original article

Interleukin-1 accounts for intrarenal Th17 cell activation during ureteral obstruction

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Interleukin 17A-secreting T-helper 17 (Th17) cells are pathogenic in inflammatory kidney diseases, but their intrarenal regulation is poorly understood. In order to better define Th17 cell dynamics during interstitial inflammation, we utilized the mouse unilateral ureteral obstruction model to analyze inflammatory cell subtypes by multicolor flow cytometry and cell sorting and by effects on *in vitro*-generated Th17 cells. Interleukin 17A expression localized to CCR6$^+$CCR4$^{+/-}$CD4$^+$ T-cells and progressively increased in obstructed kidneys. The number of CCR6$^+$CD4$^+$ T-cells increased over 10-fold by 72 h, were enriched for interleukin 17A production, and were highly proliferative based on *in vivo* bromodeoxyuridine incorporation. Secreted products of leukocytes isolated from obstructed kidneys enhanced the interleukin 17A production of *in vitro*-generated Th17 cells. This Th17-enhancing activity was identified as interleukin-1 produced by renal dendritic cells and monocytes. The *in vivo* validity of these findings was confirmed in mice lacking the interleulin-1 receptor and in mice treated with a recombinant interleukin-1 receptor antagonist, each of which exhibited reduced intrarenal Th17 activity compared with control mice. Thus, the inflamed kidney accumulates CCR6$^+$ Th17 cells that undergo activation and proliferation. Production of interleukin 1 family cytokines by resident dendritic cells and infiltrating monocytes enhances intrarenal Th17 activation in acute kidney injury.

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Acute kidney injury (AKI) is characterized by increased interstitial lymphocytes, mononuclear phagocytes, and neutrophils.[1,2] Obstruction and ischemia represent 30–50% of AKI and frequently progress to chronic kidney disease.[3–5] During AKI and glomerulonephritis, CD4$^+$ T-helper (Th) cells participate in renal inflammation and fibrosis.[6–10] Naïve Th cells differentiate into functionally distinct subsets distinguished by signature effector cytokines.[11,12] Th type 1 (Th1) cells produce interferon-gamma (IFN-γ) and are linked with tissue-destructive inflammation, whereas Th2 cells secrete interleukin (IL)-4, -5, and -13 and, under some conditions, are anti-inflammatory.[11,12] Recently some disease processes, originally believed to be Th1 mediated, were shown to be partly or predominantly driven by a proinflammatory CD4$^+$ T-cell subtype, Th17, which is characterized by the production of IL-17 family cytokines.[13–15] Interleukin-17A, the best characterized and most potent of these, stimulates neutrophil migration to sites of inflammation and amplifies effects of other proinflammatory cytokines on fibroblasts, epithelial, and mesangial cells.[13,16,17] IL-17A-producing cells are pathogenic in Crohn's disease, multiple sclerosis, rheumatoid arthritis,[15] glomerulonephritis,[9,18,19] and AKI.[20,21]

Th17 cells differ from Th1 and Th2 cells by cytokine profile, chemokine receptor expression, and mechanisms of differentiation and activation.[14] In humans, Th17 cells express CCR2, CCR4, CCR6, and CXCR4,[22,23] whereas in mouse they preferentially express CCR6, CCR4, and CCR7.[24] Although Th17 cells express some trafficking receptors in a tissue-specific manner, CCR6 appears to be uniformly expressed.[23] Th17 differentiation is incompletely understood but transforming growth factor-β, IL-6, IL-21, and IL-23 are implicated in both humans and mouse.[15,25–27] IL-23 promotes the expansion of established Th17 populations but does not induce Th17 differentiation in naïve T-cell precursors.[28] IL-1 family cytokines are also critically required for early Th17 cell programming and for Th17 cell-mediated autoimmunity.[14,29] In synergy with IL-6 and IL-23, IL-1 regulates Th17 cell differentiation and maintains IL-17A expression in Th17 effectors.[30] IL-1 also regulates activation

of previously differentiated (effector memory) Th17 cells during tissue-specific inflammation and autoimmunity[31]—a process that may be of specific relevance to Th17 cell involvement in renal inflammation.

Recently published studies have demonstrated the pathogenic significance of IL-17A-producing cells in diverse renal diseases.[17,18,21,32–39] Drawing upon our prior observations demonstrating the presence of renal effector memory Th17 cells in mouse unilateral ureteral obstruction (UUO),[20] the present study sought to define molecular species and their cellular sources that underlie the presence of activated Th17 cells in the obstructed kidney, the key phenotypic characteristics of these cells, especially as they relate to inflammatory processes, and the functional significance of the identified pathway accounting for the Th17 cell accumulation in the obstructed kidney.

RESULTS

IL-17A is increasingly expressed in the renal cortex following UUO and is localized to CD4^{+} T cells

Total leukocytes (CD45-enriched cells), prepared from mouse kidneys at 24, 48, and 72 h following UUO and stimulated with a low concentration of anti-CD3 antibody, produced IL-17A that was of higher concentration from cells of obstructed kidneys compared with non-obstructed (control) kidneys, and increased progressively over time (Figure 1a). The largest increment in anti-CD3-inducible IL-17A occurred between 24 and 48 h. Cortical tissue from individual kidneys was subjected to quantitative RT-PCR (qRT-PCR; Figure 1b), demonstrating a progressive increase in IL-17A mRNA in obstructed kidneys between 24 and 72 h, and confirming *in situ* IL-17A expression following UUO. Quantitative RT-PCR of magnetic bead-separated CD45^{+} and CD45^{−} cells from kidney digests indicated that IL-17A mRNA was confined to the CD45^{+} leukocyte-enriched fractions (Figure 1c). Fluorescence-activated cell sorting (FACS) of 72-h kidney digests into four individual fractions based on expression of CD45, the Th marker CD4, and the dendritic cell (DC) marker CD11c demonstrated that IL-17A mRNA was localized to the CD4^{+} fraction of obstructed kidneys (Figure 1d). Thus, consistent with our previous findings,[20] a subset of T cells within obstructed but not control kidneys are primed to secrete IL-17A in high amounts following low-level T-cell receptor stimulation. Furthermore, a progressive increase in intrarenal expression of IL-17A occurs within 72 h of UUO and is localized to CD4^{+} leukocytes.

Renal Th17 cells preferentially express CCR6 and undergo progressive accumulation and proliferation in obstructed kidneys
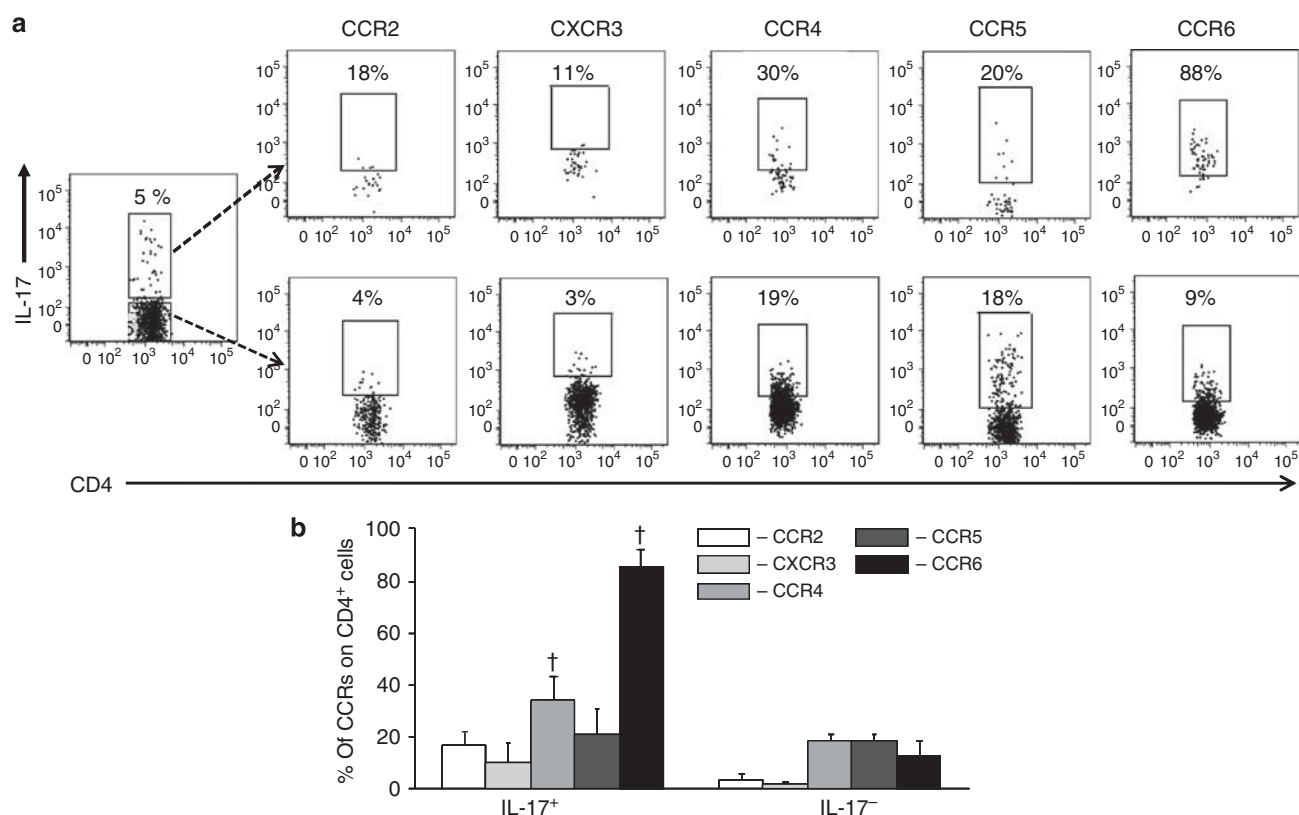
Chemokine receptor expression was examined as a means to identify T-cell sub-populations enriched for Th17 activity. Combined surface and intracellular staining of anti-CD3-stimulated cells of 72-h obstructed kidney cells was analyzed by multicolor flow cytometry. Cells were surface stained for
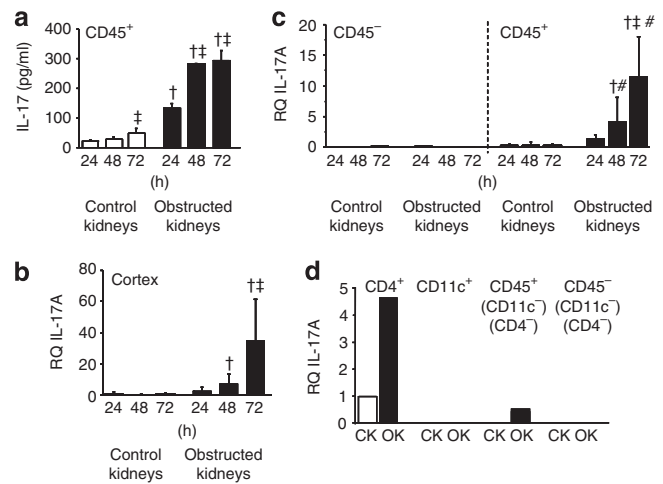
Figure 1 | Interleukin (IL)-17A expression in obstructed kidneys. Mice were subjected to unilateral ureteral obstruction (UUO) by left ureteral ligation for 24, 48, and 72 h ($n = 3$–5 per group). (**a**) Production of IL-17A in culture supernatant of CD45-enriched cells from obstructed (OK) and nonobstructed (control, CK) kidneys following 24 h culture with low-dose anti-CD3 stimulation. (**b**) Relative quantity (RQ) of IL-17A mRNA in whole cortex of OK and CKs measured by quantitative RT-PCR (qRT-PCR). (**c**) RQ of IL-17A mRNA in CD45-depleted (CD45^{−}) and CD45-enriched (CD45^{+}) cells of OK and CK measured by qRT-PCR. (**d**) RQ of IL-17A mRNA in fluorescence-activated cell sorting (FACS)-purified cell populations from CK and OK prepared 72 h after UUO. Sorted cell populations were CD4^{+} (CD4 T cells), CD11c^{+} (dendritic cells), CD45^{+} (CD4^{−}, CD11c^{−}; all other leukocytes), and CD45^{−} (CD4^{−}, CD11c^{−}; nonleukocyte kidney cells). Results are presented as means ± s.d. [†]$P < 0.05$ compared with equivalent result for control kidneys; [‡]$P < 0.05$ compared with 24-h OKs; [#]$P < 0.05$ compared with equivalent result for CD45^{−} cells.

CD45, CD4, and one of several chemokine receptors (CCR2, CCR4, CCR5, CCR6, CXCR3), and then intracellularly stained for IL-17A (Figure 2a and b). IL-17A^{+}CD4^{+} cells were most readily distinguishable from IL-17A^{−}CD4^{+} cells by frequency of CCR6 expression ($> 88\%$ versus $< 9\%$ in this experiment, one of three performed). CCR4 expression was also more frequent on IL-17A^{+}CD4^{+} cells. Combined CD4/CCR6/CCR4 staining indicated that IL-17A^{+} cells constituted 30% of CCR6^{+}CCR4^{−} and 23% of CCR6^{+}CCR4^{+} CD4^{+} T cells but were rare among the CCR6^{−} subpopulations (Figure 3a). IL-17A staining level was highest among the CCR6^{+}CCR4^{−} cells. Quantitative RT-PCR of FACS-purified CD4^{+}CCR6^{+} and CD4^{+}CCR6^{−} cells from 72-h obstructed and control kidneys confirmed that IL-17A mRNA was most readily detectable in CD4^{+}CCR6^{+} cells (Figure 3b and Supplementary Figure S1 online). Importantly, whereas CD4^{+}/CCR6^{+} cells were present within control kidneys and could be successfully purified, IL-17A mRNA was undetectable in these cells.

Subsequently, CCR6 expression ($\pm$ CCR4) was used to analyze the dynamics of Th17 cells and other CD4^{+} T cells within obstructed kidneys. Total CD4^{+}CCR6^{+}CCR4^{+} and CD4^{+}CCR6^{+}CCR4^{−} cell numbers were compared for individual obstructed and control kidneys at 24, 48, 72,

Figure 2 | Chemokine receptor expression of interleukin (IL)-17$^+$ and IL-17$^-$ CD4$^+$ T cells from obstructed kidney (OK). CD45-enriched cells from 72-h OKs were cultured for 24 h with low-dose anti-CD3 stimulation, and then surface stained for CD45, CD4, and one of several cytokine receptors (CCR2, CXCR3, CCR4, CCR5, CCR6), followed by intracellular staining for IL-17A and analysis by flow cytometry. (**a**) Representative dot plots showing frequency of chemokine receptor expression on IL-17A$^+$ and IL-17A$^-$ sub-populations following gating on CD45$^+$ CD4$^+$ cells. (**b**) Graphical representation of results expressed as mean ± s.d. for frequencies of chemokine receptor expression on IL-17A$^+$ CD4$^+$ and IL-17A$^-$ CD4$^+$ cells obstructed. $^†P < 0.05$ compared with an equivalent result for IL-17A$^-$ CD4$^+$ cells.

and 96 h post UUO (Figure 4a). The numbers of both increased early (24 h) in obstructed kidneys and continued to increase, although at a slower rate, up to 96 h. The proliferative activity of CCR6$^+$ and CCR6$^-$ Th cells accumulating in obstructed kidneys was compared by *in vivo* bromodeoxyuridine (BrdU) labeling for 72 h after UUO (Figure 4b and c). BrdU incorporation was detected in greater proportions of both CD4$^+$ T-cell subsets in obstructed compared with control kidneys. However, the proportion of BrdU$^+$ cells among the CD4$^+$CCR6$^+$ subset of obstructed kidneys was almost twice that of CD4$^+$CCR6$^-$ cells, indicating a greater rate of proliferation.

Renal leukocyte populations secrete Th17-activating factors following UUO

As we had previously observed that liposomal clodronate administration before UUO resulted in loss of intrarenal CD4$^+$ T cell priming for IL-17A production,[20] we hypothesized that intrarenal Th17 cell activity following UUO is promoted by one or more factors produced locally by cells of the mononuclear phagocyte system. To investigate further, conditioned media (CM) were prepared from CD45$^+$ and CD45$^-$ cells of obstructed and control kidneys 24, 48, and

72 h post UUO and were added to *in vitro*-generated Th17 cells during restimulation with anti-CD3 antibody. IL-17A production was quantified intracellularly by flow cytometry (Figure 5a and Supplementary Figure S2 online) and in supernatants by enzyme-linked immunosorbent assay (ELISA) (Figure 5b). The results indicated that CM from CD45$^+$ cells of obstructed kidneys promoted IL-17A production by CD4$^+$ T cells. This IL-17A-enhancing effect on IL-17A secretion was progressively greater for medium from CD45$^+$ cells prepared following 24, 48, and 72 h of UUO (Figure 5a and b) and was also observed in the absence of T-cell receptor stimulation (Supplementary Figure S3 online). The effect was absent for CM from all other fractions. These observations confirmed the production by intrarenal leukocytes of one or more soluble factors capable of enhancing Th17 cell activation. The expression of four candidate mediators—IL-1α, IL-1β, IL-23, and IL-6—was analyzed by ELISA in CM of CD11c$^+$ (DC-enriched), CD45$^+$CD11c$^-$ (non-DC leukocytes), and CD45$^-$ (non-leukocyte) cells of obstructed and control cells 72 h post UUO (Figure 6). Increased secretion of IL-1α and IL-1β was observed for both DC-enriched and DC-depleted leukocytes from obstructed kidneys. Increased production of IL-23 at this time point was observed for
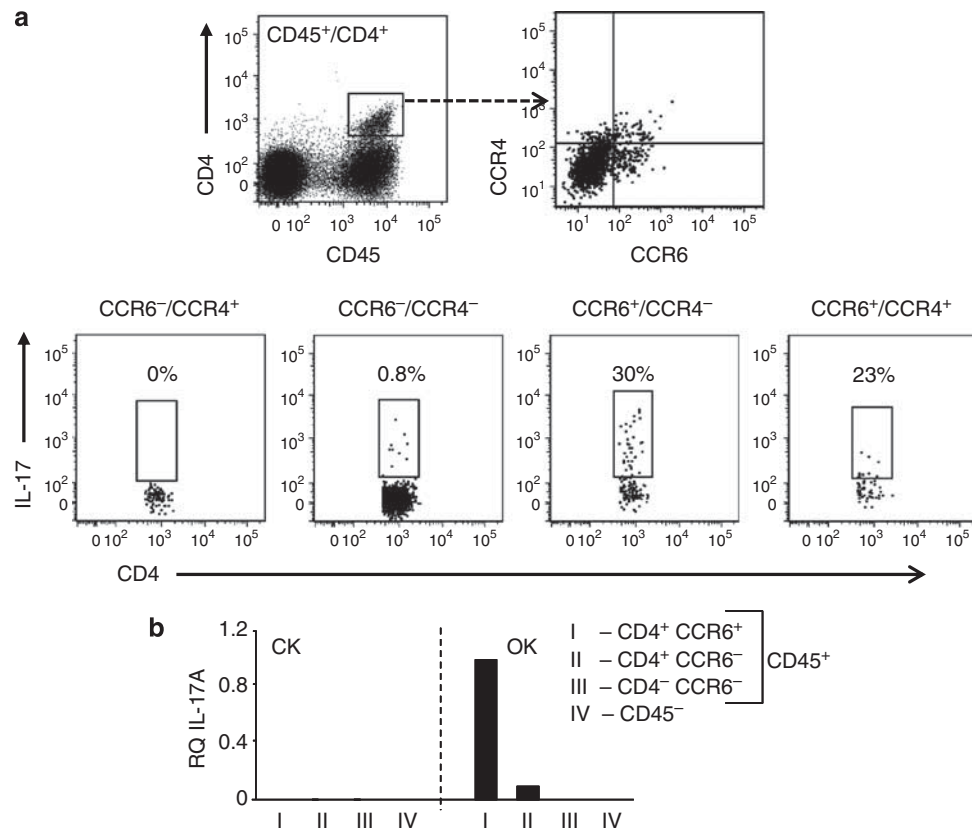
Figure 3 | CD4$^+$CCR6$^+$ T cells are the predominant source of interleukin (IL)-17A in obstructed kidneys (OKs). (**a**) Representative dot plots showing the frequencies of IL-17A intracellular staining among four populations of CD45$^+$CD4$^+$ T cells from 72-h OK: CCR6$^-$CCR4$^+$, CCR6$^-$CCR4$^-$, CCR6$^+$CCR4$^-$, and CCR6$^+$CCR4$^+$. (**b**) Relative quantity (RQ) of IL-17A mRNA in four fluorescence-activated cell sorting (FACS)-purified cell populations from control (CK) and OK 72 h after unilateral ureteral obstruction (UUO): CD4$^+$CCR6$^+$, CD4$^+$CCR6$^-$, CD4$^-$CCR6$^-$ (all CD45$^+$), and CD45$^-$ (see Supplementary Figure S1 online for illustration of sorting strategy and purity).

DC-depleted leukocytes alone, and increased IL-6 was predominantly confined to nonleukocyte renal cells. Consistent results were also obtained by qRT-PCR carried out on the same cell populations analyzed without *in vitro* culture (Supplementary Figure S4a online). Moreover, addition of CM from CD11c$^+$ cells from obstructed kidneys to *in vitro*-generated Th17 induced a similar enhancing effect on IL-17A production to that observed for total CD45$^+$ cells (Supplementary Figure S4b and c online).

IL-1 is responsible for intrarenal Th17-enhancing activity following UUO

The contribution of IL-1α/β and IL-23 to the Th17-enhancing effect of CM from CD45$^+$ cells of obstructed kidney was examined using blocking antibodies against the receptors for IL-1α/β and IL-23 (Figure 7). The induction of IL-17A production by CM of CD45$^+$ cells from obstructed kidney was abolished by blockade of IL-1 receptor (IL-1R1/CD121a) but not by blockade of IL-23 receptor. To confirm the role of IL-1 in intrarenal Th17 activation, UUO was carried out for 72 h in IL-1R1 knockout and wild-type mice with Th17 activity analyzed by intracellular staining (Figure 8a–c) and ELISA (Figure 8d) of anti-CD3-stimulated cells from obstructed kidneys. Intra-renal Th1 responses were

similarly compared using assays for IFN-γ. The results revealed reduced Th17 activity in obstructed kidneys of IL-1R1 knockout animals in the form of reduced mean fluorescence intensity of anti-IL-17A staining among the IL-17A$^+$ cells (Figure 8c) and reduced concentration of IL-17A in culture supernatants (Figure 8d). In contrast, obstructed kidneys from IL-1R1 knockout animals had increased Th1 activity (Figure 8a–d). The obstructed and control kidneys of IL-1R1 knockout mice did not have reduced frequency of CD4$^+$ or CD4$^+$CCR6$^+$ T cells (Supplementary Figure S5 online). The results suggested that signaling through the IL-1R1 is unnecessary for intrarenal accumulation of CD4$^+$CCR6$^+$ cells following UUO, but regulates the proportion of Th cells that are competent for IL-17A secretion.

To elucidate the contribution of IL-1 to both proliferation and activation of intrarenal Th17 cells in genetically normal animals, groups of mice were administered the recombinant IL-1R1 antagonist (IL-1Ra), anakinra, or vehicle for 72 h post UUO along with continuous *in vivo* BrdU labeling. IL-1Ra administration did not result in an overall reduction in the proportionate accumulation of CD45$^+$ leukocytes in obstructed kidneys (Figure 9a) or in the proportion of intrarenal CD45$^+$ that were BrdU$^+$ (data not shown).

Nevertheless, the proportion of $CD4^+$ T cells among the $CD45^+$ cells was reduced in obstructed kidneys of IL-1Ra-treated mice—primarily due to reduced proportion of $CD4^+BrdU^+$ cells (Figure 9b). Intracellular staining of anti-CD3-stimulated cells from obstructed kidneys demonstrated that, for $BrdU^+$, but not for $BrdU^-$ $CD4^+$ T cells, IL-1Ra administration was associated with reductions in the proportion that were $IL-17A^+$, as well in the mean

fluorescence intensity of anti-IL-17A staining among the $IL-17A^+$ cells (Figure 9c). Reduced Th17 activity in obstructed kidneys of IL-1Ra-treated mice was also demonstrated by IL-17A ELISA of anti-CD3-stimulated culture supernatants and by qRT-PCR of renal cortical tissue for IL-17A mRNA (Figure 9d). In contrast to results for IL-1R1-KO mice, IL-1Ra therapy was not associated with evidence of enhanced intrarenal Th1 activity (Supplementary Figure S6a online). Analyses of splenic T cells indicated that there was no extrarenal effect of IL-1Ra on proliferation rate of $CD4^+$ T cells or on anti-CD3-stimulated production of IL-17A and IFN-γ (Supplementary Figure S6b and c online). Histological analysis of the kidneys from this experiment demonstrated a modest protective effect of IL-1Ra therapy against tubular dilatation and atrophy but not against interstitial inflammatory cell infiltration (Supplementary Figure S7 online). In keeping with Jones *et al.*,[40] this observation indicated that IL-1 family cytokines and their associated enhancement of intrarenal Th17 activity are not the predominant drivers of renal damage in this model.
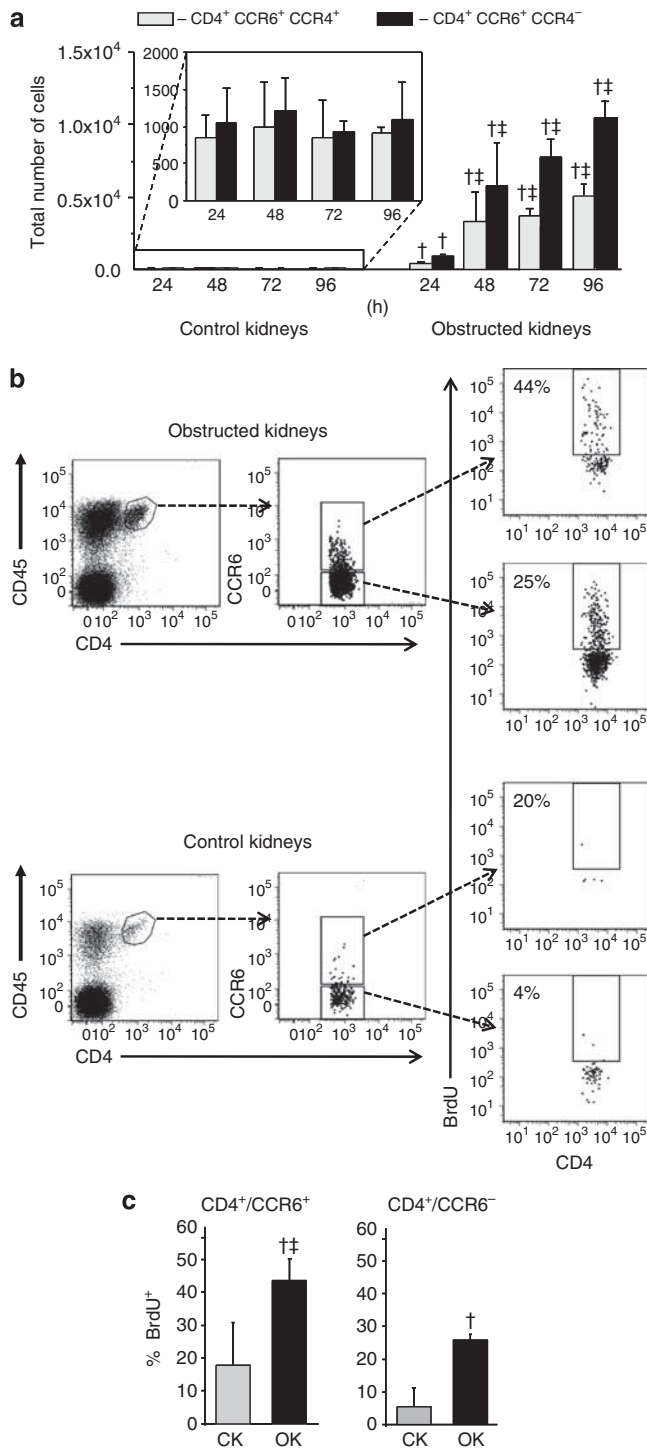
Th17-enhancing IL-1 is produced by DCs and monocytes in obstructed kidney

To more clearly identify cellular sources of Th17-enhancing activity following UUO, four individual myeloid cell populations were sorted to high purity by FACS from 72-h obstructed and control kidneys based on surface expression of CD11b, CD11c, F4/80, and Ly6C. These populations were designated as follows: (1) $F4/80^+$ DCs ($CD11b^+CD11c^+F4/80^+Ly6C^-$); (2) $F4/80^-$ DCs ($CD11b^+CD11c^+F4/80^-Ly6C^-$); (3) inflammatory monocytes ($CD11b^+CD11c^-Ly6C^{hi}$); (4) macrophages ($CD11b^+CD11c^-Ly6C^{lo}$) (Supplementary Figure S8 online). Coculture of $F4/80^+$ DCs, $F4/80^-$ DCs, and inflammatory monocytes (but not macrophages) from obstructed kidneys with restimulated, *in vitro*-generated Th17 cells resulted in enhanced IL-17A production (Figure 10a). Of interest, CM from total $CD45^+$ cells and from the sorted myeloid cell sub-populations did not enhance IFN-γ production by restimulated *in vitro*-generated Th1

Figure 4 | Accumulation and proliferation of $CCR6^+$ T-helper (Th) cells in obstructed kidneys (OKs). (**a**) Calculated total numbers of $CCR6^+CCR4^+CD4^+$ and $CCR6^+CCR4^-CD4^+$ cells in control (CK) and OKs between 24 and 96 h following unilateral ureteral obstruction (UUO), based on viable cell counts and flow cytometry of whole-kidney digests. $^\dagger P < 0.05$ compared with equivalent result for CKs; $^\ddagger P < 0.05$ compared with an equivalent result at 24 h. (**b**) Kidney cells suspensions prepared following 72 h of UUO and continuous bromodeoxyuridine (BrdU) ingestion via drinking water were surface stained for CD45, CD4, and CCR6, followed by intracellular staining for BrdU. Representative dot plots are shown illustrating the frequency of $BrdU^+$ cells among the $CCR6^+$ and $CCR6^-$ $CD4^+$ T cells from OKs and CKs. (**c**) Graphical representation of analysis illustrated in **b**. Results are expressed as mean ± s.d. of the percent $BrdU^+$ among $CCR6^+CD4^+$ and $CCR6^-CD4^+$ T cells for individually analyzed CK and OK ($n = 5$). $^\dagger P < 0.05$ compared with an equivalent result for CKs; $^\ddagger P < 0.05$ compared with an equivalent result for $CD4^+CCR6^-$ cells.
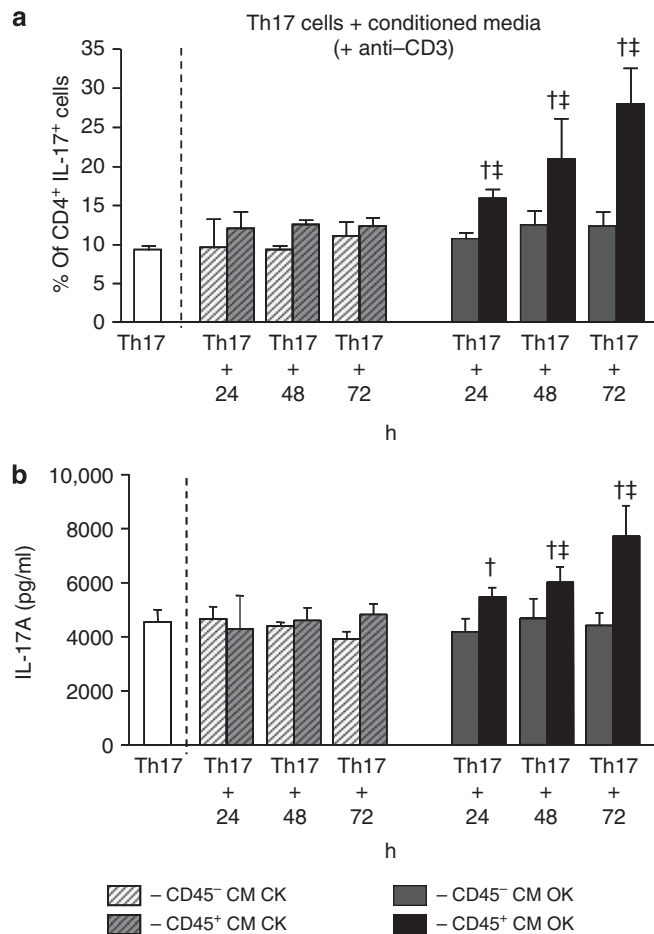
Figure 5 | **Leukocytes from obstructed kidney (OK) release T-helper 17 (Th17)-cell-activating factors.** *In vitro*-generated mouse Th17 cells were restimulated by overnight culture with 0.1 µg/ml anti-CD3 antibody in the absence (white columns) or presence (all other columns) of conditioned media (CM) from CD45-depleted ($CD45^-$) or CD45-enriched ($CD45^+$) cells of control (CK) or OK following 24, 48, and 72 h of unilateral ureteral obstruction (UUO). Interleukin (IL)-17A production by the restimulated Th17 cells under all conditions was measured by intracellular flow cytometry expressed as percent $IL-17^+$ among the total $CD4^+$ cells (**a**) (see Supplementary Figure S2 online for examples of staining) and by assay of supernatants for IL-17A concentration expressed as pg/ml (**b**). All results are presented as mean ± s.d. of three individual samples. $^\dagger P < 0.05$ compared with an equivalent CK CM addition, $^\ddagger P < 0.05$ compared with an equivalent $CD45^-$ CM addition.

cells (Supplementary Figure S9 online). Quantitative RT-PCR analysis and ELISA of culture supernatants from the sorted populations confirmed elevated IL-1α expression by inflammatory monocytes and both $F4/80^+$ and $F4/80^-$ DCs (Figure 10b and c). A similar expression pattern was observed for IL-23, whereas IL-6 expression was found to be highest in macrophages purified from the obstructed kidney (Supplementary Figure S10 online). Taken together, the results indicate that IL-1α and/or IL-1β represent important intrarenal Th17-enhancing factors and are produced within the obstructed kidney both by resident (DCs) and infiltrating (monocytes) myeloid cell populations.
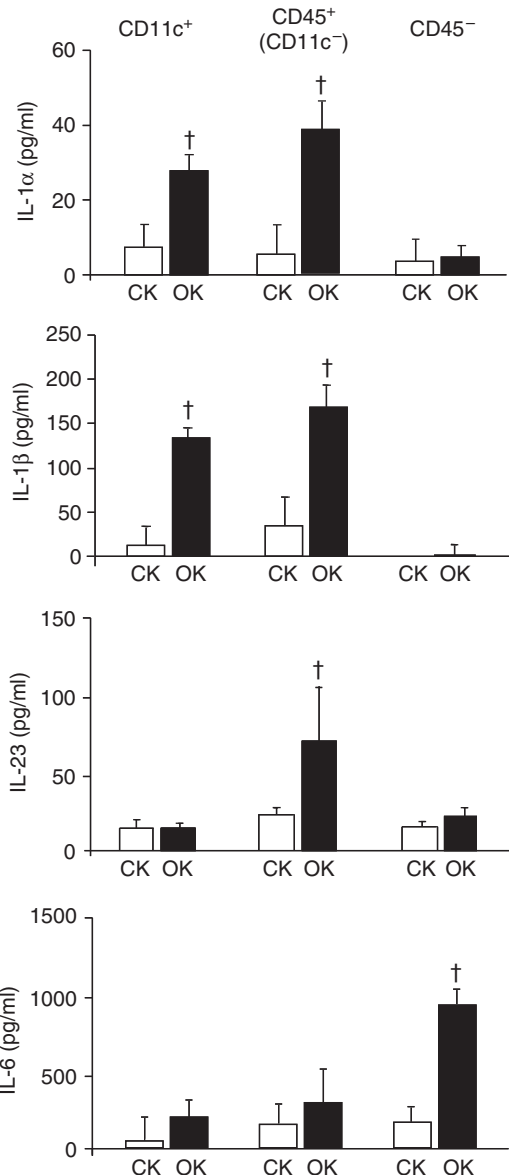
Figure 6 | **Secretion of candidate T-helper 17 (Th17)-activation factors by leukocyte and nonleukocyte cell populations of obstructed kidneys (OKs).** Cytokine (interleukin (IL)-1α, IL-1β, IL-23, and IL-6) concentrations in culture supernatants of cell populations from control (CK) and OK prepared following 72 h of unilateral ureteral obstruction (UUO) by magnetic column separations and placed in culture overnight. Cell populations were dendritic cell (DC)-enriched ($CD11c^+$), non-DC leukocytes ($CD45^+CD11c^-$), and nonleukocyte renal cells ($CD45^-$). Results are expressed as mean ± s.d. (pg/ml) for three individual supernatants from each group of sorted cells. $^\dagger P < 0.05$ compared with an equivalent CK sample.

DISCUSSION

To our knowledge, the present study is the first to identify the basis for activation of Th17 cells within the injured kidney, namely, locally produced IL-1 originating from DCs and monocytes. Moreover, using mice deficient in IL-1R1 and administration of IL-1Ra, we demonstrate the non-redundant role of IL-1 as a mediator of Th17 cell activation and
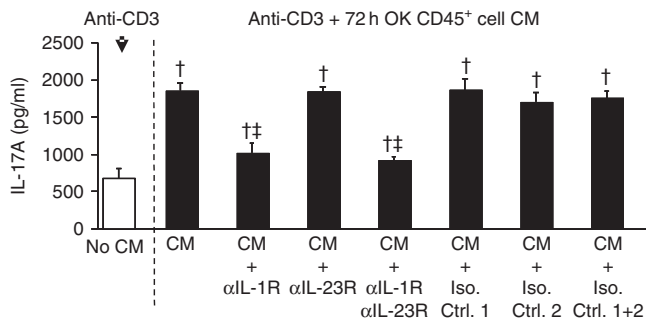
Figure 7 | Increased interleukin (IL)-17A production by in vitro-generated T-helper 17 (Th17) cells is inhibited by IL-1R1 blockade. IL-17A concentrations in culture supernatants from in vitro-generated Th17 cells stimulated for 24 h with 0.1 μg/ml anti-CD3 in the absence (white column) or presence (all other columns) of conditioned medium (CM) from total leukocytes (CD45+ cells) of 72-h obstructed kidneys (OKs) with and without the addition of blocking antibodies or relevant isotype control antibodies (Iso. Ctrl.). The following blocking antibodies were used: anti-IL-1R1 (2.5 μg/ml) and/or anti-IL-23 receptor (5 μg/ml). Results are expressed as mean ± s.d. (pg/ml) for triplicate wells of each condition. $^{†}P < 0.05$ compared with no CM control sample; $^{‡}P < 0.05$ compared with the result for relevant isotype control.

proliferation *in vivo* following UUO. As such, our studies provide novel insights regarding the nature of the processes that enable the accumulation and activation of Th17 cells in the injured kidney.

In recent years, important roles for Th17 cells, their dominant secreted product, IL-17A, and the factors responsible for their differentiation and activation have been demonstrated in a range of kidney diseases.[18,19] Increased intrarenal leukocyte expression of IL-17A has been reported in human and rodent transplant rejection, lupus nephritis, and crescentic glomerulonephritis, as well as in human nephrotic syndrome and rodent ischemia–reperfusion injury.[17,21,32–36,39,41,42] Deficiency or blockade of IL-17A ameliorates the severity of kidney disease in rodent models of ischemia–reperfusion injury, nephrotoxic serum nephritis, and anti-myeloperoxidase glomerulonephritis,[17,21,37] whereas the presence and/or frequency of intrarenal IL-17-producing lymphocytes correlates with disease severity in human lupus nephritis and chronic transplant rejection.[36,39]

Of interest, this recent literature indicates that the potential cellular sources for pathogenic IL-17A within the
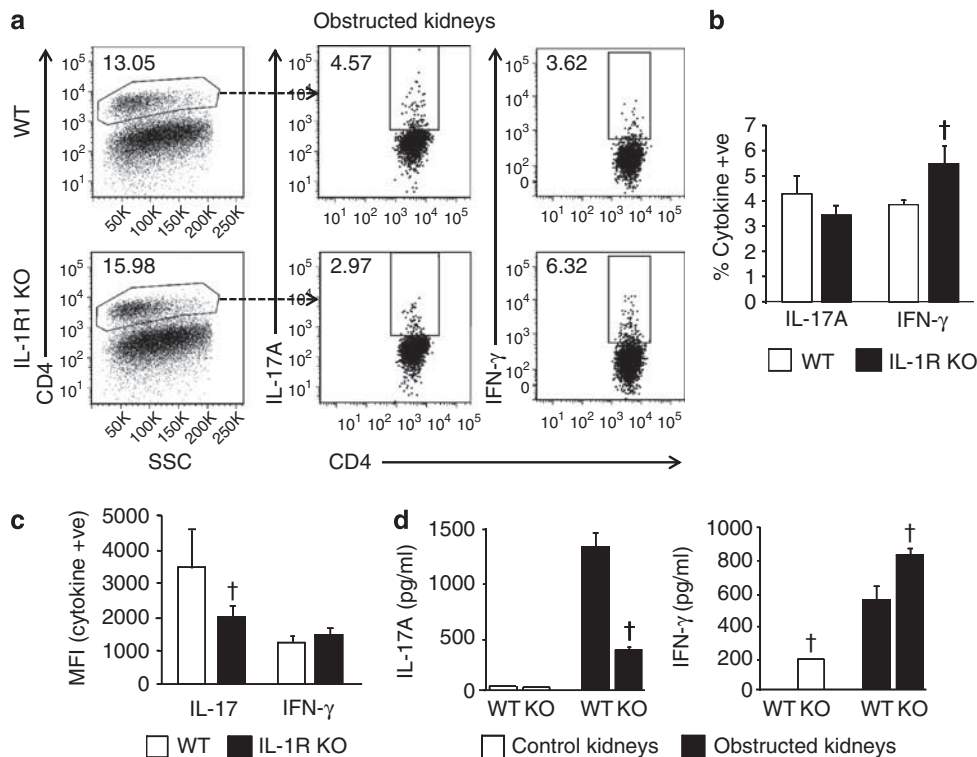
Figure 8 | Diminished renal T-helper 17 (Th17) activity in obstructed kidneys of IL-R1-deficient mice. Wild type (WT) and IL-1R1 knockout (KO) mice were subjected to unilateral ureteral obstruction (UUO) for 72 h. Whole-kidney cell suspensions (a–c) and total CD45+ leukocytes (d) from obstructed kidneys (OKs) were stimulated overnight with 1.0 μg/ml anti-CD3 antibody. (a) Examples of flow cytometry analysis of IL-17A and interferon (IFN)-γ intracellular staining of CD45+CD4+. The percentage of analyzed cells with positive staining for CD4 (left plots), IL-17A (middle plots), and interferon-gamma (IFN-γ) (right plots) are shown. (b) Graphical representation of intracellular cytometry analyses for IL-17A and IFN-γ expressed as mean ± s.d. of the percent of CD45+/CD4+ T cells that were cytokine-positive (+ve) for individual OKs (n = 5 per group). (c) Graphical representation of intracellular cytometry analyses for IL-17A and IFN-γ expressed as mean ± s.d. of the mean fluorescence intensity (MFI) of intracellular cytokine staining among the cytokine-positive CD45+CD4+ T cells for individual OKs. (d) IL-17A and IFN-γ enzyme-linked immunosorbent assay results from culture supernatants of anti-CD3–stimulated total CD45+ leukocytes from 72 h control (CK) and OKs of IL-1R1 WT and KO mice. Results are expressed as mean ± s.d. (pg/ml) for triplicate samples from each condition. $^{†}P < 0.05$ compared with an equivalent result for WT. SSC, side scatter.
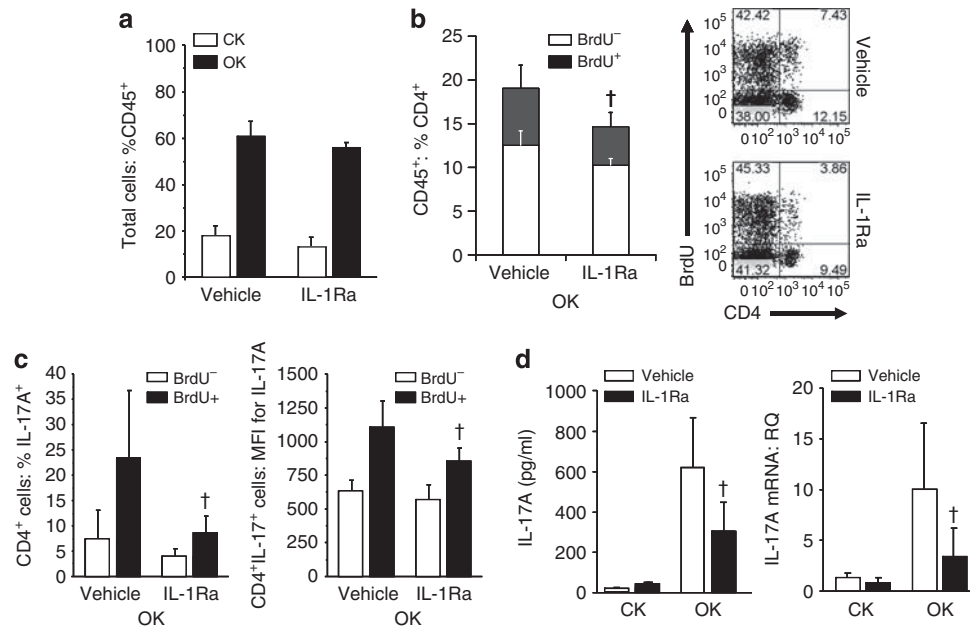
Figure 9 | Diminished proliferation and activation of renal T-helper 17 (Th17) cells in obstructed kidneys (OKs) of mice treated with IL-1R antagonist (IL-1Ra). Groups of mice ($n = 6$) subjected to unilateral ureteral obstruction (UUO) for 72 h were continuously exposed to bromodeoxyuridine (BrdU) via drinking water and received daily intraperitoneal injections of vehicle or IL-1Ra. (**a**) Proportions of leukocytes (CD45$^+$) among total cell suspensions from nonobstructed control kidneys (CKs) and obstructed kidneys (OKs) of the two groups by flow cytometric analysis. (**b**) Left: proportions of nonproliferative (BrdU$^-$) and proliferative (BrdU$^+$) CD4$^+$ T cells among the CD45$^+$ leukocytes from OKs of the two groups by combined surface and intracellular flow cytometric analysis. Right: examples of flow cytometric dot plots used to coanalyze CD4 and BrdU staining following gating on CD45$^+$ cells of OKs. (**c**) Magnetic column-separated CD45$^+$ cells from OK were stimulated overnight with low-dose anti-CD3 antibody and were analyzed by surface and intracellular flow cytometry for the proportions of IL-17A$^+$ cells (left) and the mean fluorescence intensity of IL-17A staining (right) among the bromodeoxyuridine (BrdU)$^-$ and BrdU$^+$ CD4$^+$ T cells. (**d**) Culture supernatants from anti-CD3–stimulated CD45$^+$ cells of CK and OK from the two groups were analyzed by enzyme-linked immunosorbent assay for IL-17A production (left). Whole renal cortex samples of CK and OK from the two groups were analyzed by quantitative RT-PCR (qRT-PCR) for relative quantity (RQ) of IL-17A mRNA (right). All results are presented as mean ± s.d. †$P < 0.05$ compared with an equivalent result for the vehicle-treated group. MFI, mean fluorescence intensity.

kidney extend beyond conventional CD4$^+$ T cells to include neutrophils,[21] CD4$^-$CD8$^-$ (double-negative) T cells,[33,34] and γδ-T cells.[43] Nevertheless, our own prior observations in UUO[20] and those of others in glomerulonephritis[17,44,45] and transplant rejection[39] confirm that diverse forms of progressive renal immune/inflammatory disease are associated with interstitial accumulation of bona fide Th17 cells expressing markers of effector memory and activated phenotypes. In the current study, we confirm that UUO produces a progressive increase in IL-17A expression within obstructed renal cortex over 72 h and, using magnetic cell separation and high-level purification by FACS, we sequentially localize IL-17A mRNA to CD45$^+$ leukocytes, CD4$^+$ T cells, and, finally, the CCR6$^+$CD4$^+$ T-cell subfraction. The observation indicates a degree of intrinsic activation of Th17 cells, as detectable IL-17A transcript was absent in equivalent fractions from non-obstructed kidneys. Given the presumed non-antigen-specific nature of obstruction-associated inflammatory injury, this finding is in keeping with so-called 'bystander activation' of effector memory Th17 cells, although T-cell receptor specificity for renal autoantigens remains an intriguing possibility.[46,47] In keeping with the recent findings of Turner et al.[45] in nephrotoxic serum nephritis, we find that intrarenal Th17 cells in UUO are predominantly CCR6$^+$.

At 72 h, up to one-third of CD4$^+$CCR6$^+$ cells were capable of rapid IL-17A production upon low-level T-cell receptor stimulation. Notably, the number of CD4$^+$CCR6$^+$ Th cells increased over 10-fold in obstructed kidneys between 24 and 96 h following UUO, with a high proportion of these having incorporated BrdU by 72 h. Although this does not formally identify the site of T-cell proliferation, the lower rate of BrdU incorporation in the equivalent subset from the non-obstructed kidneys provides evidence that intrarenal proliferation is also an important mechanism of Th17 cell accumulation in AKI.

Recently, a role has emerged for IL-23 in the differentiation and activation of pathogenic Th17 cells and other IL-17A-producing leukocytes in rodent models of glomerulonephritis and ischemia–reperfusion injury and in human antineutrophil cytoplasmic antibody-associated vasculitis.[17,21,32,38,48] In other autoimmune and inflammatory conditions, IL-1 has been identified as an important local mediator of pathogenic Th17-cell activation.[29,43] In the current study, we demonstrate the secretion of soluble Th17-enhancing activity by cells from obstructed kidneys. This activity was localized initially to CD45$^+$ intrarenal leukocytes (both CD11c$^+$ and CD11c$^-$) and next to F4/80$^+$ DCs, F4/80$^-$ DCs, and Ly6C^{hi} (inflammatory) monocytes,
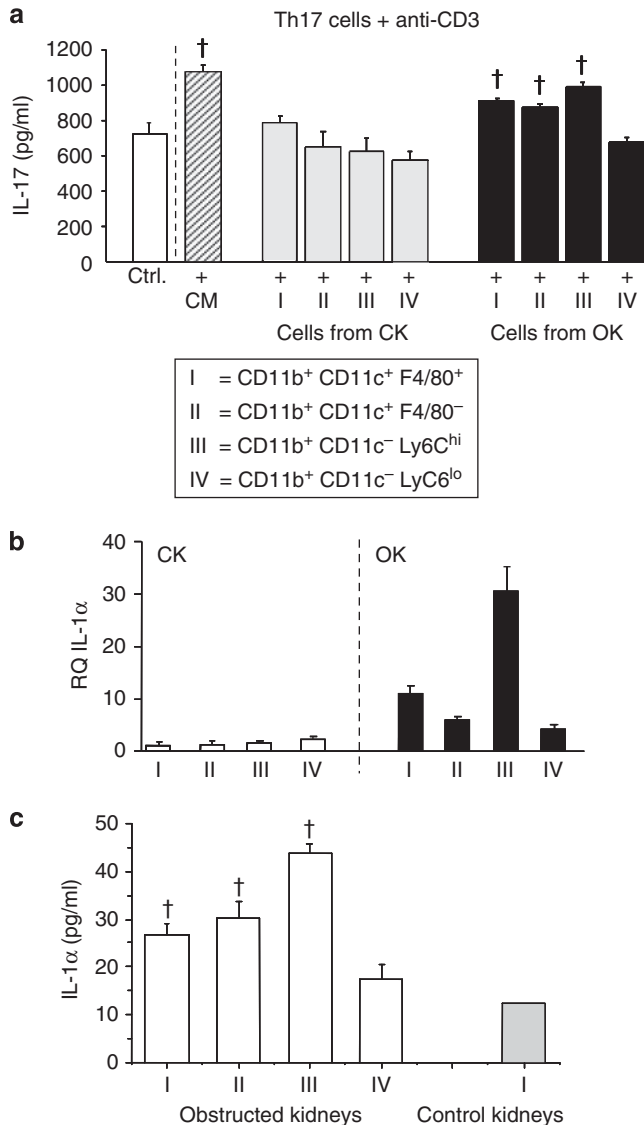
Figure 10 | Release of T-helper 17 (Th17) activation factors by multiple renal myeloid cell populations following unilateral ureteral obstruction (UUO). (a) Interleukin (IL)-17A enzyme-linked immunosorbent assay results for supernatants of *in vitro*-generated Th17 cells that were stimulated for 24 h with anti-CD3 in the presence of control medium (Ctrl.) or conditioned medium (CM) from total CD45$^+$ leukocytes of 72-h obstructed kidneys (OKs) or were cocultured with four different myeloid cell populations fluorescence-activated cell sorting (FACS)-purified from control kidney (CK) and OK 72 h after UUO ($n = 5$). The surface marker characteristics of the four myeloid cell populations are listed in the box. Staining and purity of sorted cells are presented in Supplementary Figure S6 online. $^†P < 0.05$ compared with Ctrl. (b) Quantitative RT-PCR result showing relative quantity (RQ) of IL-1α mRNA in FACS-purified cell populations expressed as fold expression relative to population I from CKs. (c) IL-1α enzyme-linked immunosorbent assay result for supernatants of populations I–IV (OKs) and population I (CKs) placed in culture in equal numbers for 24 h following FACS purification. $^†P < 0.05$ compared with the result for CKs population I.

although not to Ly6C^{lo} macrophages. Interestingly, although increased expression of both IL-1α/β and IL-23 were observed in DCs and monocytes from obstructed kidneys, IL-1 family cytokines, functioning via IL-1R1, were found to be entirely responsible for this *in vitro* Th17-activating effect. The *in vivo* relevance of this finding was validated in IL-1RI-deficient mice in which Th17 cell activity was reduced in obstructed kidneys compared with wild-type animals despite comparable, or greater, accumulation of CD4$^+$CCR6$^+$ T cells. Similarly, mice treated with IL-1Ra following UUO demonstrated reduced intrarenal Th17 activity. In this experiment, concomitant BrdU labeling indicated that IL-1R blockade reduced Th17 activity among proliferative (BrdU$^+$) Th cells from obstructed kidneys. In contrast, BrdU incorporation and IL-17A production by splenic Th cells was unaffected. Thus, we convincingly show that both activation and proliferation of intrarenal Th17 cells are positively regulated by IL-1. Although this does not rule out a role for IL-23 in other aspects of the triggering of Th17 cells during intrarenal inflammation, it does emphasize the significance of IL-1 in specifically promoting Th17-cell activation and expansion at sites of localized 'sterile' inflammation.[49,50]

Given the growing interest in clinical application of IL-1 antagonists and blocking antibodies to inflammatory diseases,[50,51] the potential to target pathogenic renal Th17 responses by this strategy merits consideration. Notably, Timoshanko *et al.*[52] observed attenuation of experimental glomerulonephritis in mice lacking IL-1R1, IL-1α, or IL-1β, and Furuichi *et al.*[53] demonstrated reduced early severity of renal ischemia–reperfusion injury in IL-1α/β-deficient mice, as well as exacerbated injury in mice genetically lacking IL-1Ra. In UUO, Jones *et al.*[40] observed reduced intrarenal pro-fibrotic activity at 7 (but not 14) days in IL-1R1-deficient compared with wild-type mice, although T-cell infiltration and Th activity was not analyzed in this study. In our own experiments using IL-1Ra during the initial 72 h following UUO, we observed only a mild protective effect against tubular dilatation and atrophy by histological analysis, with no overt reduction in the extent of interstitial cellular infiltrates. Furthermore, despite the inhibition of Th17 activity, neutrophil infiltration of obstructed kidneys at this time point was not reduced when analyzed by flow cytometry (data not shown). Thus, although UUO has proven to be of value as a model system in which to evaluate the dynamics and activation factors for intrarenal Th17 cells during kidney injury, our data suggest that IL-1 blockade and its associated inhibition of localized Th17 activity may be of limited benefit in obstructive renal injury. As Kitching and Holdsworth[18] have recently highlighted, cross-regulation of Th17 and Th1 cells during inflammation could result in augmentation of harmful Th1 responses following specific Th17 targeting. However, although we observed increased numbers of CD4$^+$IFN-γ^+ T cells in obstructed kidneys of IL-1R1-KO compared with wild-type mice, this phenomenon was not reproduced in IL1Ra-treated mice in which a trend toward reduced Th1 activity in obstructed kidneys was

observed in the form of lower anti-CD3-stimulated IFN-γ production (see Supplementary Figure S6a online). In this experiment, it was not possible to accurately co-stain for intracellular BrdU and IFN-γ (data not shown). Thus, although our findings clearly document the enhancing effect of IL-1 on intrarenal Th17 activation and proliferation, its role in regulating intrarenal Th1 (as well as other Th subsets) remains unclear. In the broader sense, the lack of robust benefit of IL-1 blockade and intrarenal Th17 suppression in UUO likely reflects the general observation that therapeutic approaches for inflammatory diseases based on targeting of a single cytokine/chemokine have been less successful than a multi-pronged approach. In contrast, antigen-driven, immune-mediated kidney diseases in which Th17 cells have been shown to have a major pathogenic role (e.g., antineutrophil cytoplasmic antibody-associated vasculitis and crescentic glomerulonephritis) may prove to be more responsive to IL-1 blockade either alone or in combination with inhibition of other Th17-differentiation and -activation factors such as IL-23, IL-6, and transforming growth factor-β1. It should also be noted that Th17 cells represent only one discrete target for locally produced IL-1 within the kidney and, as shown by Timoshanko et al.[52] in chimeric mice, the responses of non-immune, resident renal cells to IL-1 contribute significantly to tumor necrosis factor production and glomerular injury in a model of crescentic glomerulonephritis.

In summary, we have used the UUO model as an 'incubator' of localized Th17 cell accumulation within the inflamed kidney. Our findings favor the following conclusions: (1) Effector memory CD4^{+} T cells preprogrammed for IL-17A production accumulate in substantial numbers within injured kidney through chemokine receptor-specific recruitment and localized proliferation. (2) Even without antigenic priming, acute renal inflammation induces local activation of Th17 cells, of which IL-1, produced by DCs and inflammatory monocytes, is a key mediator. Our findings will be of value in better defining the dynamics and pathogenic properties of intrarenal Th17 cells, their dependence upon activation factors secreted locally within the kidney by cells of the mononuclear phagocyte system, and the targets available for inhibiting their effector functions during kidney disease.

MATERIALS AND METHODS

Experimental animals

Eight- to twelve-week-old female C57BL/6 (B6) mice were purchased from Harlan Laboratories UK (Bicester, UK). Mice genetically deficient in IL-1R1 on a B6 background, originally from Jackson Laboratories, Bar Harbor, ME, were bred on-site. Animals were housed in specific pathogen-free facilities. Procedures were carried out under license from the Irish Department of Health and Children and approved by the NUI Galway Animal Ethics Committee.

Reagents

Antibodies, culture media, buffer solutions, ELISA reagents, and other materials used in the study are detailed in Supplementary Methods online.

Cell cultures

Cells were cultured in 96-well round-bottom plates at 10^6 cells/ml with other additions as described for individual experiments. For ELISA of secreted products, supernatants were withdrawn at 24 h. For surface and intracellular staining of cultured cells, Brefeldin A (GolgiPlug 1 μl/ml, BD Biosciences, Oxford, UK) was added 8 h before analysis. For preparation of CM, magnetic column-purified cells were placed in culture at 10^6 cells/ml for 24 h, following which media were withdrawn and frozen at −20 °C before being used at 1:1 ratio with fresh medium as described for individual experiments.

UUO and preparation of kidney cell digests

Mouse UUO with preparation of cell suspensions between 24 and 96 h later by collagenase/DNase digestion was conducted as previously described (see also Supplementary Methods online).[20,54] In some experiments, mice received a bolus of BrdU intraperitoneally at the time of surgery, followed by continuous exposure to BrdU-containing water for 72 h. In some experiments, groups of mice also received the IL-1R antagonist (IL-1Ra), anakinra, at a concentration of 100 mg/kg i.p. in 250 μl of phosphate-buffered saline or phosphate-buffered saline alone by intraperitoneal injection at 0, 24, and 48 h following UUO.

Flow cytometry, fluorescence-activated cell sorting, and magnetic column separation of kidney cells

Kidney cells suspended in 100 μl aliquots of FACS buffer were incubated with 3–5 fluorochrome-labeled and/or biotinylated antibodies for 30 min at 4 °C, followed by washing in FACS buffer and, if necessary, incubation with fluorochrome-labeled streptavidin for 30 min at 4 °C and then resuspended in 4% paraformaldehyde in phosphate-buffered saline. Intracellular staining was carried out using the Cytofix/Cytoperm and BrdU detection kits (BD Biosciences) according to the manufacturer's instructions. Analysis was performed using a BD Biosciences FACSCanto flow cytometer and FlowJo software (TreeStar, Olten, Switzerland).

For FACS, cell suspensions from 3–5 kidneys were resuspended in FACS sorting buffer with fluorochrome- and biotin-labeled monoclonal antibodies for 30 min at 4 °C, and then washed, filtered through a 40-μm mesh and sorted using a BD Biosciences FACSAriaII sorter with purity determined by post-sort analysis.

Magnetic column separations of kidney cell suspensions were carried out in MACS buffer by manufacturer-recommended protocols using MS columns and an OctoMACS separator (Miltenyi Biotec, Auburn, CA). For individual experiments, positive and/or negative column fractions were retained, washed in MACS buffer, and used for culture and/or qRT-PCR. In some experiments, cell suspensions were first subjected to anti-CD11c magnetic column separation, and then the negative fractions were subjected to anti-CD45 magnetic column separation to sequentially prepare CD11c^{+}, CD45^{+}CD11c^{−}, and CD45^{−} cell fractions.

Cultures with *in vitro*-generated Th17 and Th1 cells

Th17- and Th1-skewed mouse CD4^{+} T-cell cultures were generated over 10 days from splenocytes of healthy adult mice using standard protocols (see Supplementary Methods online for details). For restimulation experiments, CD4^{+} T cells were repurified from differentiation cultures by magnetic column separation and then plated in fresh culture medium 10^6 cells/ml with or without anti-CD3 (1.0 or 0.1 μg/ml) for 24 h. In individual experiments, conditioned or control media were added at a 1:1 ratio with fresh medium. In some experiments, previously optimized concentrations

of blocking antibodies or relevant isotype control antibodies were also added. In others, FACS-purified cells from non-obstructed and obstructed kidneys were added at a 1:10 ratio. After 24 h, concentrations of IL-17A and IFN-γ were measured in culture supernatants by ELISA.

RNA preparation and qRT-PCR

Total RNA was isolated from whole kidney or sorted cell populations using RNeasy Mini or Micro kits (Qiagen, Valencia, CA; see also Supplementary Methods online). RNA was converted to cDNA using the High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems, Carlsbad, CA). Equal amounts of cDNA were analyzed by qRT-PCR using the TaqMan Master Mix and commercial primer/probe sets for mouse GAPDH, IL-1α, IL-1β, IL-17A, IL-6, and IL-23(p19) on a StepOne Plus Real Time PCR System (all from Applied Biosystems). Relative quantifications were performed using the comparative C_T method with normalization to GAPDH and results expressed as fold difference relative to a relevant control sample.

Statistical analysis

Individual experiments were carried out between two and six times to ensure reproducibility. For culture experiments, between three and six replicates were initiated and individually analyzed for each condition. Data are presented as mean ± s.d. Statistical comparisons between individual groups were performed by unpaired, two-tailed *t*-tests using Microcal Origin V6.0 software (Northampton, MA) with significance assigned at $P < 0.05$.

DISCLOSURE

KHGM is a cofounder and minority shareholder in Opsona Therapeutics, a start-up company involved in the development of anti-inflammatory therapeutics.

SUPPLEMENTARY MATERIAL

Figure S1. The gating strategy and purity of fluorescence-activated cell sorting from 72-h obstructed kidneys for the purpose of analyzing interleukin (IL-17A) mRNA expression is shown.
Figure S2. Examples of intracellular flow cytometric analysis of *in vitro*-generated T-helper 17 (Th17) cells for interleukin (IL)-17A following anti-CD3 stimulation with and without exposure to conditioned media from CD45-depleted (CD45⁻) and CD45-enriched (CD45⁺) cells of obstructed kidneys following 24, 48, and 72 h of unilateral ureteral obstruction (UUO).
Figure S3. Soluble products of CD45⁺ cells of obstructed kidneys induce increased interleukin (IL)-17A secretion by *in vitro*-generated T-helper 17 (Th17) cells in the absence of anti-CD3 stimulation.
Figure S4. (A) qRT-PCR of various magnetic column-enriched kidney cell populations for IL-1-α, IL-1-β, IL-23 p19 and IL-6.
(B) Enhancement of IL-17A production of *in vitro*-generated Th17 cells by conditioned medium of CD11c+ cells of 72 h obstructed kidney.
Figure S5. CD4⁺ CCR6⁺ T cells are not proportionally diminished in obstructed kidneys of IL-1R knockout (KO) mice compared with wild type.
Figure S6. (A) Anti-CD3-stimulated IFN-gamma production and cortical mRNA expression from kidneys of vehicle- and IL-1Ra-treated

mice. (**B**, **C**) *In vivo* BrdU incorporation and stimulated IL-17A and IFN-gamma production by T-cells from spleens of vehicle- and IL-1Ra-treated mice.
Figure S7. Histological scoring for tubulointerstitial injury in control and obstructed kidneys of vehicle- and IL-1Ra-treated mice.
Figure S8. The gating strategy and purity of fluorescence-activated cell sorting from 72-h obstructed kidneys for the purpose of isolating individual myeloid cell populations is shown.
Figure S9. Renal myeloid cell populations do not enhance interferon-gamma (IFN-γ) production by *in vitro*-generated T-helper 1 (Th1) cells following unilateral ureteral obstruction (UUO).
Figure S10. Quantitative RT-PCR results showing relative quantities (RQs) of IL-23 (p19) and IL-6 mRNA in fluorescence-activated cell sorting (FACS)-purified myeloid cell populations from control (CK) and obstructed (OK) kidneys following 72 h of unilateral ureteral obstruction (UUO).
Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

REFERENCES

1. Bajwa A, Kinsey G, Okusa M. Immune mechanisms and novel pharmacological therapies of acute kidney injury. *Curr Drug Targets* 2009; **10**: 1196–1204.
2. Friedewald JJ, Rabb H. Inflammatory cells in ischemic acute renal failure. *Kidney Int* 2004; **66**: 486–491.
3. Schrier RW, Wang W, Poole B *et al.* Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. *J Clin Invest* 2004; **114**: 5–14.
4. Venkatachalam MA, Griffin KA, Lan R *et al.* Acute kidney injury: a springboard for progression in chronic kidney disease. *Am J Physiol - Renal Physiol* 2010; **298**: F1078–F1094.
5. Chevalier RL, Forbes MS, Thornhill BA. Ureteral obstruction as a model of renal interstitial fibrosis and obstructive nephropathy. *Kidney Int* 2009; **75**: 1145–1152.
6. Ysebaert DK, De Greef KE, De Beuf A *et al.* T cells as mediators in renal ischemia/reperfusion injury. *Kidney Int* 2004; **66**: 491–496.
7. Tapmeier TT, Fearn A, Brown K *et al.* Pivotal role of CD4+ T cells in renal fibrosis following ureteric obstruction. *Kidney Int* 2010; **78**: 351–362.
8. Burne MJ, Daniels F, El Ghandour A *et al.* Identification of the CD4+ T cell as a major pathogenic factor in ischemic acute renal failure. *J Clin Invest* 2001; **108**: 1283–1290.
9. Summers SA, Steinmetz OM, Li M *et al.* Th1 and Th17 cells induce proliferative glomerulonephritis. *J Am Soc Nephrol* 2009; **20**: 2518–2524.
10. Heymann F, Meyer-Schwesinger C, Hamilton-Williams EE *et al.* Kidney dendritic cell activation is required for progression of renal disease in a mouse model of glomerular injury. *J Clin Invest* 2009; **119**: 1286–1297.
11. Mucida D, Cheroutre H, Sidonia F *et al.* The many face-lifts of CD4 T helper cells. *Adv Immunol* 2010; **107**: 139–152.
12. Farrar JD, Asnagli H, Murphy KM. T helper subset development: roles of instruction, selection, and transcription. *J Clin Invest* 2002; **109**: 431–435.
13. Moseley TA, Haudenschild DR, Rose L *et al.* Interleukin-17 family and IL-17 receptors. *Cytokine Growth Factor Rev* 2003; **14**: 155–174.
14. Mills KHG. Induction, function and regulation of IL-17-producing T cells. *Eur J Immunol* 2008; **38**: 2636–2649.
15. Bettelli E, Oukka M, Kuchroo VK. TH-17 cells in the circle of immunity and autoimmunity. *Nat Immunol* 2007; **8**: 345–350.
16. Woltman AM, De Haij S, Boonstra JG *et al.* Interleukin-17 and CD40-Ligand synergistically enhance cytokine and chemokine production by renal epithelial cells. *J Am Soc Nephrol* 2000; **11**: 2044–2055.
17. Paust H-J, Turner J-E, Steinmetz OM *et al.* The IL-23/Th17 axis contributes to renal injury in experimental glomerulonephritis. *J Am Soc Nephrol* 2009; **20**: 969–979.
18. Kitching AR, Holdsworth SR. The emergence of Th17 cells as effectors of renal injury. *J Am Soc Nephrol* 2011; **22**: 235–238.
19. Turner J-E, Paust H-J, Steinmetz OM *et al.* The Th17 immune response in renal inflammation. *Kidney Int* 2010; **77**: 1070–1075.
20. Dong X, Bachman LA, Miller MN *et al.* Dendritic cells facilitate accumulation of IL-17 T cells in the kidney following acute renal obstruction. *Kidney Int* 2008; **74**: 1294–1309.
21. Li L, Huang L, Vergis AL *et al.* IL-17 produced by neutrophils regulates IFN-γ mediated neutrophil migration in mouse kidney ischemia-reperfusion injury. *J Clin Invest* 2010; **120**: 331–342.

22. Kryczek I, Banerjee M, Cheng P et al. Phenotype, distribution, generation, and functional and clinical relevance of Th17 cells in the human tumor environments. *Blood* 2009; **114**: 1141–1149.

23. Acosta-Rodriguez EV, Rivino L, Geginat J et al. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat Immunol* 2007; **8**: 639–646.

24. Nakae S, Iwakura Y, Suto H et al. Phenotypic differences between Th1 and Th17 cells and negative regulation of Th1 cell differentiation by IL-17. *J Leukoc Biol* 2007; **81**: 1258–1268.

25. Acosta-Rodriguez E, Napolitani G, Lanzavecchia A et al. Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol* 2007; **8**: 942–949.

26. Stockinger B, Veldhoen M. Differentiation and function of Th17 T cells. *Curr Opin Immunol* 2007; **19**: 281–286.

27. Manel N, Unutmaz D, Littman D. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORγt. *Nat Immunol* 2008; **9**: 641–649.

28. Volpe E, Servant N, Zollinger R et al. A critical function for transforming growth factor-beta, interleukin 23 and proinflammatory cytokines in driving and modulating human T(H)-17 responses. *Nat Immunol* 2008; **9**: 650–657.

29. Sutton C, Brereton C, Keogh B et al. A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. *J Exp Med* 2006; **203**: 1685–1691.

30. Chung Y, Chang SH, Martinez GJ et al. Critical regulation of early Th17 cell differentiation by interleukin-1 signalling. *Immunity* 2009; **30**: 576–587.

31. Liu H, Rohowsky-Kochan C. Regulation of IL-17 in human CCR6+ effector memory T cells. *J Immunol* 2008; **180**: 7948–7957.

32. Ooi JD, Phoon RKS, Holdsworth SR et al. IL-23, not IL-12, directs autoimmunity to the Goodpasture antigen. *J Am Soc Nephrol* 2009; **20**: 980–989.

33. Zhang Z, Kyttaris VC, Tsokos GC. The role of IL-23/IL-17 axis in lupus nephritis. *J Immunol* 2009; **183**: 3160–3169.

34. Crispín JC, Oukka M, Bayliss G et al. Expanded double negative T cells in patients with systemic lupus erythematosus produce IL-17 and infiltrate the kidneys. *J Immunol* 2008; **181**: 8761–8766.

35. Shao X, Yang X, Zhao X et al. The prevalence of Th17 cells and FOXP3 regulate T cells (Treg) in children with primary nephrotic syndrome. *Ped Nephrol* 2009; **24**: 1683–1690.

36. Wang Y, Ito S, Chino Y et al. Laser microdissection-based analysis of cytokine balance in the kidneys of patients with lupus nephritis. *Clin Exp Immunol* 2010; **159**: 1–10.

37. Gan P-Y, Steinmetz OM, Tan DSY et al. Th17 cells promote autoimmune anti-myeloperoxidase glomerulonephritis. *J Am Soc Nephrol* 2010; **21**: 925–931.

38. Nogueira E, Hamour S, Sawant D et al. Serum IL-17 and IL-23 levels and autoantigen-specific Th17 cells are elevated in patients with ANCA-associated vasculitis. *Nephrol Dial Transplant* 2010; **25**: 2209–2217.

39. Deteix C, Attuil-Audenis V, Duthey A et al. Intragraft Th17 infiltrate promotes lymphoid neogenesis and hastens clinical chronic rejection. *J Immunol* 2010; **184**: 5344–5351.

40. Jones LK, O'Sullivan KM, Semple T et al. IL-1RI deficiency ameliorates early experimental renal interstitial fibrosis. *Nephrol Dial Transplant* 2009; **24**: 3024–3032.

41. Hsieh HG, Loong CC, Lui WY et al. IL-17 expression as a possible predictive parameter for subclinical renal allograft rejection. *Transplant Int* 2001; **14**: 287–298.

42. Van Kooten C, Boonstra JG, Paape ME et al. Interleukin-17 activates human renal epithelial cells *in vitro* and is expressed during renal allograft rejection. *J Am Soc Nephrol* 1998; **9**: 1526–1534.

43. Sutton CE, Lalor SJ, Sweeney CM et al. Interleukin-1 and IL-23 induce innate IL-17 production from γδ T cells, amplifying Th17 responses and autoimmunity. *Immunity* 2009; **31**: 331–341.

44. Steinmetz OM, Turner J-E, Paust H-J et al. CXCR3 mediates renal Th1 and Th17 immune response in murine lupus nephritis. *J Immunol* 2009; **183**: 4693–4704.

45. Turner J-E, Paust H-J, Steinmetz OM et al. CCR6 recruits regulatory T cells and Th17 cells to the kidney in glomerulonephritis. *J Am Soc Nephrol* 2010; **21**: 974–985.

46. Macconi D, Chiabrando C, Schiarea S et al. Proteasomal processing of albumin by renal dendritic cells generates antigenic peptides. *J Am Soc Nephrol* 2009; **20**: 123–130.

47. Rees A. Cross dendritic cells anger T cells after kidney injury. *J Am Soc Nephrol* 2009; **20**: 3–5.

48. Edgerton C, Crispín JC, Moratz CM et al. IL-17 producing CD4+ T cells mediate accelerated ischemia/reperfusion-induced injury in autoimmunity-prone mice. *Clin Immunol* 2009; **130**: 313–321.

49. Sims JE, Smith DE. The IL-1 family: regulators of immunity. *Nat Rev Immunol* 2010; **10**: 89–102.

50. Mills KHG, Dunne A. Immune modulation: IL-1, master mediator or initiator of inflammation. *Nat Med* 2009; **15**: 1363–1364.

51. Dinarello CA. Blocking IL-1 in systemic inflammation. *J Exp Med* 2005; **201**: 1355–1359.

52. Timoshanko JR, Kitching AR, Iwakura Y et al. Contributions of IL-α and IL-β to crescentic glomerulonephritis in mice. *J Am Soc Nephrol* 2004; **15**: 910–918.

53. Furuichi K, Wada T, Iwata Y et al. Interleukin-1-dependent sequential chemokine expression and inflammatory cell infiltration in ischemia-reperfusion injury. *Crit Care Med* 2006; **34**: 2447–2455.

54. Dong X, Swaminathan S, Bachman LA et al. Antigen presentation by dendritic cells in renal lymph nodes is linked to systemic and local injury to the kidney. *Kidney Int* 2005; **68**: 1096–1108.