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

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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-producing neutrophils was markedly increased in IL-10 knockout mice compared with wild-type mice. However, IFN-γ neutralization had no impact on renal dysfunction, suggesting IFN-γ-independent mechanisms of tissue injury 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: 4904–4911.
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 reg-
ulation 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-
SPIE(p 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 nephropathy (15).
It is possible that production of IL-10 by dendritic cells is an en-
dogenous 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 examine the role of dendritic cell IL-10, we employed a con-
ditional cell ablation approach in which a mixed bone marrow
chimera was created containing hematopoietic cells equally de-
formed to 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-
duation 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 C57BL/6 mice and IL-10
KO mice (B6.129P2-Ii10tm1Cgn/J) and CD11c-DTRtg mice (B6.PV-Tg
Ifgax-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
from two doses of 600 rad, 4 h apart) and then injected with 10 million donor
bone marrow cells by tail vein. These chimeric mice were used for
Experiments at 14 wk of age. Four sets of bone marrow chimera mice were
generated: wild-type (WT) mice reconstituted with WT bone marrow
or CD11c-DTRtg bone marrow and WT mice reconstituted with equal
amounts of WT and CD11c-DTRtg bone marrow or IL-10 KO and CD11c-
DTRtg bone marrow. Animals were used according to protocols approved
by the Institutional Animal Care and Use Committee of The Pennsylvania
State University College of Medicine (Hershey, PA).

Drug and IFN-γ Ab administration and renal function
assessment

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 (D2072B,
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 Ab retrieval was performed using 10 mM
sodium citrate buffer. Immunohistochemistry for neutrophils was performed
using rat anti-mouse neutrophil-specific primary Ab (Ly-6G, clone IA18,
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 membrane was 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 (Amerham Biosciences, Pis-
catayow, 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 (HL-3), F4/80 (BM8),
CD11b (M1/70, 7/4) (AbD Serotec, Oxford, U.K.), Ly-6G (IA8, Bioresearch,
San Diego, CA), CD4 (GK1.5), CD8 (53-6.7), B220 (RA3-6B2), NK1.1
(PK136), CD3 (145-2C11), PDCA-1 (927), and IFN-γ (XMGI2.2).

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 FACSCalibur and analyzed using CellQuest (BD Phar-
mingen) or WinMDI 2.8 free software (http://facs.scripps.edu/software.
hml). Renal dendritic cells (CD45+CD11c+) from single-cell suspensions
of the kidney were sorted by flow cytometry using a MoFlo (Beckman
Coulter, Brea, CA) cell sorter (purity >90%).

Quantification of mRNA by real-time PCR

Total RNA was extracted from kidneys or 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′-AGG AAG GGC
GAC GCC GAG CAG ATT GCT-3′; reverse: 5′-TGG AGC CTG GTC
AGC TGG TCA CAG GTC-3′), IL-10R2 (forward: 5′-GCT
AGC TCT AGG AAT GAT TC-3′; reverse: 5′-ATT CCT CTG CA A GTT
CCA C-3′), IL-10 (forward: 5′-CCA AGC ATG CCA GAA GAT AA-3′;
reverse: 5′-AGG G GA GAA ATC GAT GAC AG-3′), β-actin (forward:
5′-TGT TAC CAA CTG CCA CGA CA-3′; reverse: 5′-GGG GTG TTG
AGA AGC TTG CTC ACC A-3′), CCL2 (forward: 5′-ATG CAG GCT CTC
GTC ATG-3′; reverse: 5′-GCT TGA GGT GGT TGT TGA-3′), CXCL1 (for-
ward: 5′-GCT GGG ATT CAC TCT AAC AA-3′; reverse: 5′-GGG GAA
CAT CTT GA-3′), CCL1 (forward: 5′-ATG CAG GCT CTC GTC
ATG-3′; reverse: 5′-GCT TGA GGT GGT TGT TGA-3′), ICAM-1 (for-
ward: 5′-AGA TCA CAT CTC GGG TG-3′; reverse: 5′-CCT CAG
AGC GAA ACA AG-3′). The amplification specificity

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 (D2072B,
Diazyme Laboratories, Poway, CA).

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 earlier and 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 nephropathy. 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/)
fluorescence intensity of IFN-γ expression, was similar in the different groups of mice, consistent with previous observations in renal ischemic injury (12).

Neutrophils contain IFN-γ and release it upon activation (40, 41). IFN-γ neutralization is reported to attenuate kidney injury in renal ischemic injury (13). To determine the significance of IFN-γ in cisplatin nephrotoxicity, we examined renal function in WT and IL-10 KO mice treated with cisplatin in the presence of an IFN-γ–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-γ 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-γ.

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. 6). 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, 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 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 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 (56), 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 autoimmunity (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.

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