In acute kidney injury, which is a significant cause of morbidity and mortality, cytokines and leukocytes promote inflammation and injury. We examined the pathogenic role of IL-17A in cisplatin-induced acute kidney injury. Intrarenal IL-17A mRNA transcription and protein expression were increased in wild-type mice after cisplatin-induced renal injury. An important role for IL-17A in the nephrotoxicity of cisplatin was demonstrated by observing protection from cisplatin-induced functional and histological renal injury in Il17a−/− and Rorγt−/− mice, as well as in mice treated pre-emptively with anti—IL-17A antibodies. Both renal injury and renal IL-1β and IL-17A production were attenuated in Asc−/− and Tlr2−/− mice, suggesting that cisplatin induces endogenous TLR2 ligand production and activates the ASC-dependent inflammasome complex, resulting in IL-1β and injurious IL-17A production. Neutrophils and natural killer cells are the likely targets of these pathways, because combined depletion of these cells was strongly protective; anti—IL-17A antibodies had no additional effect in this setting. Although IL-17A can also be produced by CD4+ and γδ T cells, IL-17A from those cells does not contribute to renal injury. Cisplatin-induced injury was unchanged in γδ T-cell–deficient mice, whereas Il17a−/−/CD4+ T cells induced similar injury as did wild-type CD4+ T cells on transfer to cisplatin-injected Rag1−/− mice. These studies demonstrate an important role for TLR2, the ASC inflammasome, and IL-17A in innate leukocytes in cisplatin-induced renal injury. (Am J Pathol 2014, 184: 1411–1418; http://dx.doi.org/10.1016/j.ajpath.2014.01.023)
IL-17A. Binding of surface-expressed TLRs, including TLR2, results in expression of pro–IL-1β. Subsequent activation of the inflammasome, an intracellular multiprotein complex that contains a nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin, domain like receptor (NLRP), and an adaptor protein called apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), results in the generation of mature IL-1β. Although this pathway leads to the production of IL-17A in models of infectious diseases,\(^8,9\) its relevance in autoimmunity and kidney disease is not known. Interestingly, many of the leukocytes that promote AKI can produce IL-17A.\(^5\) CD4\(^+\) T cells mediate cisplatin-induced AKI,\(^10,11\) but a role for Th17 cells has not been defined. Although pathogenic roles for neutrophil-derived IL-17A,\(^12\) γδ T cells,\(^13\) and natural killer (NK1.1) cells\(^14\) have been suggested in ischemic AKI, their role in cisplatin-induced AKI is less well defined.

In the present studies, we explored the role of IL-17A in cisplatin-induced AKI. We found that both functional and histological renal injury were dependent on IL-17A and were attenuated by depletion IL-17A. Activation of the innate pattern recognition receptor TLR2 and the ASC inflammasome was required for the production of IL-17A in the kidney and subsequent renal injury. We also demonstrated that, rather than IL-17A produced by CD4\(^+\) T cells, innate cells were the effector cells mediating cisplatin-induced AKI.

Materials and Methods

Experiment Design and Ethics

Male mice (8 to 10 weeks old) were used in experiments. C57BL/6 wild-type (WT) mice were purchased from Monash University Animal Services (Melbourne, Australia). Il17a\(^−/−\),\(^15\) Rorγ\(^t−/−\),\(^16\) Th2\(^−/−\),\(^17\) Asc\(^−/−\), and Tcrd\(^−/−\) mice from the Jackson Laboratory (Bar Harbor, ME) were all bred at the Monash Medical Centre Animal Facility (Clayton, VIC, Australia). Recombinant activation gene—deficient (Rag I\(^−/−\)) mice were bred at the Walter and Eliza Hall Institute (Melbourne, Australia). Cisplatin (15 mg/kg) (Sigma-Aldrich, St. Louis, MO) was injected intraperitonally. Experiments ended at either 24 or 96 hours. Neutralizing or depleting monoclonal antibodies against IL-17A, neutrophils (anti-Ly6G), and NK1.1 cells (anti-NK1.1) and isotype control antibodies were purchased from Bio X Cell (West Lebanon, NH). Antibodies (100 μg) were dissolved in 200 μL saline and injected intraperitonally before cisplatin administration (day –1) and again at 24 hours (day 1) and 72 hours (day 3) after cisplatin-induced injury.

Studies adhered to the National Health and Medical Research Council of Australia guidelines for animal experimentation.

Assessment of Renal Injury

Kidney sections were fixed in buffered formalin for 24 hours and embedded in paraffin wax. Tubular injury was assessed on PAS-stained sections, coded so that the person scoring slides was masked to the group from which the sections originated. Scoring was performed as described previously.\(^18−20\) Tubular injury was determined by assessing tubular epithelial cell loss, tubular necrosis, accumulation of cellular debris, and tubular cast formation. Injury was scored according to the percentage of affected tubules, as viewed under high-power microscopy, on a five-point scale: 0, normal; 1, ≤10% to 25%; 2, 26% to 50%; 3, 51% to 75%; and 4, ≥76%. Blood urea nitrogen (BUN) was measured by autoanalyzer on serum collected at the end of experiments.

Interstitial CD4\(^+\) and Neutrophil Staining

Kidney sections were fixed in periodate–lysine–paraformaldehyde for 4 hours, washed with 20% sucrose solution, and frozen in liquid nitrogen. A three-layered immunoperoxidase technique as described previously\(^21,22\) was used to stain for T cells and neutrophils on 6-μm tissue sections. The primary antibodies used were GK1.5 (anti-mouse CD4; ATCC, Manassas, VA) and RB6–8C5 (anti–Gr-1; DNAX, Palo Alto, CA) for neutrophils. The secondary antibody used was rabbit anti-rat biotin (BD Biosciences, San Jose, CA; North Ryde, Australia). A minimum of 20 consecutive interstitial fields were assessed per animal, and results were expressed as cells per high-power field.

Intrarenal Cytokine mRNA Expression and Intrarenal IL-17A and IL-1β Protein

To assess mRNA expression of IL-17A, CCL5, CCL2, CXCL1, CXCL2, and CCL20, RNA was extracted from whole kidney as described previously and was measured by quantitative real-time PCR.\(^21,22\) Gene-specific oligonucleotides were synthesized (Life Technologies, Carlsbad, CA; Melbourne, Australia) as described previously.\(^21,22\) Expression was standardized to 18S (housekeeping gene) before being expressed as a fold change relative to control or cisplatin-injected mice. To measure IL-17A and IL-1β protein, kidneys were placed in 5% fetal calf serum and homogenized. Homogenates were centrifuged at 167,700 × g for 15 minutes. Supernatants were isolated and assessed for cytokine production of IL-17A (R&D Systems, Minneapolis, MN) and IL-1β (Elisa Kit, Victoria, Australia).

Leukocyte Isolation and Flow Cytometry

Kidneys were finely minced, then digested with 5 mg/mL collagenase D (Roche Diagnostics, Indianapolis, IN) and 100 μg/mL DNase I (Roche Diagnostics) in Hanks’ balanced salt solution (37°C, 20 minutes) (Sigma-Aldrich). Kidney cells were dissociated with a 1000-μL pipette tip and incubated for 5 minutes. This step was repeated before cells were gently passed through a 20-gauge syringe until smooth and washed in PBS (with 0.5% bovine serum albumin, 2 mmol/L EDTA). Cells were filtered using a 40-μm
cell strainer (BD Biosciences), erythrocytes were lysed, and the CD45<sup>+</sup> leukocyte population was isolated using magnetic-activated cell sorting with mouse CD45 MicroBeads (Miltenyi Biotec, Auburn, CA; Bergisch Gladbach, Germany). For IL-17A intracellular cytokine staining, CD45<sup>+</sup> renal cells were activated by incubation with 50 ng/mL phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich) and 1 μg/mL ionomycin (Sigma-Aldrich) in X-VIVO 20 medium (37°C, 4 hours) (Lonza, Walkersville, MD). After 30 minutes, 10 μg/mL brefeldin A (Sigma-Aldrich) was added. Cells were washed and stained for 20 minutes at 4°C with fluochrome-conjugated antibodies: anti-mouse CD45 (Pacific Blue, 30-F11; BioLegend, San Diego, CA), anti-mouse CD4 (phycoerythrin, GK1.5; eBioscience, San Diego, CA), and anti-mouse Ly6G (APC, 1A8; BD Biosciences). Intracellular IL-17A staining (fluorescein isothiocyanate, eBio17B7; eBioscience) was performed using a BD Cytofix/Cytoperm kit (BD Biosciences). Samples were acquired on a FACSCanto II instrument using FACSDiva software version 6.1.2 (BD Biosciences), and flow cytometric data were analyzed using FlowJo software version 8.8.6 (TreeStar, Ashland, OR). Fluorescence – 1 controls were used to ensure specificity in data analysis; doublets were excluded by forward- and side-scatter profile.

For CD4<sup>+</sup> T cell transfer studies, CD4<sup>+</sup> T cells were isolated from spleens of WT or Il17a<sup>−/−</sup> mice by magnetic-activated cell sorting using L3T4 MicroBead antibody (Miltenyi Biotec, Auburn, CA; Bergisch Gladbach, Germany). We injected 3 × 10<sup>6</sup> CD4<sup>+</sup> T cells (from WT or Il17a<sup>−/−</sup> mice) intravenously into Rag1<sup>−/−</sup> mice and waited 3 weeks to facilitate reconstitution, according to published protocols.11

Statistical Analysis
Student’s t-test was used for analysis of two groups and one-way analysis of variance (Tukey’s post hoc test) for more than two groups of data using GraphPad Prism software version 6.0 (GraphPad Software, San Diego, CA). P < 0.05 was considered significant.

Results
Kidney IL-17A Expression Is Increased after Cisplatin-Induced Injury

We have previously shown that, after cisplatin administration, renal inflammation peaks after 24 hours and substantial kidney injury has occurred by 96 hours.18 We therefore injected WT mice with cisplatin and measured kidney expression of IL-17A and related chemokines at 24 and 96 hours after injection. Kidney mRNA for IL-17A peaked at 24 hours and had returned to baseline levels by 96 hours (Figure 1A). CCL20, an important chemokine in CD4<sup>+</sup> T cell infiltration, was also increased at 24 hours after cisplatin injection (Figure 1B). The expression of two key neutrophil chemoattractants, CXCL1 (Figure 1C) and CXCL2 (Figure 1D), peaked at 24 and 96 hours after cisplatin administration, respectively.

Figure 1  Kidney IL-17A mRNA expression increases after cisplatin administration in WT mice. A: Relative to unadministered mice, kidney IL-17A expression was increased at 24 hours after cisplatin injections, but returned to baseline by 96 hours. B: Expression of the key Th17-attracting chemokine CCL20 peaked at 96 hours. C and D: Kidney expression of two key neutrophil chemoattractants, CXCL1 (C) and CXCL2 (D), peaked at 24 and 96 hours after cisplatin administration, respectively. Data are expressed as means ± SEM. n = 8 (untreated; treated, 96 hours). *P < 0.05, **P < 0.01, and ***P < 0.001.

Figure 2  Functional and histological renal injury decreases in the absence of IL-17A. A and B: At 96 hours after cisplatin administration, functional renal injury measured by serum BUN (A) and histological injury score (B) was decreased in Il17a<sup>−/−</sup> mice, relative to WT mice. C–F: Representative low- and high-power photomicrographs of tubulointerstitial injury in WT (C and E) and Il17a<sup>−/−</sup> (D and F) mice. Data are expressed as means ± SEM. n = 8 (Il17a<sup>−/−</sup>) or 9 (WT). **P < 0.01; ***P < 0.001.
Th17 cell recruitment, peaked at 96 hours after cisplatin-induced injury (Figure 1B). Of two key neutrophil chemoattractants, CXCL1 peaked at 24 hours, whereas CXCL2 was higher at 96 hours (Figure 1, C and D).

Endogenous IL-17A and RORγt Mediate Functional and Histological Renal Injury

WT and Il17a−/− mice were administered cisplatin, and kidney injury was assessed 96 hours later. BUN and histological renal injury were diminished in the absence of IL-17A (Figure 2, A and B). The marked tubular necrosis and injury consistently observed in WT mice after cisplatin administration was reduced in the absence of endogenous IL-17A (Figure 2, C and F). To define the role of RORγt, the key transcription factor that induces IL-17A, we injected WT and Rorγt−/− mice with cisplatin and assessed renal injury 96 hours later. Compared with WT mice, there was a decrease in functional and histological renal injury in Rorγt−/− mice (Figure 3).

To assess the potential of IL-17A inhibition in AKI, we administered anti–IL-17A antibodies (or isotype control antibodies) to WT mice 1 day before cisplatin-induced injury, with further doses 1 and 3 days after cisplatin. Mice treated with anti–IL-17A antibodies showed less functional and histological renal injury after 96 hours (Figure 4).

TLR2 Activation and the ASC-Dependent Inflammasome Complex Regulate Kidney IL-17A Production and Injury

Because both the inflammasomes and TLR2 have been implicated in IL-17A production, we administered cisplatin to WT, Asc−/−, and Tlr2−/− mice. After 96 hours, one kidney was homogenized and the supernatants were assessed by enzyme-linked immunosorbent assay for IL-17A and IL-1β production. Compared with WT mice, production of kidney IL-17A and IL-1β was decreased in both Asc−/− and Tlr2−/− mice (Figure 5, A and B). Renal injury was decreased in both Asc−/− and Tlr2−/− mice (Figure 5, C–G), indicating that TLR2 binding and ASC activation are required both for production of IL-17A within the kidney and for development of injury.

Both Neutrophils and CD4+ T Cells Produce IL-17A, and Neutrophil Recruitment Is IL-17A Dependent

Subsequently, we injected WT mice with cisplatin and assessed IL-17A production by intrarenal leukocytes (Figure 6, A–C). Both innate (Ly6G+ neutrophils) and adaptive (CD4+ T cells) immune cells produced cells produced IL-17A; approximately 30% of the IL-17A—producing cells were neutrophils and 15% were CD4+ T cells. Compared with cisplatin-injected WT mice, neutrophil recruitment in Il17a−/− mice was diminished in the absence of IL-17A at 24 hours (Figure 6, D–F), but interstitial CD4+ T-cell recruitment was unchanged (Figure 6, G–I). IL-17A deficiency did not result in significant changes to intrarenal expression of CXCL1, CXCL2, CCL2, or CCL5 mRNA 24 hours after cisplatin (Table 1). Thus, although both neutrophils and CD4+ T cells in the injured kidney produce IL-17A, only neutrophil recruitment requires endogenous cisplatin-induced IL-17A production.
Cisplatin-Induced AKI Occurs Independently of γδ T Cells and of IL-17A Produced by T Cells

To determine whether γδ T cells are required for AKI, we administered cisplatin to WT and Tcrd−/− mice (which lack γδ T cells). There was no difference in functional and histological renal injury between WT and Tcrd−/− mice after 96 hours (Figure 7, A and B). T cells are important mediators of renal injury after cisplatin administration, and Rag1-deficient mice lack adaptive immune cells. We reconstituted the T-cell compartment of Rag1−/− mice with CD4+ T cells from WT or Il17a−/− mice and then injected the Rag1−/− mice with cisplatin. WT CD4+ T cells enhanced renal injury, compared with cisplatin-injected unreconstituted Rag1−/− mice; reconstitution with Il17a-deficient T cells resulted in similar increases in functional and histological injury (Figure 7, C and D).

Innate Effector Cells Propagate IL-17A–Induced AKI

To investigate whether the effects of IL-17A inhibition result solely from decreased renal neutrophil recruitment, we treated three groups of WT mice with cisplatin. The first group received isotype control antibodies alone, the second group underwent neutrophil depletion using an anti-Ly6G antibody with an additional isotype control antibody, and the third group underwent neutrophil depletion (anti-Ly6G antibodies) and IL-17A inhibition using an anti–IL-17A antibody. Although there was a decline in functional and histological injury in mice treated with anti-Ly6G antibody, this reached statistical significance only after the additional administration of an anti–IL-17A antibody (Figure 8, A and B). These results indicate that the effects of IL-17A inhibition extend beyond limiting the pathogenic effects of neutrophils.

Because NK cells also can produce IL-17A, we investigated their contribution to cisplatin-induced AKI using three groups of cisplatin-injected mice. The first group received control antibodies, the second group underwent depletion of NK cells and neutrophils (receiving both anti-NK1.1 and anti-Ly6G antibodies), and the third group, in addition to being depleted of NK cells and neutrophils, also underwent IL-17A inhibition. Although functional and histological renal injury diminished after treatment with anti-NK1.1 and anti-Ly6G, the addition of anti–IL-17A therapy did not offer additional therapeutic protection (Figure 8, C and D). These results indicate that innate immune cells are the key drivers of IL-17A–mediated AKI.

Discussion

In our present studies, we demonstrated that innate IL-17A is a significant contributor to cisplatin-induced AKI. Neutralizing endogenous IL-17A significantly attenuated renal functional and histological damage, thus identifying anti–IL-17A–based therapies as potential treatments for human drug-induced AKI. Activation of TLR2 and the ASC inflammasome were required for generation of IL-17A within the kidney. The injurious effects of IL-17A resulted from recruitment of innate effector cells, rather than to IL-17A produced from CD4+ T cells or γδ T cells.

The demonstration of the important role for IL-17A in cisplatin-induced AKI has clinical implications. Several IL-17A neutralizing antibodies tested in clinical practice show great promise for treating psoriasis, psoriatic arthritis, and ankylosing spondylitis. IL-17A serum levels are increased in patients with glomerulonephritis and correlate with disease severity in patients with autoimmune anti-neutrophil cytoplasmic antibody–associated vasculitis and lupus nephritis. However, anti–IL-17A monoclonal antibodies have not yet been tested in the treatment of kidney diseases. Given that pre-emptive treatment with anti–IL-17A antibody protected mice from cisplatin-induced AKI, this therapy may be useful in clinical AKI. The occurrence of AKI is often predictable, so preventive treatment with anti–IL-17A therapy could improve clinical outcomes.

Innate pattern recognition receptors, including TLRs and the inflammasome, trigger inflammation in response to both invading pathogens and endogenous molecules released from injured tissue. Activation of the inflammasome results in the production of active IL-1β and IL-18, important mediators of inflammation whose production and release is tightly
controlled. Before activation of the inflammasome, an initiating signal provided by TLR binding is required, resulting in the generation of pro–IL-1β and pro–IL-18 (often referred to as signal 1). Subsequent activation of the inflammasome results in the generation of active IL-1β and IL-18, which then induces the downstream generation of IL-17A.9 In the present study, intrarenal IL-17A production was diminished in Asc–/– mice, which were also protected from renal injury. Similarly, Tlr2–/– mice demonstrated decreased renal IL-1β and IL-17A production, with renal protection. These results suggest that findings reported in models of infection are also relevant in inflammatory renal disease.8,9

It is likely that, when cisplatin administration triggers cell necrosis, endogenous TLR2 ligands are produced that bind TLR2.27,28 The resultant ASC-dependent inflammasome activation leads to the IL-17A production that mediates injury. The adaptor protein ASC is involved in two inflammasome complexes, the NLRP3 and AIM2,26 and further studies are needed to determine the relative contribution of each complex in this process. Although we have identified IL-17A as a potential therapeutic target, our findings also help identify additional upstream targets.

The role of CD4+ T cells producing IL-17A in autoimmune and inflammatory renal disease has been well studied. In a planted antigen model of glomerulonephritis, Th17 cells induce proliferative glomerulonephritis by recruiting neutrophils to glomeruli.32 Similarly pathogenic roles for IL-17A have been demonstrated in CD4+ T-cell–dependent models of rapidly progressive glomerulonephritis.15,30 CD4+ T cells play a role in cisplatin-induced renal injury; CD4+ T cell–deficient mice are protected from cisplatin-induced AKI, whereas reconstitution with CD4+ T cells restores injury.11 In a previous study, Rag1–/– mice were protected from injury after cisplatin administration,20 Reconstitution of the CD4+ T cell compartment before cisplatin injections restored the pattern and intensity of injury. However, although kidney expression of the key Th17 chemokine CCL20 increased after cisplatin administration, the accumulation of interstitial CD4+ T cells was not altered in the absence of IL-17A. Furthermore, reconstituting Rag1–/– with IIf17a–/– deficient CD4+ T cells resulted in injury similar to that seen when WT CD4+ T cells were used. The results confirm that IL-17A produced by CD4+ T cells is not important in cisplatin-induced AKI. Recently, γδT cells have been identified as important in several IL-17A–mediated diseases,34 including glomerulonephritis induced by a planted foreign antigen.35 Because Tcrd–/– mice develop less injury after undergoing ischemic AKI,34 we

Table 1 Intrarenal Chemokine mRNA Expression at 24 Hours after Cisplatin-Induced Renal Injury in WT and IIf17a–/– Mice

<table>
<thead>
<tr>
<th>Kidney mRNA</th>
<th>WT (RQ)</th>
<th>IIf17a–/– (RQ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCL1</td>
<td>1.0 ± 0.4</td>
<td>1.9 ± 0.6</td>
</tr>
<tr>
<td>CXCL2</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>CCL2</td>
<td>1.0 ± 0.1</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>CCL5</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SEM. n = 10 (WT); n = 9 (IIf17a–/–). P > 0.05.

RQ, relative quantification.
Figure 7  Cisplatin-induced renal injury is not mediated by γδ T cells or CD4+ T cells that produce IL-17A. A and B: Compared with renal injury observed in WT mice injected with cisplatin, functional (A) and histological (B) injury did not differ in Tcrd−/− mice, which lack γδ T cells. C and D: Subsequently, we reconstituted Rag1−/− mice with CD4+ T cells; when reconstitution was complete, mice were administered cisplatin. Although renal injury was increased in Rag1−/− mice reconstituted with WT CD4+ T cells compared with unreconstituted Rag1−/− (dashed lines), functional (C) and histological (D) injury in Rag1−/− mice reconstituted with Il17a−/−CD4+ T cells compared with WT CD4+ T cells was similar to that seen when WT cells were used. Data are expressed as means ± SEM. n = 8 (WT, A and B); n = 9 (Tcrd−/−); n = 7 (Rag1−/− unreconstituted (dashed lines); Rag1−/− mice receiving Il17a−/− CD4+ T cells; and Rag1−/− mice receiving WT CD4+ T cells).

Figure 8  Inhibition of both neutrophils and NK cells protects WT mice from cisplatin-induced renal injury. A and B: WT mice treated with control antibodies and cisplatin developed significant functional (A) and histological (B) renal injury. Pre-emptive neutrophil depletion with anti-Ly6G, followed by cisplatin, resulted in a trend toward attenuated injury; however, a combination of neutrophil depletion and anti-IL-17A neutralization before cisplatin injections significantly attenuated renal injury. C and D: WT mice treated with control antibodies and cisplatin developed significant functional (C) and histological (D) renal injury. Pre-emptive treatment of WT mice with anti-Ly6G and anti-NK1.1 antibodies offered significant protection, which was not enhanced after additional IL-17A neutralization. Data are expressed as means ± SEM. n = 6 (A and B, white and gray bars); n = 7 (A and B, solid bars; C and D). *P < 0.05, **P < 0.01, and ***P < 0.001.

postulated that γδ T cells contribute to cisplatin-induced AKI. However, deleting γδ T cells did not alter functional and histological renal injury.

The inflammatory effects of IL-17A are exerted not only through adaptive immune cells, but also through innate immune cells (neutrophils, in particular). In response to microbial infection, IL-17A produced within 4 hours enhances the production of neutrophil-attracting chemokines, thus stimulating neutrophil recruitment. Although early IL-17A−induced neutrophil recruitment is important in host defense after bacterial and fungal infections, IL-17A−directed neutrophil recruitment can be injurious. After cisplatin injections, fewer neutrophils were seen in kidneys of Il17a−/− mice, compared with WT mice, although changes in CXCL1 and CXCL2 expression were unclear. These results indicate that IL-17A is required for neutrophil recruitment after cisplatin administration.

Pre-emptive neutrophil depletion with anti-Ly6G antibody partially attenuated renal injury, although this did not reach statistical significance unless IL-17A was also inhibited, suggesting that the action of IL-17A inhibition extends beyond prevention of neutrophil recruitment. Data on the role of neutrophils in cisplatin-induced renal injury are somewhat conflicting. It has been shown that interstitial neutrophil recruitment increases after cisplatin administration, although administration of a single dose of anti-Gr-1 antibody did not attenuate renal injury. However, Cxcr2−/− deficient mice, which lack the receptor for CXCL1 and CXCL2, were protected from cisplatin-induced AKI. Thus, it would seem likely that effective neutrophil depletion offers some protection (although not complete protection) from cisplatin-induced AKI and that this effect is, at least partially, IL-17A dependent.

We also found that the renal protection observed after depletion of neutrophils and NK1.1 cells was not further enhanced after IL-17A neutralization. In kidney biopsies from humans with acute tubular necrosis, NK1.1+ cells form part of the inflammatory milieu, and NK1.1+ cells drive kidney injury after ischemia–reperfusion. In cisplatin-induced AKI, numbers of NK1.1+ cells increased with disease progression, although inhibition did not significantly attenuate renal injury. These results, together with our present findings, suggest that inhibiting neutrophils or NK1.1+ cells alone is insufficient to protect from cisplatin-induced AKI; however, inhibition of both neutrophils and NK1.1+ cells offers protection, which is most likely mediated through IL-17A.

In conclusion, IL-17A mediates cisplatin-induced AKI, and IL-17A blockade has therapeutic promise. Activation of TLR2 and the ASC-dependent inflammasome complex is required for generation of renal IL-17A and renal injury.
The mechanism of IL-17A–induced injury involves a combination of innate effector cells, rather than IL-17A–producing CD4\(^+\) T cells or γδ T cells.

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