interstitium covered by α-SMA-expressing cells (Figure 5E). In IL-1RI−/− mice, the reduction in α-SMA mRNA expression fell just short of statistical significance (ANOVA, P = 0.054).

Renal tubulointerstitial apoptosis and myofibroblast accumulation

Apoptosis is important in the development of renal fibrosis due to obstruction. Immunohistochemistry revealed significant apoptosis, determined by cleaved caspase-3 positive tubular and interstitial cells, in obstructed kidneys from WT mice (Figure 5A and G). IL-1RI−/− mice were protected from apoptosis, with fewer cleaved caspase-3-positive cells present in the tubulointerstitium of obstructed kidneys (Figure 5C), but IL-1β deficiency did not result in reduced apoptosis (Figure 5B). As a marker for epithelial myofibroblast transformation, α-SMA was assessed (Figure 5D–F, H and I). Compared with WT mice, Macrophage and neutrophil infiltration is unaffected in the absence of either IL-1β or IL-1RI

Seven days after the induction of UUO, WT mice exhibited a significant infiltrate of F4/80+ macrophages in the tubulointerstitium (Figure 6A and D). The extent of this macrophage infiltrate was unaltered in the absence of either IL-1β or IL-1RI (Figure 6B–D). To confirm this negative finding, intrarenal expression of F4/80 mRNA was similar in all three groups of mice at 7 days (Figure 6E). Neutrophil accumulation was also unaffected by IL-1β or IL-1RI deficiency (WT 2.9 ± 1.0, IL-1β−/− 2.4 ± 1.2, IL-1RI−/− 3.5 ± 0.71 cells/hpf).
Expression of pro-inflammatory cytokines and adhesion molecules in IL-1β−/− and IL-1RI−/− mice

As IL-1 can induce other proinflammatory mediators, proinflammatory cytokines and adhesion molecules were studied in obstructed kidneys from WT, IL-1β−/− and IL-1RI−/− mice (Table 2). Induction of these mRNA species was observed in the obstructed kidney (compared with the contralateral kidney, except for MIF mRNA, paradoxically decreased). IL-1β or IL-1RI deficiency did not affect expression of TNF, MIF or IL-18 mRNA. Compared with obstructed kidneys from WT mice, IFN-γ mRNA expression was decreased in obstructed kidneys from both IL-1β−/− and IL-1RI−/− mice. Consistent with the lack of the effect of endogenous IL-1 on macrophage accumulation, in obstructed kidneys, mRNA for E-selectin, ICAM-1 and VCAM-1 was unaffected by the absence of either IL-1β or IL-1RI.

Changes in IL-1/IL-1R family gene expression in IL-1β−/− and IL-1RI−/− mice

To assess whether up- or downregulation of IL-1α, IL-1RI or IL-1Ra in IL-1β−/− mice explains the more significant protection in IL-1RI−/− mice, expression of IL-1α, IL-1β, IL-1RI and IL-1Ra was assessed in IL-1β−/− and IL-1RI−/− mice (Table 3). No compensatory changes that would account for this difference were observed. In obstructed kidneys of IL-1β−/− mice, neither IL-1α nor IL-1RI was further up-regulated. IL-1Ra, potentially protective in UUO, was not downregulated in IL-1β−/− mice. In obstructed kidneys of IL-1RI−/− mice, IL-1β was not upregulated compared with WT. IL-1α mRNA was not significantly increased in IL-1RI−/− mice over WT, but in IL-1RI−/− mice, mRNA for IL-1Ra was increased over WT values.

Catch-up in interstitial fibrosis at 2 weeks in IL-1RI−/− mice

To determine whether IL-1RI deficiency results in lasting protection from interstitial fibrosis, IL-1RI−/− mice were studied 2 weeks after UUO. Histologically, fibrosis had progressed in WT mice and appearances in IL-1RI−/− mice were similar to WT (Figure 7A–D). This catch-up in disease was confirmed by measurement of indices of renal fibrosis. With the exception of a trend to reduced α1(1) procollagen mRNA expression (P = 0.16), values were similar in 2-week obstructed kidneys in WT and IL-1RI−/−
mice (Figure 8). Analysis of other mediators of fibrosis and inflammation (Table 4) showed no difference in TGF-β mRNA, α-SMA accumulation or adhesion molecule expression. Scoring of infiltrating F4/80+ macrophages was identical, but compared with obstructed WT kidneys at 2 weeks, F4/80 mRNA was reduced in IL-1RI−/− mice. The reduced IFN-γ mRNA observed at 1 week was no longer apparent and both MIF and IL-18 mRNA were increased in IL-1RI−/− mice.

Discussion

Interactions between IL-1 and the IL-1RI are important in inflammatory renal disease. Human data suggest that polymorphisms in the IL-1 gene cluster, specifically, the variable-number-of-tandem-repeats polymorphism in the gene encoding IL-1Ra and a single-nucleotide polymorphism in the IL-1α promoter, were associated with an increased risk of end-stage renal failure [27]. We hypothesized that IL-1 and the type I IL-1 receptor contribute to experimental progressive renal disease. As we expected, mRNA for the IL-1 family, both proinflammatory (IL-1α, IL-1β and IL-1RI) and anti-inflammatory (IL-1Ra), were upregulated by 7 days after UUO. Previous studies have demonstrated upregulation of IL-1β and IL-1RI at an mRNA and/or protein level [17], and in vitro studies have demonstrated that renal fibroblasts produce both IL-1α and IL-1β [12]. The current studies tested the hypotheses that these interactions are important in obstructive uropathy-induced renal fibrosis. They demonstrate that IL-1RI plays a profibrotic role in obstructive uropathy by promoting TGF-β1 mRNA expression and its downstream effects, including the induction of CTGF and expression of type I...
collagen mRNA and protein, findings supported by reduced apoptosis in IL-1RI−/− mice. IL-1 can affect macrophages, particularly by enhancing their recruitment into the kidney, and in overtly immune-mediated experimental renal injury, IL-1 does affect macrophages. However, our studies found no effects on macrophage or neutrophil infiltration or expression of adhesion molecules, confirming that IL-1 acts on TGF-β, independent of effects on immune cells.

The current studies, supporting a profibrotic role for the IL-1RI in renal fibrosis, are concordant with in vitro studies [10–13] and in vivo studies using IL-1Ra [17]. There are several mechanisms by which this can occur, including adhesion molecule induction, enhancement of inflammatory macrophage recruitment, macrophage activation or induction of TGF-β, well recognized as an important profibrotic growth factor [28]. Some studies in experimental renal disease have suggested that IL-1/IL-1RI interactions are pathogenetic due to their effects on leukocyte recruitment and accumulation [17,29–31]. In our studies, no change in macrophage infiltration was observed. At least one other study has inhibited IL-1 and found no alterations in the recruitment of macrophages, unless other cytokines were concurrently neutralized [32]. It is likely that the nature and intensity of the disease (in the current studies, complete ureteric obstruction) can engage other mediators of leukocyte recruitment in an IL-1RI-independent manner. IL-1 does have the capacity to promote macrophage accumulation and increase the expression of adhesion molecules, and deficiency of leukocyte adhesion molecules limits fibrosis in newborn mice [33]. However, E-selectin, ICAM-1 or VCAM-1 mRNA expression was unchanged in the absence of IL-1β or IL-1RI. Not all studies have reported that endogenous IL-1 affects adhesion molecules, suggesting that the effects of IL-1 on adhesion molecules may be stimulus specific. For example, in MRL/lpr mice, IL-1 neutralization did not alter ICAM-1 expression [34]. The effects of IL-1/IL-1RI on the production of other proinflammatory molecules revealed that the expression of TNF, MIF and IL-18 mRNA was unaffected, but IFN-γ mRNA expression was decreased. While IFN-γ activates macrophages to enhance inflammation, potentially increasing injury and downstream fibrosis, it can also antagonize TGF-β-induced collagen gene expression [35]. The net effect of changes in IFN-γ expression, particularly in renal diseases independent of adaptive immune responses, is uncertain [36–38].

In vitro studies using primary cultures of human proximal tubular cells or cortical fibroblasts suggest that IL-1β’s profibrotic effects are TGF-β mediated [10,13]. IL-1α could promote fibrosis by enhancing epithelial myofibroblast transformation in a TGF-β-dependent manner [8]. The current studies demonstrate that in vivo, IL-1/IL-1RI interactions promote TGF-β, CTGF and α-SMA gene expression consistent with these and other in vitro studies [8–10,13]. In addition, reduced apoptosis in IL-1RI−/− mice is likely to have been due to reduced TGF-β [39]. IL-1β has been previously demonstrated in infiltrating cells and in tubular cells in both experimental and human renal disease [40–42]. A number of cell types, both immune cells and resident tissue cells, have the capacity to express IL-1α, IL-1β and the IL-1R. Functional studies using chimeric mice in experimental glomerulonephritis demonstrated that interactions between leukocyte-derived IL-1β and renal tissue cell-expressed IL-1RI are important in renal injury [43,44]. From our studies it is not possible to ascertain the dominant cellular source of IL-1 in UUO, but IL-1’s effects are likely to be through IL-1RI on both tubular cells and fibroblasts.

IL-1RI-deficient mice were more protected than IL-1β−/− mice. There were no changes in IL-1α or IL-1Ra mRNA in IL-1β−/− mice, suggesting that IL-1β−/− mice did not develop compensatory changes in the developmental absence of IL-1β. Incidentally, IL-1RI−/− mice did show IL-Ra increased expression, but as the IL-1RI is the

**Table 4.** Fibrotic and inflammatory parameters after 2 weeks in kidneys subjected to unilateral ureteric obstruction in genetically normal wild-type (WT) mice and IL-1R−/− mice

<table>
<thead>
<tr>
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<th>WT</th>
<th>IL-1R−/−</th>
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<tr>
<td>TGF-β1 mRNA</td>
<td>57.1 ± 28.0</td>
<td>54.9 ± 30.0</td>
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<td>TGF-β2 mRNA</td>
<td>59.8 ± 30.6</td>
<td>46.8 ± 30.1</td>
</tr>
<tr>
<td>TGF-β3 mRNA</td>
<td>47.3 ± 22.9</td>
<td>31.3 ± 22.0</td>
</tr>
<tr>
<td>α-SMA (%)</td>
<td>52.8 ± 20.0</td>
<td>35.9 ± 18.5</td>
</tr>
<tr>
<td>Macrophages (0–3+)</td>
<td>2.7 ± 0.2</td>
<td>2.7 ± 0.7</td>
</tr>
<tr>
<td>F4/80 mRNA</td>
<td>7.0 ± 0.8</td>
<td>4.4 ± 0.7a</td>
</tr>
<tr>
<td>TNF mRNA</td>
<td>10.9 ± 1.6</td>
<td>12.4 ± 2.0</td>
</tr>
<tr>
<td>Neutrophils (c/hpf)</td>
<td>1.6 ± 1.2</td>
<td>2.6 ± 1.2</td>
</tr>
<tr>
<td>IL-1α mRNA</td>
<td>1.49 ± 0.23</td>
<td>2.18 ± 0.33b</td>
</tr>
<tr>
<td>MIF mRNA</td>
<td>142 ± 35</td>
<td>221 ± 47a</td>
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<tr>
<td>IFN-γ mRNA</td>
<td>5.34 ± 2.44</td>
<td>4.63 ± 4.22</td>
</tr>
<tr>
<td>E-selectin mRNA</td>
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<td>ICAM-1 mRNA</td>
<td>27.3 ± 6.3</td>
<td>24.2 ± 6.9</td>
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<tr>
<td>VCAM-1 mRNA</td>
<td>99.3 ± 14.5</td>
<td>119 ± 30.6</td>
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Except as indicated, all values are expressed as mean ± SD, arbitrary units. 
A P < 0.01, B P < 0.001.

Fig. 8. Collagen accumulation in obstructed kidneys 2 weeks after undergoing unilateral ureteric ligation. Two weeks after unilateral ureteric ligation, collagen accumulation in IL-1R−/− mice was similar to that in C57BL/6 wild-type (WT) mice assessed by picrosirius red staining (A), biochemical assessment of collagen accumulation (B) and expression of mRNA for the α1 chain of type I procollagen (C, P = 0.16). Dotted lines represent values from contralateral kidneys.
only signalling receptor for IL-1α and IL-1β, this change is unlikely to be biologically significant. IL-1α is expressed in diseased human kidneys [42], has pro-inflammatory effects on renal tubular cells [45] and may induce epithelial myofibroblast transformation [8]. Taking these data into account, the probable explanation for the greater protection in mice deficient in IL-1RI is that IL-1α plays a pathogenic role in disease.

To determine the absolute requirement for IL-1RI, the effects of IL-1RI deficiency were assessed 2 weeks after UUO. By this time, IL-1RI−/− mice had developed similar disease to that of WT mice, suggesting significant catch-up in injury. Although there was a trend towards reduced α1(I) procollagen mRNA, collagen accumulation and histological injury were similar, as was the expression of TGF-β isoforms. These findings demonstrate that, at least in this model of fibrosis involving an ongoing and pervasive fibrotic stimulus (i.e. ureteric obstruction with ongoing mechanical stress and inflammation), other pathways that lead to TGF-β production and fibrosis are able to, with time, compensate for the absence of IL-1/IL-1R interactions.

IL-1 has the capacity to influence adaptive immune responses, which can themselves cause fibrosis. Although reducing inflammation by modulating adaptive immune responses reduces collagen accumulation [26], renal fibrosis induced by UUO is T cell (and adaptive immunity) independent [46]. The potential for deletion/inhibition of IL-1 or IL-1RI to modulate the direction of the adaptive immune response, while important in some renal diseases [29,43,47], is not relevant to these studies. The current studies examine UUO-induced renal fibrosis and cannot necessarily be generalized to all forms of progressive renal disease. However, there is functional evidence in other in vivo models of progressive renal disease and other organ systems that IL-1 is important in fibrosis. This evidence includes IL-1Ra offering protection from progressive renal disease in pathological adaptive immune responses affecting the kidney [30], improvement in experimental pulmonary fibrosis after IL-1Ra [48] and radiation-induced dermal fibrosis [49]. In summary, in renal fibrosis induced by UUO, interactions between endogenous IL-1 (IL-1α and IL-1β) and the IL-1RI are profibrotic. Their effects are mediated through the production and effects of TGF-β and not by enhancing macrophage recruitment, but at least in UUO, the protective effects of IL-1RI deficiency do not persist when fibrosis progresses.

Supplementary data

Supplementary data are available online at http://ndt.oxfordjournals.org.

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Conflicts of interest statement. None declared.

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