Endogenous IL-10 Attenuates Cisplatin Nephrotoxicity: Role of Dendritic Cells

Raghu Kempegowda Tadagavadi and William Brian Reeves

J Immunol published online 15 September 2010
http://www.jimmunol.org/content/early/2010/09/15/jimmunol.1000383

Subscription Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Endogenous IL-10 Attenuates Cisplatin Nephrotoxicity: Role of Dendritic Cells

Raghu Kempegowda Tadagavadi and William Brian Reeves

Sterile inflammation is associated with tissue injury and organ failure. Recent studies indicate that certain endogenous cytokines and immune cells may limit tissue injury by reducing immune-mediated inflammatory responses. Cisplatin is a commonly used anticancer chemotherapeutic agent but causes acute kidney injury and dysfunction. In a recent study, we showed that renal dendritic cells attenuate cisplatin-induced kidney injury by reducing inflammation. In this study, we investigated the effect of endogenous IL-10 and dendritic cell IL-10 in cisplatin-mediated kidney injury. Cisplatin treatment caused increases in renal IL-10R1 expression and STAT3 phosphorylation. In response to cisplatin treatment, IL-10 knockout mice showed more rapid and greater increases in blood urea nitrogen and serum creatinine compared with wild-type mice, indicating that endogenous IL-10 ameliorates kidney injury in cisplatin nephrotoxicity. Renal infiltration of IFN-γ in cisplatin nephrotoxicity. Renal dendritic cells showed high expression of IL-10 in response to cisplatin treatment. We further investigated the effect of dendritic cell-derived IL-10 in cisplatin nephrotoxicity using a conditional cell ablation approach. Mixed bone marrow chimeric mice lacking IL-10 in dendritic cells showed moderately greater renal dysfunction than chimeric mice positive for IL-10 in dendritic cells. These data demonstrate that endogenous IL-10 reduces cisplatin nephrotoxicity and associated inflammation. Moreover, IL-10 produced by dendritic cells themselves accounts for a portion of the protective effect of dendritic cells in cisplatin nephrotoxicity. The Journal of Immunology, 2010, 185: 000–000.
press pathogenic T cell responses both in vivo and in vitro (7, 26).
In general, dendritic cells present under steady-state conditions are
known for their ability to inhibit inflammation by various mecha-
nisms including production of IL-10, TGF-β, or IDO and regu-
lation of Tregs (15, 16, 20, 28, 29). In contrast, dendritic cells can
also initiate immunity or inflammatory tissue injury in response to
pathogens or products of cell death (30). Monocytes in an inflam-
matory milieu can differentiate into inflammatory dendritic cells and
mediate inflammation. Studies in different models of inflammation,
including transplantation, sepsis, reperfusion injury, and cytotoxicity,
suggest that tissue-resident dendritic cells possess anti-inflammatory
functions (3, 7, 15, 31). In addition, recent studies also indicate that
IL-10 produced by dendritic cells themselves or by cells under the
influence of dendritic cells ameliorates inflammatory immune re-
 sponses (3, 7, 20, 32).
Using mice that express the simian diphtheria toxin (DT) receptor
(DTR) driven by CD11c promoter (CD11c-DTRtg), we showed that
dendritic cells protect the kidney from cisplatin nephrotoxicity (15).
It is possible that production of IL-10 by dendritic cells is an
edogenous protective mechanism in cisplatin nephrotoxicity. To test
this hypothesis, we investigated the actions of endogenous IL-10
and dendritic cell-derived IL-10 in cisplatin nephrotoxicity. To
evaluate the role of dendritic cell IL-10, we employed a con-
tinuous cell ablation approach in which a mixed bone marrow
chimera was created containing hematopoietic cells equally de-
derived from CD11c-DTRtg and IL-10 knockout (KO) mice (33).
DT treatment in these mice depletes IL-10–positive dendritic cells,
leaving behind dendritic cells negative for the IL-10 gene. Our
results indicate that endogenous IL-10 protects mice from cisplatin
nephrotoxicity. Although dendritic cells showed significant atten-
uation of kidney injury, only a portion of this protection could be
attributed to IL-10 produced by dendritic cells.

Materials and Methods

Mice
Experiments were performed using 8–10-wk-old C57BL6 mice and IL-10
KO mice (B6.129P2-Ii10tm1Cgn/J) and CD11c-DTRtg mice (B6.FVB-Tg
Itgax-DTR/GFP 57Lan/J) harboring a transgene encoding a simian DTR/
GFP fusion protein under the transcriptional control of mouse CD11c
promoter. For making bone marrow chimeras, 6–8-wk-old donor mice
were euthanized with sodium pentobarbital, and the femurs were removed
and flushed with DMEM medium containing 10% FBS to obtain bone
marrow cells. Six-week-old recipient mice were exposed to
acute kidney injury was induced in mice by a single i.p. injection of
cisplatin (20 mg/kg body weight). Dendritic cells were ablated in chimeric
mice by i.p. injection of DT (4 ng/gm body weight) twice, 24 h before and
24 h after cisplatin injection. For experiments to determine the role of IFN-γ
in cisplatin nephrotoxicity, IFN-γ–neutralizing Ab (100 μg/mouse) or
isotype control Ab (eBioscience, San Diego, CA) was injected i.p. 1 h
before cisplatin injection (13). Renal function was determined by mea-
suring blood urea nitrogen (BUN; VITROS DT60 II chemistry slides, Ortho
Clinical Diagnostics, Rochester, NY) and serum creatinine (DZ072B;
Diazyme Laboratories, Poway, CA).

IL-10 quantification
Serum IL-10 levels were measured using an IL-10 ELISA kit (R&D
Systems, Minneapolis, MN).

Immunostaining
Formalin-fixed kidney tissue sections were stained for neutrophils using
Ly-6G Ab as described before (15). Briefly, kidney tissue sections of 5 μm
thickness were deparaffinized, and Ag retrieval was performed using 10 mM
sodium citrate buffer. Immunohistochemistry for neutrophils was performed
using a streptavidin–biotin–anti-mouse neutrophil-specific primary Ab (Ly-6G, clone 1A8,
BD Biosciences, San Jose, CA). Five ×20 original magnification fields were
examined in each kidney section for quantification of neutrophils.

Western blot analysis
Kidneys were homogenized in lysis buffer, separated on 10% SDS-PAGE,
and then transferred onto polyvinylidene difluoride membranes. After
blocking, the membranes were incubated with rabbit anti–p-STAT3 and anti-
STAT3 Ab (Cell Signaling Technology, Boston, MA) followed by HRP-
conjugated goat anti-rabbit Ab. After washing, proteins on the membrane
were detected using ECL detection reagent (Amersham Biosciences, Pic-
cataway, NJ).

Flow cytometry
Single-cell suspensions of kidneys were prepared for flow cytometry as
described before (15). Briefly, kidneys were minced into fragments of 1
mm³ and digested with 2 mg/ml collagenase D and 100 U/ml DNase I for
45 min. The digested kidneys were passed through 100-μm followed by
40-μm mesh. RBCs in the resulting renal suspension were lysed using
RBC lysis buffer (Sigma-Aldrich, St. Louis, MO).

Renal cells were treated with rat anti-FcR from 2.4G2 hybridoma su-
perantigen to block FcRs and then stained using the following fluorochrome-
labeled Abs: anti-CD45 (clone 30-F11), CD11c (HL3), F4/80 (BM8),
CD11b (M1/70, 7/4) (AbD Serotec, Oxford, U.K.), Ly-6G (1A8, Biologic,
San Diego, CA), CD4 (GK1.5), CD8 (53-6.7), B220 (RA3-6B2), NK1.1
(FK136), CD3 (145-2C11), PDCA-1 (927), and IFN-γ (XMGl.2). Unless
otherwise mentioned, the Abs were purchased from BD Pharmingen (San
Diego, CA) or eBioscience. Intracellular cytokine staining was performed
using Cytofix/Cytoperm reagent (BD Biosciences). Flow cytometry
was performed on a FACSAcurCalibur and analyzed using CellQuest
(BD Pharmingen) or WinMDI 2.8 free software (http://facs.scripps.edu/software.
hml). Renal dendritic cells (CD45+CD11c+) from single-cell suspensions
of the kidneys were sorted by flow cytometry using a MoFlo (Beckman
Coultier, Brea, CA) cell sorter (purity ≥95%).

Quantification of mRNA by real-time PCR
Total RNA was extracted from kidneys of renal dendritic cells and reverse
transcribed using the Omniscript reverse transcription kit (Qiagen, Valencia,
CA) and random primers as described before (9). The cDNA was amplified
using the SYBR Green PCR amplification kit (Qiagen) in the Applied
Biosystems 7700 sequence detection system (Applied Biosystems, Foster
City, CA). The primers used were: IL-10R1 (forward: 5'-GGG GAA GAA
ATC GAT GAC AG-3', reverse: 5'-ACT CAA GG-3'), CXCL1 (forward:
5'-ACT CAA CAT TCA CGG TGC TG-3', reverse: 5'-GGG GTG TTGA
-3'), CXCL2 (forward: 5'-ATG CAG GTC CCT GTC-3', reverse: 5'-GCC
TCT AGG ATT CAC CTC AAG AA-3'), IL-10R2 (forward: 5'-GCC
AGC TCT GAG AAT GAT TC-3', reverse: 5'-ATT GTT CCT CA A GGT
-3'), CXCL3 (forward: 5'-GCC AAC CT TAC GAG GAA ATT AG-3',
reverse: 5'-GCC AGA GAA AAT GAT GAG AC-3'), β-actin (forward: 5'-TGT
TAC CAA CTG GCA GGA CA-3', reverse: 5'-GGG GTG TGGA CTG
TCT ACA AA-3'), CXCL10 (forward: 5'-GCC TGG ATT CAT CTC AAG
-3'), IL-10 (forward: 5'-GCC AAC CT TAC GAG GAA ATT AG-3',
reverse: 5'-GAG GGA AAT GAT GAG AC-3'), IL-10 (forward: 5'-GCC
AAC CT TAC GAG GAA ATT AG-3', reverse: 5'-GAG GGA AAT GAT GAG
AC-3'). The amplification specificity
was confirmed by melting-curve analysis. Quantitative
levels of different mRNA were normalized to β-actin expression.

Statistical analysis
Results were expressed as mean ± SE. Data were analyzed using two-
tailed t test or one-way ANOVA with Bonferroni analysis. A p value <0.05
was considered significant.

Results
Cisplatin increases renal IL-10R expression and STAT3 phosphorylation
The IL-10R is a heterodimer complex composed of two subunits,
R1 and R2 (34). IL-10R1 binds selectively to IL-10 independent
of IL-10R2 and is generally rate limiting to IL-10R formation (35). However, IL-10R2 binding to the IL-10/IL-10R1 complex is required for efficient signaling through the members of STAT family (36). In this study, we investigated the serum IL-10 concentration, renal IL-10, IL-10R1, and IL-10R2 expression, and STAT3 phosphorylation in response to cisplatin treatment (Fig. 1). Mice treated with cisplatin showed an initial decrease in serum IL-10 at 24 h, followed by an increase at 48 h and 72 h compared with mice treated with saline (Fig. 1A). Mice injected with cisplatin showed a dramatic upregulation of IL-10R1 but not of IL-10 or IL-10R2 in kidneys at 24 h compared with saline-treated mice (Fig. 1B). The basal level of IL-10R2 expression in the kidney was much higher (~200-fold) than for IL-10R1, but did not change after cisplatin treatment. In addition, kidneys from mice treated with cisplatin showed significant phosphorylation of STAT3 at 24 h and 48 h (Fig. 1C). Phosphorylation of STAT3 was almost absent in saline-treated kidneys. These results are consistent with activation of IL-10R signaling in the kidney after cisplatin treatment and a possible role for endogenous IL-10 in cisplatin nephrotoxicity.

**Endogenous IL-10 ameliorates cisplatin nephrotoxicity**

Certain renal pathologies are ameliorated by exogenous or endogenous IL-10 (18, 23–25). Exogenous administration of IL-10 attenuates cisplatin nephrotoxicity (8). However, the role of endogenous IL-10 in modulating cisplatin-induced kidney injury is unknown. Having determined that cisplatin treatment causes upregulation of IL-10R1 and phosphorylation of STAT3, we next investigated the role of endogenous IL-10 in the pathogenesis of cisplatin-mediated acute renal failure. WT and IL-10 KO mice were treated with cisplatin and renal function was assessed by measuring the levels of BUN and serum creatinine. As shown in Fig. 2, WT mice treated with cisplatin showed minimal increases in the levels of BUN and serum creatinine (Fig. 2A) at 24 h with more dramatic increases at 48 h and 72 h. In comparison with WT mice, IL-10 KO mice treated with cisplatin showed greater increases in the levels of BUN and serum creatinine. WT and IL-10 KO mice treated with saline had comparable basal levels of BUN and serum creatinine. These findings indicate that endogenous IL-10 production is protective in cisplatin nephrotoxicity. In cisplatin nephrotoxicity, a number of cytokines and chemokines are upregulated in the kidney and contribute to renal dysfunction (9, 37). IL-10 is known to inhibit the production of different adhesion molecules, cytokines, and chemokines. Therefore, we investigated the impact of the deletion of endogenous IL-10 on the expression of adhesion molecules and chemokines during cisplatin nephrotoxicity. IL-10 KO mice treated with cisplatin showed increased expression of ICAM-1, CCL2, CXCL1, and CXCL10 compared with WT mice treated with saline (Fig. 2C), indicating that endogenous IL-10 reduces renal inflammation induced by cisplatin.

**IL-10 attenuates renal infiltration of neutrophils**

Acute sterile inflammation instigates infiltration of neutrophils and monocytes into injured tissues (3, 15). In our earlier studies of cisplatin nephrotoxicity, we demonstrated both early and profound infiltration of neutrophils into kidneys, followed by monocytes at later stages of renal injury (15). IL-10 inhibits monocyte and neutrophil infiltration and their production of inflammatory cytokines (7, 18–25). Because the absence of endogenous IL-10 exacerbated kidney injury and increased the expression of CXCL10 and CCL2, potent neutrophil and monocyte chemoattractants, we examined renal infiltration of leukocytes in WT and IL-10 KO mice 48 h after cisplatin injection. The number of neutrophils in kidneys of IL-10 KO mice treated with saline was comparable to that of saline-treated WT mice (Fig. 3A). However, IL-10 KO mice treated with cisplatin showed a large influx of neutrophils into kidneys. This observation is consistent with an earlier observation in renal ischemia reperfusion injury that endogenous IL-10 attenuates kidney injury and infiltration of neutrophils (38). Immunohistochemical staining of renal sections for neutrophils confirmed the findings obtained by flow cytometry (Fig. 3B, 3C). Cisplatin treatment had no impact on monocyte infiltration in IL-10 KO mice compared with WT mice at 48 h. The numbers of T cells, B cells, NK cells, and plasmacytoid dendritic cells in saline- or cisplatin-treated IL-10 KO mice were also comparable to WT mice. Likewise, renal resident macrophages and dendritic cell numbers were not dramatically altered in WT or IL-10 KO mice treated with either saline or cisplatin.

IFN-γ plays a critical role in the pathogenesis of acute kidney injury (12, 13, 39). In renal ischemia reperfusion injury, neutrophils produce IFN-γ and mediate kidney injury. In this study, we investigated IFN-γ expression in neutrophils that infiltrated into kidney at 48 h after cisplatin treatment (Fig. 4A, 4B). IL-10 KO mice treated with saline showed very low numbers of IFN-γ–positive neutrophils and were comparable to WT mice treated with saline. Cisplatin-treated WT mice showed a moderate increase in IFN-γ–expressing neutrophils compared with WT mice treated with saline. Compared with cisplatin-treated WT mice, IL-10 KO mice treated with cisplatin showed an even greater increase in renal IFN-γ–positive neutrophils. Although the number of IFN-γ–positive neutrophils was increased in the IL-10 KO mice, the IFN-γ content of individual neutrophils, as judged by the mean

![FIGURE 1.](http://www.jimmunol.org/) Cisplatin-induced changes in serum IL-10 levels, renal IL-10R1 expression, and renal STAT3 phosphorylation. A. Blood collected from WT mice at different time intervals after cisplatin injection was analyzed for IL-10 by ELISA. *p < 0.05 versus 24 h and 72 h cisplatin; †p < 0.05 versus all other groups. n = 3–6. B. Kidneys from WT mice harvested 24 h after saline or cisplatin treatment were used to determine the expression of IL-10, IL-10R1, and IL-10R2 by real time RT-PCR. †p < 0.05 versus saline. n = 3–4. C. Kidneys from WT mice obtained 24 h and 48 h after saline or cisplatin treatment were used to determine phosphorylation of STAT3 by Western blot analysis. n = 4.
fluorescence intensity of IFN-\(\gamma\) expression, was similar in the different groups of mice, consistent with previous observations in renal ischemic injury (12).

Neutrophils contain IFN-\(\gamma\) and release it upon activation (40, 41). IFN-\(\gamma\) neutralization is reported to attenuate kidney injury in renal ischemic injury (13). To determine the significance of IFN-\(\gamma\) in cisplatin nephrotoxicity, we examined renal function in WT and IL-10 KO mice treated with cisplatin in the presence of an IFN-\(\gamma\)-neutralizing Ab or isotype control Ab (Fig. 4C). Consistent with the results in Fig. 2, cisplatin-treated IL-10 KO mice sustained more severe renal failure than WT mice. However, neutralization of IFN-\(\gamma\) had no impact on renal function in either strain of mice. Thus, in contrast to renal ischemic injury (12, 13), cisplatin nephrotoxicity and the effects of IL-10 on cisplatin nephrotoxicity are independent of IFN-\(\gamma\).

Cisplatin upregulates renal dendritic cell expression of IL-10

Dendritic cells form an abundant population of leukocytes in the kidney and are known to attenuate nephrotoxic nephritis and cisplatin nephrotoxicity in mice (7, 15). IL-10 is an anti-inflammatory cytokine produced by many cell types, including dendritic cells (19, 20). We have shown recently that depletion of dendritic cells in the CD11c-DTRtg system exacerbates cisplatin nephrotoxicity (15). This pattern of response to cisplatin in dendritic cell-depleted mice is similar to that observed in IL-10 KO mice (Fig. 2). We had also shown recently that dendritic cell depletion results in an increase in neutrophil influx, similar to that observed in the absence of IL-10 (Fig. 3). These observations raise the possibility that the production of IL-10 by dendritic cells in response to cisplatin treatment is responsible for the protective effect of dendritic cells in cisplatin nephrotoxicity. We sorted renal dendritic cells from saline- or cisplatin-treated mice 24 h postinjection and measured the expression of IL-10 by real-time RT-PCR (Fig. 5). Renal dendritic cells from cisplatin-treated mice showed a 10-fold increase in IL-10 expression as compared with saline-treated mice.

Dendritic cell IL-10 provides partial protection in cisplatin nephrotoxicity

Dendritic cells have been reported to produce IL-10 and attenuate inflammation in allergic asthma, endotoxin-induced uveitis, and is-
chemia reperfusion injury of the liver (3, 20, 32). Having determined that dendritic cells and endogenous IL-10 protect kidneys from cisplatin nephrotoxicity, and renal dendritic cells express IL-10 in response to cisplatin treatment, we investigated the role of dendritic cell IL-10 production in the attenuation of cisplatin nephrotoxicity. We used a conditional cell ablation method to determine the effect of dendritic cell IL-10 in cisplatin nephrotoxicity (Fig. 6A). In this technique, equal numbers of IL-10 KO and CD11c-DTRtg mice bone marrow cells are injected into irradiated WT mice. Postinjection of DT, these mixed chimeric mice selectively lack dendritic cell-derived IL-10. First, as a control to determine the effect of 50% dendritic cell depletion on cisplatin nephrotoxicity, we made mixed chimeric mice containing hematopoietic cells equally derived from WT and CD11c-DTRtg mice and WT to WT chimeric mice. These mixed chimeric mice were injected with DT and cisplatin, and renal function was determined by measuring BUN and serum creatinine. Depletion of 50% of dendritic cells in mixed chimeric mice resulted in a similar degree of renal dysfunction as nonablated WT to WT chimeric mice as determined by the levels of BUN and serum creatinine (68 ± 31.6 versus 75.3 ± 17.9 mg/dl; 0.5 ± 0.1 versus 0.6 ± 0.1 mg/dl, respectively at 48 h). These findings also indicate that 50% depletion of dendritic cells in mixed chimeric mice, by itself, does not significantly impact on cisplatin nephrotoxicity. Next, to determine the role of dendritic cell-derived IL-10 in cisplatin nephrotoxicity, we injected mixed chimeric mice containing IL-10 KO and CD11c-DTRtg–derived hematopoietic cells with cisplatin or cisplatin and DT and compared the extent of renal dysfunction with dendritic cell-depleted and nondepleted CD11c-DTRtg to WT chimeric mice (Fig. 6B). DT was injected twice, 24 h before and 24 h after cisplatin injection. Mixed chimeric mice depleted of IL-10–producing dendritic cells showed a moderate increase in BUN and serum creatinine (Fig. 6B) as compared with nondepleted mice at 48 h, but not at 24 h, after cisplatin treatment. In contrast, consistent with our recent report (15), dendritic cell-depleted CD11c-DTRtg to WT chimeric mice showed severe renal dysfunction compared with nondepleted mice. These results indicate that IL-10 of dendritic cell origin accounts for some, but not all, of dendritic cell-mediated protection against cisplatin nephrotoxicity.

**Discussion**

Studies from many laboratories over the past decade have firmly established the role of inflammation in the pathogenesis of renal diseases of various origins, including ischemic and toxic kidney injury. Renal cells and resident leukocytes, in response to ischemic or toxic insults, secrete a wide range of chemokines and cytokines (9, 10, 12, 13, 37). These mediators of inflammation upregulate the expression of adhesion molecules and attract different populations of leukocytes that include neutrophils, macrophages, T cells, NK cells, and dendritic cells, which may further exacerbate injury by producing soluble mediators of inflammation (9, 11–13, 42, 43). Concurrent with the induction of a stress-activated inflammatory response, many agents with anti-inflammatory properties (e.g., adenosine, NO, netrin-1, IL-10, and heme oxygenase) are produced that may prevent tissue injury or help in tissue repair/remodeling subsequent to injury in different organs and tissues, including the kidneys (2–8). IL-10 is a multifunctional anti-inflammatory cytokine that has been reported to attenuate different...
renal pathologies (18, 23–25). Our earlier studies using a cell ablation mouse model established that renal dendritic cells protect the kidneys from cisplatin-mediated injury. In this study, we investigated the role of endogenous IL-10 in cisplatin nephrotoxicity using IL-10 KO mice. We also explored the role of IL-10 produced by dendritic cells in cisplatin nephrotoxicity. Our findings indicate that endogenous IL-10 and dendritic cell IL-10 protect mice from cisplatin nephrotoxicity.

In the current study, cisplatin treatment caused an early decrease in serum IL-10, followed by an increase at later time intervals. The reason for the initial decrease in serum IL-10 is not known, but could reflect effects of cisplatin on circulating or bone marrow leukocytes. Cisplatin treatment increased renal expression of IL-10R1 but not IL-10R2, consistent with an earlier observation in LPS-stimulated neutrophils (44). The basal level of renal IL-10R2 expression was high relative to IL-10R1 expression, suggesting that IL-10R1 expression is rate limiting to IL-10 signaling. Cisplatin treatment caused marked phosphorylation of STAT3 in kidneys. Although IL-10 signals through STAT3, other cytokines that are known to increase in renal injury, such as IFN-γ, also may have contributed to STAT3 phosphorylation in response to cisplatin treatment. Taken together, these findings indicate a possible function for endogenous IL-10 in cisplatin nephrotoxicity. This role was further established in studies using IL-10 KO mice, which demonstrated a marked increase in cisplatin-induced renal dysfunction and renal inflammation in the absence of endogenous IL-10. Endogenous IL-10 has also been shown to be protective in other forms of kidney injury, such as ischemia-reperfusion injury and immune-complex glomerulonephritis (22, 38) and in injury to other organs, such as liver (3, 45), heart (46), lung (21), and intestine (47).

Neutrophils are mobilized to sites of tissue injury under the influence of chemokines and represent the hallmark of inflammation and tissue damage. The extent of neutrophil infiltration into the kidney correlates with the magnitude of kidney injury (13, 15). Neutrophil infiltration was determined using the Ly-6G Ab rather than the commonly used Gr-1 Ab because the latter detects both monocytes and neutrophils (15, 48). Infiltration of neutrophils, but not other leukocyte populations, was more abundant in IL-10 KO mice compared with WT mice, consistent with our earlier observations (15). These neutrophils were positive for IFN-γ. Although the number of IFN-γ–positive neutrophils was increased in the absence of IL-10, the IFN-γ content of individual neutrophils and the percentage of IFN-γ–positive neutrophils were similar to WT mice. Neutrophils contain stores of IFN-γ that are released in response to stimulation (40, 41). In this regard, IFN-γ has been shown to aggravate kidney injury (13, 39). Mice negative for IFN-γ in hematopoietic cells showed attenuation of kidney injury in renal ischemia reperfusion injury. However, IFN-γ appears to play little role in cisplatin nephrotoxicity based on the lack of an effect of IFN-γ neutralization on renal dysfunction. With regard to neutrophil infiltration, we do not know if the reduction in

FIGURE 6. Dendritic cell-derived IL-10 partially mediates dendritic cell attenuation of cisplatin nephrotoxicity. A. Approach used to determine the contribution of dendritic cell IL-10 in cisplatin nephrotoxicity. Treatment of mixed bone marrow chimeric mice reconstituted with bone marrow from CD11c-DTRtg mice and IL-10 KO mice with DT causes depletion of IL-10 expressing dendritic cells leaving behind with only IL-10–deficient dendritic cells. B. WT mice were reconstituted with bone marrow from CD11c-DTRtg mice (n = 5) or a mixture of CD11c-DTRtg and IL-10 KO bone marrow (n = 10–13) and were treated with cisplatin or DT and cisplatin. Blood collected at 24 h and 48 h after DT treatment was analyzed for BUN and serum creatinine.
neutrophil influx in the presence of endogenous IL-10 accounts for the protection against cisplatin kidney injury. Likewise, further studies are required to determine if the decrease in neutrophil influx resulted from a direct effect of IL-10 on neutrophils or from either an IL-10–induced decrease in the production of neutrophil attractants or an indirect result of decreased tissue damage. We note that the expression of the neutrophil chemokine KC is dramatically increased in both ischemic (49) and cisplatin-induced kidney injury (37) and that endogenous IL-10 limited the increase in CXCL1 expression.

Our reported studies indicate that conventional dendritic cells protect mice from cisplatin nephrotoxicity (15). Likewise, Lech et al. (50) found that resident dendritic cells protect against renal ischemic injury, perhaps due to activation of the single Ig IL-1–related receptor. Injection of bone marrow–derived dendritic cells has been reported to aggravate ischemic kidney injury (13). In our hands, injection of bone marrow–derived dendritic cells did not alter cisplatin nephrotoxicity (data not shown). However, bone marrow–derived dendritic cells cocultured with cisplatin-treated renal epithelial cells showed increased expression of MHC class I, MHC II, CD80, and CD86, whereas these activation markers were not affected on renal dendritic cells by cisplatin treatment in vivo (data not shown) (15). These results suggest that bone marrow–derived and tissue–resident dendritic cells may differ with respect to their anti-inflammatory properties. These observations also invite caution regarding the interpretation of studies that use cultured dendritic cells in in vivo models.

Under steady-state conditions, dendritic cells suppress inflammation by various mechanisms including production of IL-10 (20, 51). In response to apoptotic cell uptake, dendritic cells secrete more IL-10 and less proinflammatory cytokines (52, 53). In contrast, dendritic cells encountering endogenous ligands of necrotic cells produce proinflammatory cytokines (54). However, in vivo, the response of tissue–resident dendritic cells to dying cells is not clear. In allergic asthma and endotoxin–induced uveitis, dendritic cells produce IL-10 and ameliorate inflammation (20, 32). Recently, hepatic dendritic cells were shown to produce IL-10 and attenuate sterile inflammation of the liver (3). As dendritic cells are known to produce IL-10, we first examined the renal dendritic cell production of IL-10 after cisplatin treatment. IL-10 expression by renal dendritic cells was increased 10–fold after cisplatin treatment compared with saline–treated mice. However, we could not detect any difference in IL-10 expression in whole kidneys obtained from saline– or cisplatin–treated mice. The latter observation might be due to substantial dilution of mRNA of renal dendritic cells by mRNA from other renal cells. In this regard, renal dendritic cells constitute <0.1% of total kidney cells.

Establishing a direct link between dendritic cell IL-10 and cisplatin nephrotoxicity requires a system in which dendritic cells lack the capacity to produce IL-10. This can be achieved either by conditional gene ablation or conditional cell ablation (33, 55). The conditional cell ablation approach we employed has the advantages of speed, lower cost, and, because the ablation is only temporary, a lower likelihood for the development of compensatory pathways compared with conditional gene ablation (33, 55). This method has been used to investigate the function of different secreted factors or molecules of dendritic cells, including IL-15 (50), B cell activating factor, macrophage migration inhibition factor (55), and MHC II (57) in normal immune homeostasis, immunity, and tolerance. Injection of DT into chimeric mice having leukocytes equally derived from CD11c–DTRtg and IL-10 KO bone marrow causes depletion of CD11c–DTRtg dendritic cells, leaving behind only the IL-10 KO dendritic cells. Using this approach, we showed a protective function for dendritic cell IL-10 in cisplatin nephrotoxicity. However, considering that the attenuation of kidney injury by dendritic cell IL-10 was incomplete, other dendritic cell mechanisms must also have accounted for the protective actions of dendritic cells.

Endogenous IL-10 provides marked protection against cisplatin nephrotoxicity. It is possible that IL-10 produced by other cells, such as Tregs, protect the kidneys from cisplatin–induced nephrotoxicity (7, 58, 59). Tregs are regulated by dendritic cells through their cell surface and secreted molecules, including MHC II and ICOS–L (7, 57, 60). In support of this notion, a recent study showed a drastic reduction in Treg number after depletion of dendritic cells in mice (57). Likewise, constitutive depletion of dendritic cells produced a break in self-tolerance and a spontaneous fatal autoimmune (61). Thus, it is possible that dendritic cell regulation of Treg function, including IL-10 production, contributes to the attenuation of cisplatin nephrotoxicity. In this regard, studies in a murine model of chronic kidney disease showed attenuation of kidney injury by Tregs (59). Likewise, recent findings support a role for Treg–mediated suppression of innate immunity and amelioration of kidney injury in renal ischemia reperfusion injury and cisplatin toxicity (58, 62).

In summary, we have determined the effect of cisplatin on renal IL-10 signaling and investigated the role of endogenous IL-10 and dendritic cell–produced IL-10 in cisplatin–induced acute kidney injury. Endogenous IL-10 is protective in cisplatin nephrotoxicity and dendritic cell–derived IL-10 partially mediates dendritic cell attenuation of cisplatin nephrotoxicity. The protective role of dendritic cells and endogenous IL-10 might be linked through the regulation of Tregs. Further studies are warranted on dendritic cell regulation of Tregs in cisplatin nephrotoxicity and on IL-10 actions in acute kidney injury. Elucidation of these mechanisms may be exploited for pharmacologic or cell–based interventions to treat acute kidney injury.

Acknowledgments
We thank Ganesan Ramesh, Chris Norbury, and Rob Bonneau for support and critical review of the manuscript. We also thank Wei Wang for technical assistance and Nate Sheaffer and Dave Stanford of the Penn State Hershey Flow Cytometry Core Facility for support.

Disclosures
The authors have no financial conflicts of interest.

References